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STUDIES ON AMPHIBIAN ENDOCRINES. THE PARATHYROID GLAND OF *RANA RIDIBUNDA*

By

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(Received December 16, 1966)

Introduction

MAURER (1888) was the first to demonstrate that Amphibia possess parathyroid glands, homologous to those of higher vertebrates. Subsequently, other zoologists (THOMPSON (1911), ROMEIS (1926), WAGGENER (1929), SHAPIRO (1933), HOLMES (1934) and RIZKALLA (1964) studied these glandules in some amphibians.

The scope of this paper is to describe in detail the morphology, histology and histochemical properties of the parathyroid glands of *Rana ridibunda*, one of the commonest frogs in Hungary. This anuran animal was chosen for many reasons. So far as I am aware, the parathyroids in this frog have not hitherto been located or described. Moreover, it is of particular interest to mention that in *R. ridibunda* the anatomical position, size and number of the parathyroid glands are markedly differing from those described for other anurans.

Concerning the structure of the amphibian parathyroids, on the other hand, conflicting findings have been recorded by MAURER (1888), KRAUSE (1923), ROMEIS (1926) and WAGGENER (1929). The present studies were designed to throw light on the differences between the parathyroid glands of *R. ridibunda* (Ranidae) and those of other anuran parathyroids, with special reference to those of *Bufo regularis* (Bufonidae) (cf. RIZKALLA, 1964).

Materials and methods

Parathyroid glands from *R. ridibunda* (collected in October) were largely used. Various methods of fixation were employed. As fixatives Zenker's acetic, Zenker's formol, Susa's and Regaud's fluids were used. Following the latter fixative the material was postchromated with 4% potassium bichromate for a week at 37 °C.

Serial paraffin sections of 4—6 μ thickness were prepared. The following stains were employed: Delafield's haematoxylin-eosin for routine work; Delafield's haematoxylin, van Gieson and orcein for connective tissue; modified Mallory's stain (cf. SCHAFER, 1956) for colloid and connective tissue; Heidenhain's azan for colloid; Mayer's mucicarmine and toluidine blue for mucus: Heidenhain's iron haematoxylin for mitochondria and Sudan black B for demonstration of fat in paraffin sections.

Observations

Two parathyroid glands are found in *R. ridibunda*, each lying far about 5 mm from the longitudinal axis of the body. The gland is oval in shape, measuring about 4 mm in length and 2 mm in width. Accordingly, the parathyroids are well-visible with the naked eye.

Each parathyroid gland is situated just close to the outer border of the posterior cornu of the hyoid apparatus and roughly at the level of the larynx. Close to, and laterally to each gland there are the external jugular vein, hypoglossal nerve and the lingual artery. The vein, nerve and artery run close to one another and are always connected with the gland by connective tissue fibres. The gland extends antero-posteriorly from the connection of the lingual and faciomandibular veins till half-way of the external jugular vein. Accordingly, once the external jugular vein is clearly dissected, one cannot miss the parathyroid gland.

A very small branch from the hypoglossal nerve innervates the gland. The gland is nourished by a small branch of the lingual artery, while its blood is drained in the external jugular vein. The colour of the parathyroid gland is variable in different specimens, ranging from light red to dark brownish in colour. This variation in colour is attributed to different vascularization.

The parathyroid glands of *R. ridibunda*, like those of other amphibians, are simple in structure. They can be referred to as the most primitive type. Since parathyroid glands appear first in this class of vertebrates, they are far from the complexity of structure seen in the higher classes of vertebrates.

The parathyroid gland is surrounded by a rather thin connective tissue capsule (Fig. 2), which is unbroken except where blood vessels pass through or leave it. The capsule is formed only of collagenous fibres, showing a light brown colour when stained with orcein. Dark brown-stained elastic fibres occur only in the walls of blood vessels. Sometimes, parts of the capsule are covered by a relatively thick layer of adjoining connective tissue connecting the neighbouring blood vessels and nerves. A fatty connective tissue is of frequent occurrence in close contact with the gland, occasionally invading the parathyroid to intermingle with the subcapsular epithelial cell masses (Fig. 1).

Collagenous connective tissue fibres, deviating from the capsule, usually form projection-like trabeculae. They penetrate the epithelium of the parathyroid especially at the sites where the blood vessels enter the gland. In rare cases, trabeculae reach the central mass of the parathyroid. Such connective tissue bands are best illustrated with connective tissue stains, especially with modified Mallory's stain.

The afferent vessel penetrates the parathyroid gland in close proximity to the efferent one. Where these arteries and veins enter and leave the capsule,

Fig. 1. Photomicrograph of a section of the parathyroid gland of *R. ridibunda* showing the hilum, fatty tissue and its peripheral and central zones. ×55. *b.cp.*, blood capillary; *b.vs.*, blood vessel; *c.ts.ca.*, connective tissue capsule; *ct.z.*, central zone; *d.s.ce.*, dark-stained cells; *f.ts.*, fatty tissue; *h.*, hilum; *l.s.ce.*, light-stained cells; *p.z.*, peripheral zone

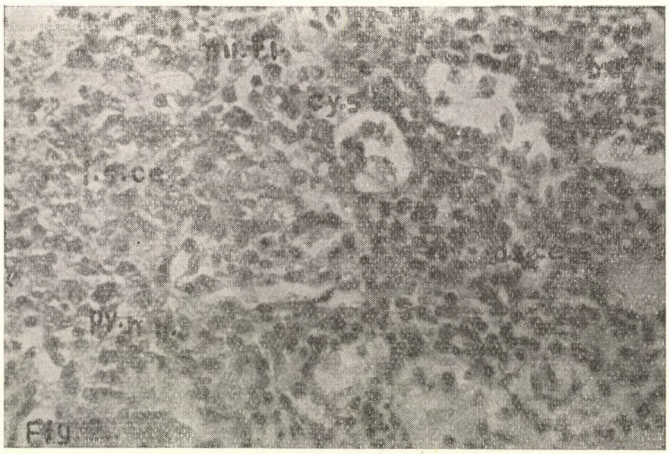

Fig. 2. Photomicrograph of a section through the parathyroid gland of *R. ridibunda* showing the difference in structure between the poorly and darkly-staining masses of cells. ×215. *b.cp.*, blood capillary; *cy.sh.*, cytoplasmic shreds; *d.s.ce.*, dark-stained cells; *l.s.ce.*, light-stained cells; *mi.fi.*, mitotic figures; *py.nu.*, pycnotic nuclei

usually there is a depression resembling a hilum in the body of the parathyroid gland (Fig. 1). As soon as the afferent vessel penetrates the capsule it divides into numerous tributaries, which in turn continue to branch and anastomose, forming a closely meshed network of capillaries throughout the whole parathyroid tissue (Figs 1, 2 and 3). Sometimes the blood capillaries anastomose to form relatively large blood sinuses.

The parathyroid gland consists first of all of epithelial cells arranged mainly in large groups and sometimes in cords which are separated by thin

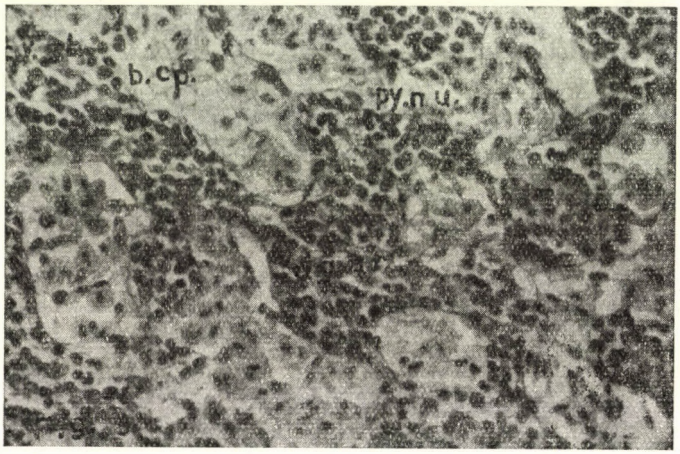

Fig. 3. Photomicrograph of a section through the parathyroid gland of *R. ridibunda* showing colloid. ×235. *b.cp.*, blood capillary; *cy.sh.*, cytoplasmic shreds; *dg.ar.*, degenerated area; *py.nu.*, pycnotic nuclei

connective tissue septa and blood vascular channels (Figs 2 and 3). The peripheral region of the gland always appears darker than the central region (Fig. 1). Such appearance is due to the peripheral region of the gland being mainly composed of cells with thin cytoplasms, and comparatively large, darkly-staining nuclei which lie close to one another. Accordingly, the microscopic appearance of the parathyroid gland of *R. ridibunda* reminds of the structure of the thymus gland of vertebrates, characterized by its dark outer cortex and light inner medulla.

The parathyroid cells present all gradations in shape, ranging from the spherical to an elongate type (Fig. 2). Cytoplasmic projections make some cells irregular in outline. The variability of cells is best seen in the central region of the gland and in close proximity to the blood vessels where the cells are large and the cytoplasm is noticeably more abundant. The shape of the nucleus is determined by the shape of the cell, some nuclei being approximately spherical, others elongated. In general, spherical nuclei are the most frequent.

The nuclear material is very conspicuous in stained preparations. The nuclear membranes appear distinct, and one or two nucleoli are found in each nucleus. Nucleoli are best demonstrated with chromatin stains, such as Heidenhains's iron haematoxylin. The chromatin occurs in granules or clumps, distributed evenly throughout the nucleoplasm (Figs 2 and 3).

Some parathyroid cells contain acidophilic colloid in the form of large granules or globules (Fig. 4). Such colloidal secretion is usually found in cytoplasmic vacuoles, appearing rosy-red or bright orange in colour and ruby red

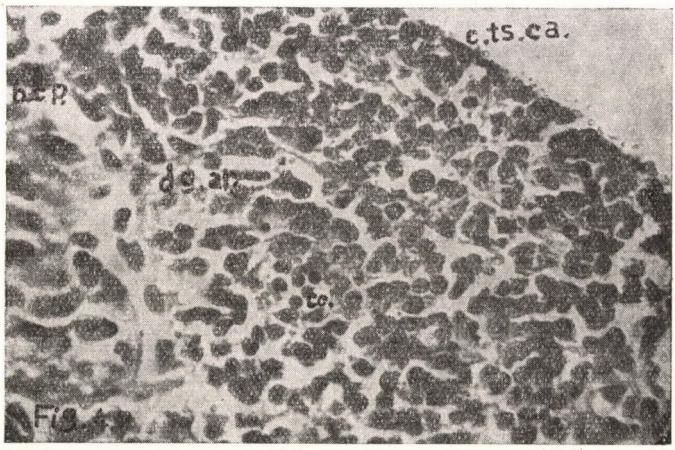

Fig. 4. Photomicrograph of a section through the parathyroid gland of *R. ridibunda* showing colloid. ×445. *b.cp.*, blood capillary; *co.*, colloid; *c.ts.ca.*, connective tissue capsule; *dg.ar.*, degenerated area

when stained with modified Mallory's stain and Heidenhain's azan stain, respectively. The colloidal material is homogeneous and highly refractive. The amount of colloid differs with the specimen, the variation being explainable by the varying physiological activity of the gland. Occasionally, colloidal globules are seen within vascular channels and this is understandable since these secretions may carry the parathyroid hormone, which must circulate in the blood stream.

A careful examination of the stained preparations has revealed a difference in the staining reaction of different parts of the central region of the parathyroid gland. Some groups of cells appear more deeply-stained than others (Figs 1 and 2). The darker nuclei are more abundant in chromatin than those poorly staining with nuclear stains. Gradations between the previous extreme two types of nuclei are also present (Fig. 2). This variation in staining does not appear to be confined to any special method of fixation or staining, since this feature is quite distinct in all preparations.

The differences in nuclear staining most probably represent different stages in the life cycle of cells. The deeply-staining nuclei thus indicate an

early stage of degeneration (Figs 2 and 3). As the degenerative process continues the pycnotic nuclei are disintegrated, showing fragmentation. On the other hand, in some parts of the parathyroid the cytoplasm shows degenerative changes, namely, vacuolization. Such cytoplasmic changes continue until the parathyroid tissue has resolved itself into an open network of faintly-staining protoplasmic strands, since a great part of the cytoplasm has been dissolved by a process of cytolysis (Fig. 3). Accordingly, an early stage in the autolysis of the parathyroid gland results in the appearance of numerous degenerating areas distributed throughout the parathyroid tissue (Figs 3 and 4).

Some parts of the parathyroid gland stain faintly, in contrast to the surrounding deeply-staining parts, reminding of the islets of Langerhans in the pancreas of vertebrates. Most probably, such masses of cells are responsible for regeneration in the parathyroid and can be referred to as growth centres. Mitotic figures are occasionally encountered in such growth centres (Fig. 3).

It is worthy of mention that no colloidal secretory granules or globules are observed to be produced by the poorly-staining masses of cells, i.e., the growth centres. The colloidal secretion is always confined to the deeply-staining masses of cells which are undergoing degenerative processes. Accordingly, the degenerated masses of cells found in the parathyroid tissue may be attributed to their colloidal production by a holocrine type of secretion.

Since the parathyroid anlage originates from the pharyngeal epithelium, it might be expected that some of the parathyroid cells retain their mucous-secreting power. Therefore, an attempt was made to test the gland for mucus with Mayer's mucicarmine and toluidine blue. Negative results were obtained.

Mitochondria are represented mainly by granules but sometimes by rods. Both are found far from the nucleus, being confined to the peripheral regions of the cell. In some of the parathyroid cells the nuclei are eccentric in position, thus the mitochondria are concentrated in the opposite poles of cells.

Positive results were obtained with Sudan black B. Fat droplets are of frequent occurrence in the cells of the peripheral region of the parathyroid tissue (Fig. 5). In the central region of the parathyroid, on the other hand, fewer cells contain fat droplets, the other being practically free from fat.

Discussion

In *R. ridibunda* the parathyroids are represented by two glands, one on each side of the body. This finding is inconsistent with that recorded for Anura. The latter generally possess four parathyroid glandules, the two of each side usually lying anterior to each other (cf. THOMPSON, 1911; ALLEN, 1920; WAGGENER, 1929 and SHAPIRO, 1933). In *Bufo* (RIZKALLA,

1964) the two parathyroid glandules of each side lie dorsal to each other, the ventral glandule being much larger than the dorsal one.

The parathyroid glands of *R. ridibunda* are relatively large in size as compared to those of other Anura, each measuring about 4 mm in length. Such large parathyroids have not been recorded before. Since in *R. ridibunda* the parathyroid number is reduced to two, it seems reasonable that the glands attain this large size as a feature of compensation. SHAPIRO (1933) claimed that in *Rana*, generally the parathyroid measures about 1 mm in length,

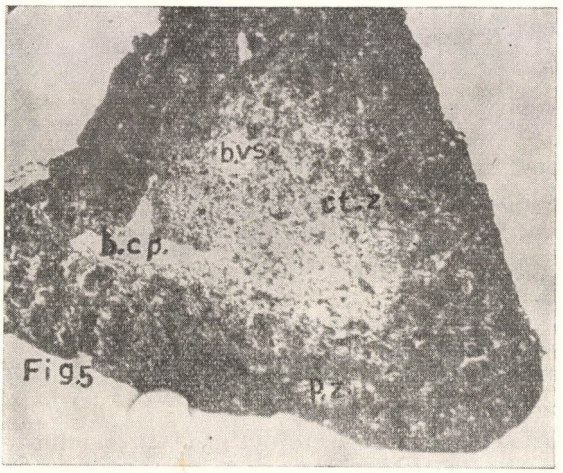

Fig. 5. Photomicrograph of a section of the parathyroid gland of *R. ridibunda* showing fat in the peripheral zone of the gland. ×65. *b.cp.*, blood capillary; *b.vs.*, blood vessel; *ct.z.*, central zone; *p.z.*, peripheral zone

while in *Xenopus* the glands are very small, ranging from 0.3 to 0.4 mm along their longitudinal axes.

The parathyroid gland of *R. ridibunda* is situated in close contact with, and medially to, both the external jugular vein and the lingual artery. SHAPIRO (1933), on the other hand, stated that in *Rana* the parathyroids lie generally between the external jugular vein and the lingual artery. Moreover, the glands are found very near to the branching of the carotid arch, *i.e.*, just in front of the carotid labyrinth *Xenopus* (SHAPIRO, 1933) the parathyroids are bounded by the carotid arch anteriorly and the systemic arch posteriorly. In *Bufo* (RIZKALLA, 1964) the parathyroids are found dorsal and ventral to both the systemic and pulmocutaneous arches, to which they are closely connected. THOMPSON (1911) and ALLEN (1920) stated that the parathyroids of anurans lie usually near the aortic arches. From the previous findings, one can come to the conclusion that the anuran parathyroids are in close association with either the arterial arches and their tributaries or the branches of the anterior venae cavae.

It is worthy of mention that the anuran parathyroid cells are characterized by their whorled arrangement (cf. THOMPSON, 1911; ROMEIS, 1926; WAGGENER, 1929 and SHAPIRO, 1933). On the contrary, the parathyroid cells in *R. ridibunda* are arranged in groups or cords which latter do not wind upon one another in spirals. Accordingly, the parathyroid of this frog resembles more closely those of urodeles than those of anurans. The parathyroid of *Bufo* (RIZKALLA, 1964) presents an intermediate condition as in some parts of the principal parathyroid the whorled arrangement is clear, while in other parts it is not. But, such whorled arrangement is usually very conspicuous in the small accessory parathyroids.

The parathyroid tissue of *R. ridibunda* is not purely epithelial as the cell masses or cords are separated by connective tissue septa as well as blood vascular channels. The connective tissue septa are more predominant in the subcapsular region than elsewhere. MAURER (1888) and KRAUSE (1923) described for the parathyroid only cell cords which were separated by vascular connective tissue. ROMEIS (1926), on the contrary, claimed that the anuran parathyroid consists of purely epithelial tissue whose interior is completely free from both connective tissue and blood vessels. A network of blood capillaries is confined to the subcapsular region.

The parathyroid of *R. ridibunda* is highly vascularized, a finding never described before for any anuran parathyroid. By using modified Mallory's stain, in some parts of the parathyroid the blood capillaries in the network are separated from one another by only a thin strand of parathyroid cells. Such complex network of blood vessels and capillaries keeps blood in close contact with the parathyroid tissue for a prolonged time.

Accessory parathyroids have been recorded for anurans by MAURER (1888), GAUPPE (1902), ROMEIS (1926), WAGGENER (1929) and RIZKALLA (1964). In *R. ridibunda* small parathyroid lobes are occasionally present in the neighbourhood of the body of the gland, but careful examination of serial sections have revealed that such glandular lobes are not independent accessory parathyroids but parts of the parathyroid gland.

Different results have been recorded concerning the fat content of the parathyroid tissue of Anura. WAGGENER (1929) and RIZKALLA (1964) obtained negative results in *Rana* and *Bufo*, respectively. However, *R. ridibunda* presents fat droplets in its parathyroid tissue, especially in the peripheral region of the gland. Such finding may be attributed to a process of cytoplasmic fatty degeneration which takes place in the cells of this region. Moreover, such cells present pycnotic nuclei indicating a process of degeneration. Fat was also demonstrated in the parathyroids of some species of *Rana* by ROMEIS (1926) and WAGGENER (1929).

SUMMARY

Rana ridibunda possesses two parathyroid glands, one on each side of the longitudinal axis of the body. The gland is situated medially and close to both the external jugular vein and lingual artery.

The gland is oval in shape and well-visible with the naked eye as it measures about 4 mm in length and 2 mm in width. The gland possesses a depression resembling a hilum, where the afferent blood vessel penetrates and the efferent one leaves the gland.

The parathyroid is formed of epithelial cells which are arranged in masses or cords. The gland is highly vascularized and the blood vessels and capillaries are distributed equally throughout the whole parathyroid tissue.

The body of the gland is usually differentiated into a darkly-staining peripheral region and a poorly-staining central one. Moreover, the latter region presents dark and light masses of cells. The darkly-staining cells indicate degeneration, while the faintly-staining masses of cells represent most probably the centres of growth responsible for regeneration in the parathyroid gland.

Some of the parathyroid cells secrete acidophilic colloidal globules and such secretory products may be the carrier of the parathyroid hormone.

The test for mucus gives negative results, whereas fat was demonstrated in the parathyroid tissue, especially in the cells of the peripheral region of the gland.

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DEMONSTRATION OF ANTIBIOTIC RESIDUES IN THE MEAT OF SLOUGHTERED ANIMALS

By

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Antibiotics are widely used for therapeutic purposes all over the world. Consequently, most people have been sensitized (GROSSKLAUS, 1967).

Several reports (KIND, 1966; KLIMA, 1966; LORENZEN, 1967; GROSSKLAUS, 1967) have been published on the reactions attributable to the consumption of foods containing antibiotic residues. Allergic conditions, such as urticaria, eczema and other dermatitides may develop and the resistance of pathogenic microorganisms may increase. The normal intestinal microflora and, consequently, the vitamin synthesis may become injured. Whilst small amounts of antibiotics have no adverse influence on the unsensitized healthy man, highly allergic organisms may develop anaphylaxis after repeated intake of antibiotics. Even lethal anaphylactic shock has been reported. Use of antibiotics in food preservation must primarily be considered from the medical point of view. In some countries, because of the adverse effects mentioned above, use of antibiotics for food preservation is prohibited (LORENZEN, 1967).

Detection of antibiotics in foods have been dealt with by several authors (KOTTER *et al.*, 1959; FREERKSEN, cit. SPROCKHOFF, 1960; SPROCKHOFF, 1960; CORETTI, 1961; GARDSIDE, cit. CORETTI, 1961; FORSCHNER, 1962; OBIGER, 1963; KIND, 1966; KLIMA, 1966; LORENZEN, 1967; GROSSKLAUS, 1967). The methods used for the detection can be divided into two groups: chemical analysis and nonspecific biological inhibition tests. Chemical analysis encounters technical difficulties and requires too much work, thus cannot be applied in the routine work. By the other method, factors inhibiting microbial growth can be demonstrated without determining the amount, identity, properties and origin of these factors. The nonspecific biological inhibition can be demonstrated with dyes indicating pH changes or reduction (KOTTER et al., 1959; SPROCKHOFF, 1960; CORETTI, 1961). If the growth of the bacterium culture used in the test, e.g., *Streptococcus thermophilus* in case of testing milk sample, is inhibited, the colour of the indicator solution (litmus 2,3,5-triphenyl-tetrazoliumchloride) remains unchanged. On the contrary, if bacteria grow undisturbed, changes in pH value, or reduction takes place which is indicated by a changed colour. Presence of the inhibitors can alternately be demonstrated with the inhibition zone which develops in agar plates inoculated with a microorganism susceptible to the inhibitor. Either the samples themselves (SPROCKHOFF, 1960; FORSCHNER, 1962; KIND, 1966; LORENZEN, 1967) or filter paper discs previously soaked in the tissue fluid are placed on the inoculated agar plate (KLIMA, 1966).

In Hungary a declaration must be enclosed to the samples submitted for laboratory examination. The declaration must reflect if the animal had been treated with antibiotic in the 5 days' period preceding its slaughtering. This declaration is important, for the efficiency of both the macroscopic meat inspection and bacteriological examination is of limited value.

Several authors have suggested that the microbiological meat control should urgently be complemented with a test for antibiotics. Thus, it seemed necessary to gather information on the antibiotic residues in the meat in Hungary and to elaborate a method for their detection.

Materials

In 1967, of the organs submitted for bacteriological examination mostly kidneys were tested for antibiotics. The organs were obtained from emergency-slaughtered, mostly sick animals. As a total, organs of 244 animals (173 cattle, 37 horses, 28 calves and 6 sheep) were tested.

Methods

To establish the suitable method, bacterium strains highly susceptible to antibiotics were used. The strains were obtained from an international collection. Four strains were used to select the most suitable of the strains suggested by different authors. The strains were as follows: *B. subtilis* ATCC 6633, *Staph. aureus* ATCC 6538, *Sarcina lutea* ATCC 9341 and *E. coli* ATCC 9637. *B. subtilis* ATCC 6633 was highly susceptible to chlortetracycline, erythromycin and streptomycin, *Staph. aureus* ATCC 6538 to chlortetracycline, erythromycin, oxytetracycline, penicillin and streptomycin, *Sarcina lutea* ATCC 9341 to chloramphenicol, chlortetracycline and penicillin, and *E. coli* to neomycin and streptomycin, as indicated in the Catalogue of the Strain Collection of the National Institute of Public Health, Budapest. Cultures grown at 37 °C overnight in a broth medium of standard composition (meat cream + yeast extract + peptone) were used as inoculum.

According to the Gardside method modified by us (1961) four pieces $10 \times 10 \times 5$ mm in size were cut from the kidney. These were frozen at −20 °C on the object-holder of the freezing microtome or in deep freezer. Agar plates of standard composition (meat cream + yeast extract + peptone medium) were inoculated, each with one of the four standard strains, by spreading two or three drops of the inoculum. Then pieces of kidney were placed on the agar surface and the plates were placed in the refrigerator at +4 °C for 4 to 4 ½ hours, and subsequently incubated at 37 °C for 18 to 20 hours.

Results

Results are summarized in Tables 1—4. Macroscopic lesions, microbiological results, pH values, isolated bacteria and the microorganisms inducing bacteraemia or septicaemia are shown separately for each animal species.

Of the 244 emergency-slaughtered animals 62 (24.6%) had been treated with antibiotics. Of the 173 cattle, 37 horses, 28 calves and 6 sheep 40 (23.1%), 8 (21.6%), 12 (42.8%) and 2 (33.3%), respectively, were treated with antibiotics just before their slaughtering.

Table 1

Results obtained with cattle

Post mortem lesions (No. of cases)	Positive cases No.	*B. subtilis*	*Staph. aureus*	*Sarcina lutea*	*E. coli*	Bacteriological result	Bacteria isolated from kidney	pH	Microbes from septicaemic cases	Microbes from bacteraemic cases
	1	+	−	−	−	V	Ae.	6.2		
Inflammation	2	+	+	+	−	B	Cl.	6.4		Clostridia
of thoracic	3	+	+	+	−	V		6.4		
organs	4	+	−	−	−	V	Ae.	< 6.0		
(26)	5	+	−	+	−	V	C., Ae., Staph.	6.2		
	6	+	+	+	+	V	Ca., Staph.	6.0		
	7	+	+	+	−	Ø		6.0		
	1	+	−	−	−	V	C., Cl.	6.2		
	2	−	+	+	−	V	C., Str.	6.4		
Adhesive	3	+	+	+	−	V	C., Cl., Str.	6.2		
peritonitis,	4	−	+	−	−	V	C., Cl., Ae.	6.2		
reticulitis	5	+	+	+	−	V	C.	6.4		
(43)	6	+	+	+	+	V	Ae.	6.8		
	7	+	+	+	−	S	C., Cl., Cor.	6.7	Corynebacteria Clostridia	
	8	+	+	+	+	V	C.	6.0		
	9	+	+	+	−	V	C.	6.2		
	10	+	−	−	−	S	C., Cl.	6.8	Clostridia, coliforms	
	1	+	+	+	−	V	Cl., Cor.	6.0		
Nephritis	2	+	−	+	−	V	Ae.	6.6		
(6)	3	+	+	+	−	V		6.8		
Mastitis (7)	1	+	+	+	+	V	C., Str.	6.0		
	1	+	+	+	−	B	C., Cl.	6.2		Streptococci, Clostrida
	2	+	−	+	−	V		6.4		
	3	+	−	+	+	V	C., Str., Staph.	6.4		
	4	+	−	+	−	V	C.	6.6		
Endometritis	5	+	+	+	−	V	C., Ae.	6.6		
and	6	+	+	+	−	V	C., Ae.	6.2		
complications	7	+	+	+	−	V	Ae.	6.0		
of calving	8	+	+	+	−	V	Ae., Staph.	6.0		
(40)	9	+	+	+	−	V	Ae., Str.	6.0		
	10	−	+	+	−	S	C., Staph.	6.0	Coliforms, clostridia, streptococci	
	11	+	+	−	−	V	Staph.	6.0		
	12	+	+	−	+	B	C., Cl.	6.0		Clostridia, coliforms
	13	+	+	−	−	V	Str.	6.8		
Arthritis	1	+	+	+	−	V	Ae., Staph.	6.0		
(13)	2	+	+	+	−	V		6.3		
Contusions, purulent and gangrenous wounds (7)	1	−	+	+	−	V	Ae.	6.0		
	1	−	+	+	−	V		< 6.0		
Other diseases	2	+	−	−	−	V	C., Staph.	< 6.0		
(21)	3	+	+	+	−	S	Ae., Staph.	< 6.0	Salmonella	
Total positive (173)	40	35	30	31	6					

Explanation: Ø, sterile; V, viscera contaminated with saprophytes; B, bacteriaemia; S, septicaemia; C, *E. coli;* Cl, Clostridia; Ae, aerobic spore-bearing germs; Str, streptococci; Staph, staphylococci; Cor, Corynebacteria

Table 2

Results obtained with horses

Post mortem lesions (No. of cases)	Positive cases No.	*B. subtilis*	*Staph. aureus*	*Sarcina lutea*	*E. coli*	Bacteriol-ogical result	Bacteria isolated from kidney	pH	Microbes from septicaemic cases
Inflammation of	1	+	+	+	—	V	C.	6.0	
thoracic organs	2	+	—	—	—	V	C.	6.0	
(3)	3	+	+	+	—	V		6.0	
Myoglobinaemia (3)	1	+	—	—	—	V		6.0	
Endometritis (1)	1	+	+	+	—	V		6.2	
Gastro-enteritis (10)	1	+	+	—	—	V	Staph.	6.6	
Contusions, purulent and gangrenous wounds (2)	1	+	+	+	—	V	C., Str.	6.0	
Other lesions (5)	1	+	+	+	—	S	Str.	6.0	Haemolyzing streptococci
Arthritis (13)									
Total	8	8	6	5	—				

Explanation, see footnote to Table 1.

In Table 5 pH values of the meat of 62 positive animals are presented. It is shown that in most of the cases pH value was below 6.4. At such values the growth of bacteria, especially that of the proteolytic ones, is inhibited.

On the basis of the intensity of inhibition caused by the antibiotic diffused from the tissue fluid of the kidney into the agar, the meat samples were divided into 3 categories. Inhibition zones not wider than 5 mm were ranked in the first category. Those showing 5—10 mm and 10—15 mm zones of inhibition were included in the 2nd and 3rd category, respectively.

As a result of the investigations, 5, 5—10 and 10—15 mm zone inhibition was observed in 54, 36 and 10% of the cases, respectively, when tested against the test strains *B. subtilis* ATCC 6633; the respective percentages were 57.5%, 22.5% and 20% for *Staph. aureus* ATCC 6538 test strain; 51.1%, 44.5% and 4.4% for *Sarcina lutea* ATCC 9341 and 83.3%, 16.7% and 0.0% for *E. coli* ATCC 9637.

Inhibition was most frequent when *B. subtilis* ATCC 6633 was used in the tests. With this test strain antibiotic was demonstrated in 50 kidney

Table 3

Results obtained with calves

Post mortem lesions (No. of cases)	Positive cases No.	*B. subtilis*	*Staph. aureus*	*Sarcina lutea*	*E. coli*	Bacteriol-ogical result	Bacteria isolated from kidney	pH	Microbes from septicaemic cases
	1	+	+	—	—	V		6.4	
	2	+	—	+	—	V		6.0	
Inflammation	3	+	+	—	—	S	C., Ae.	6.3	Proteus
of thoracic	4	+	+	+	—	V	C.	6.2	
organs	5	—	—	+	—	V		6.0	
(26)	6	—	—	+	—	V		6.0	
	7	—	—	+	—	V	C., Ae.	6.0	
	8	—	—	+	—	V		6.2	
	9	—	—	+	—	V	Ae.	6.0	
	10	—	—	+	—	V	Ae.	6.0	
	11	+	—	—	—	V	C., Ae.	6.0	
Gastro-enteritis (1)	1	—	—	+	—	V	C.	6.4	
Navel-ill (1)									
Total (28)	12	5	3	9					

Explanation, see footnote to Table 1

Table 4

Results obtained with sheep

Post mortem lesions (No. of cases)	Positive cases No.	*B. subtilis*	*Staph. aureus*	*Sarcina lutea*	*E. coli*	Bacteriol-ogical results	Bacteria isolated from kidney	pH
Gastro-enteritis (1)	1	+	—	—	—	V		6.7
Contusions, purulent and gangrenous wounds (1)	1	+	+	—	—	V	Staph.	6.7
Other (4)								
Total (6)	2	2	1					

Explanation, see footnote to Table 1

Table 5

Distribution of the positive cases by pH

	<6	6.0	6.2	6.3	6.4	6.5	6.6	6.7	6.8	Total
Cattle	3	15	7	1	6	—	3	1	4	40
Horse	1	5	1	—	—	—	1	—	—	8
Calf	1	6	2	1	2	—	—	—	—	12
Sheep	—	—	—	1	—	—	—	1	—	2

samples. With *Sarcina lutea* ATCC 9341 45 samples, with *Staph. aureus* ATCC 6538 40 samples, with *E. coli* ATCC 9637 as few as 6 samples showed antibiotic effect.

The Tables show that *E. coli* ATCC 9637 strain proved to be the least efficient in demonstrating antibiotics in the test material. The positive results obtained with this strain were overlapped by those obtained with the other 3 strains or at least with 2 of the other test strains, but not *vice versa*. On the other hand, *B. subtilis* ATCC 6633 was inhibited in 8 cases when no inhibition zone was observed with any of the other 3 strains. In 12 cases, however, no antibiotic residue was demonstrated even with this strain. As compared to the other 3 strains, efficacy of *B. subtilis* ATCC 6633 was superior in that *Staph. aureus* ATCC 6538, *Sarcina lutea* ATCC 9341 and *E. coli* ATCC 9637 strains failed to demonstrate antibiotics in 22, 17 and 56 cases, respectively. When no zone of inhibition was obtained with *B. subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341 gave positive results in all but 1 case. In one case, however, the presence of antibiotics could only be demonstrated by using *Staph. aureus* ATCC 6538 as test strain.

In spite of the demonstrable amount of antibiotics in the test materials, septicaemia and bacteraemia was found in 6 and 3 animals, respectively. Four of the 6 septicaemias were found in cattle, while the remainders were shared by one horse and one calf. As the causative agent of the septicaemias, *Corynebacterium pyogenes*, Clostridia, Salmonellae, coliform bacteria and streptococci were isolated from the organs of cattle. From organs of horses and calves haemolyzing streptococci and bacteria belonging to the Proteus genus, respectively, were demonstrated. In these cases the size of the inhibition zone ranged between 3 to 15 mm, according to the test strain used. Bacteraemia was found in 3 cattle; clostridia, streptococci and coliform microorganisms were isolated. Zone of inhibition, 2 to 8 mm in diameter, was observed in all the 3 cases. As test strains *B. subtilis* ATCC 6633, *Staph. aureus* ATCC 6538, *Sarcina lutea* ATCC 9341 were used in these tests. Presumably, the microorganisms isolated from the organs were not sensitive to the antibiotics applied and that is the reason why the treatment was unsuccessful.

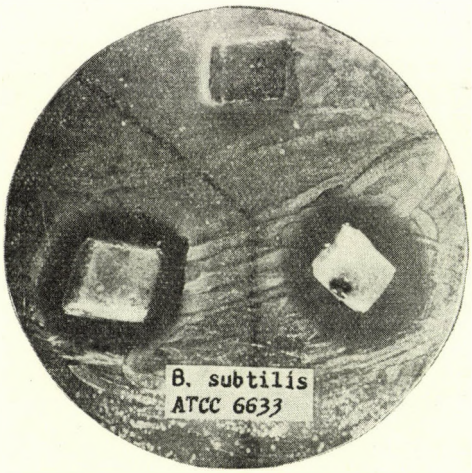

Fig. 1

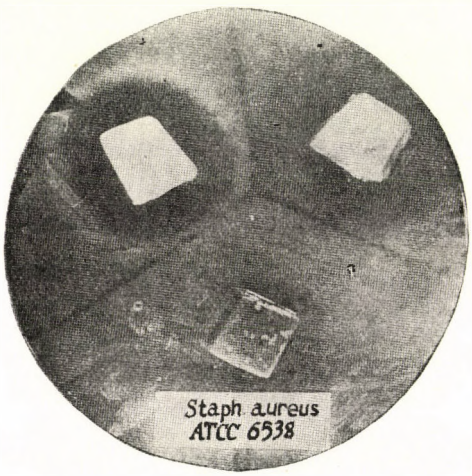

Fig. 2

The inhibition tests as carried out with the 4 test strains are illustrated in Figs 1—4.

Conclusions

1. The method elaborated by us can easily be performed in any laboratory, needs no special equipment, therefore its use for the detection of antibiotic residues might be instructed in the "Meat Control Rules".

2. Unlike some authors' opinion (SPROCKHOFF, 1960; CORETTI, 1961; KOTTER et al. 1959) the use of TTC-containing media for the demonstration

Fig. 3

Fig. 4

of antibiotic residues is unnecessary because the inhibition zone is clearly demonstrable on the meat cream + yeast extract + peptone agar without any indicator dye.

3. The Gardside method as modified by us is suitable for the detection of antibiotics in every respect. Freezing of kidney pieces can be performed on the object-holder of the freezing microtome or in a deep-freezer.

4. In accordance with several authors (SPROCKHOFF, 1960; FORSCHNER, 1962; KIND, 1966; KLIMA, 1966; LORENZEN, 1967) the tissue fluid diffused from the piece of kidney was found suitable for the demonstration of antibiotic residues.

5. Of the test strains, *B. subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341 and *Staph. aureus* ATCC 6538 can be used for the detection of the antibiotic residues. *E. coli* ATCC 9637, however, should be excluded as test strain for the detection of antibiotics used recently.

6. In agreement with other authors (KIND, 1966; KLIMA, 1966; LORENZEN, 1967) we have found that antibiotics used for therapeutic purposes just before slaughtering greatly influence the result of the microbiological meat inspection. Since pathogenic and facultative pathogenic microorganisms, or saprophytic germs in large number, can reliably be demonstrated only by the microbiological tests, which represent one of the screening stations set between the meat and the consumer, obligatory use of our method to detect antibiotic residues in the organs of emergency-slaughtered animals is suggested. The use of this test is supported by the fact that antibiotics have bacteriostatic but presumably no bactericidic action on the pathogenic microorganisms (on *B. anthracis*, *Erysipelothrix rhusiopathiae*, Salmonellae, among others), too. Therefore small numbers of bacteria may retain their viability and may multiply after the antibiotic had been inactivated. These microorganisms start multiplying after a prolonged latent period, as a rule, following 48 hours or even later. Even the growth of Salmonellae in the meat cannot be prevented by antibiotic treatment.

7. Like in other countries, in Hungary, the case histories in the declaration issued by the veterinary surgeon leave much to be desired in respect of the antibiotic treatment (LORENZEN, 1967).

8. The antibiotic residues demonstrable by this test result from antibiotics applied for therapeutic purposes; those applied as feed supplement do not reach as high a level as demonstrable in this test (KOTTER et al., 1959).

9. The method elaborated by us gives a useful aid in detecting factors inhibiting the growth of the microbes without being able to determine their amount, identity, properties or origin. The latter, however, have no influence on the final results of the microbiological meat control.

10. Since, as a result of consuming antibiotic-containing food, many people in the world have been sensitized to antibiotics, it would be desirable to pass the antibiotic-containing meat of emergency-slaughtered animals only under certain conditions. Thus, meat containing antibiotic residues and approved for consumption according to the microbiological examination might be marketed as heated meat products only, for the amount of the antibiotic is considerably reduced by heating (CORETTI, 1961). By following this procedure, allergic conditions, urticaria, eczema, dermatitis, development of resistant bacteria, alteration of the intestinal microflora and anaphylaxis induced by the repeated antibiotic intake with not fully roasted or boiled meat might be avoided (WHO, 1963).

SUMMARY

Meat of 244 emergency-slaughtered animals was investigated to detect antibiotic residue brought into the tissues for therapeutic purposes. Tests were carried out by the Gardside method as modified by us. Four pieces $10 \times 10 \times 5$ mm in size were excised from the kidneys of each animal. The pieces were frozen and put on agar plates made of standard medium and inoculated with 24 hours' cultures of standard test strains (*B. subtilis* ATCC 6633, *Staph. aureus* ATCC 6538, *Sarcina lutea* ATCC 9341 and *E. coli* ATCC 9637). After putting the frozen pieces of kidney on the plates, the media were kept in a refrigerator at $+4$ °C for 4 to 4 ½ hours then at 37 °C for 18 to 20 hours.

Antibiotic residues were demonstrated in 24.6% of the 244 cases. Since antibiotic residues in the meat have considerable influence on the result of the meat control and at the same time may be harmful for the consumer, their detection in the meat of emergency-slaughtered animals is suggested to carry out as obligatory laboratory test. Further, it is suggested that the meat containing antibiotics should not be marketed except as heated meat products.

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DIAGNOSTICAL VALUE OF THE COMPLEMENT FIXATION TEST AND THE FLUORESCENT ANTIBODY METHOD IN THE LABORATORY DIAGNOSIS OF TOXOPLASMOSIS

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The laboratory diagnosis of toxoplasmosis consumes much time and work. Direct microscopical demonstration of the causative agent in Giemsa-stained smears made from discharges or in imprint preparations made from organs, or histological demonstration in the visceral organs succeeds rarely. In most of the cases diagnosis can be made only by tedious experimental infection. The classic Sabin—Feldman test, too, involves a good deal of sources of error (BÁLINT, 1962). Therefore the possible application of the complement fixation (CF) test and the fluorescent antibody method has been the subject of our investigation.

Recently, the fluorescent antibody technique introduced by COONS and KAPLAN (1950) has widely been used in the immunology, bacteriology, biology and parasitology. GOLDMAN was the first to introduce this method in the diagnosis of toxoplasmosis for the detection of both the pathogen (GOLDMAN 1957a) and antibodies (GOLDMAN 1957b).

RUCKENBAUER et al. (1963), ITO et al. (1964, 1966) and ARCHER et al. (1966) demonstrated *Toxoplasma gondii* in peritoneal exudate and organs of both experimentally infected laboratory animals and naturally infected animals. They found the method suitable for diagnostical purposes.

Methods

To produce CF antigen, 50 mice weighing 20 g each were infected intraperitoneally with the ascitic fluids of toxoplasma-infected mice. The mice were killed on the 4th to 5th day following infection. The peritoneal cavity was irrigated with saline containing sodium citrate, and the collected fluid was centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was discarded and to the deposit citrate saline was added up to the original volume. To reveal the antigen, the suspension was shaken in a bottle with glass beads, then frozen at —20 °C for 2 hours and finally thawed in a 37 °C water bath. Freezing and thawing were repeated twice. Following the 3rd thawing the suspension was centrifuged at 10,000 r.p.m. for 30 minutes. The supernatant

was titrated with positive serum and used in proper dilution as antigen. The antigen was stored at —20 °C until used.

The micromethod was performed in a total volume of 0.5 ml. Reading was made as usual and repeated next morning. Unless haemolysis occurred at room temperature within 16—20 hours, the reaction was regarded as positive.

Following the evaluation of our preliminary experiments, in agreement with most of the authors referred to in the introduction, we used the direct immunofluorescence. This method is simpler and faster and involves fewer sources of errors than the indirect method.

To produce fluorescent conjugate, blood was taken from rabbits naturally infected with toxoplasma. Serum samples with a CF titre of 1 : 800 were pooled. The globulin fraction was precipitated from the pool with ammonium sulphate and conjugated with fluorescein-isothiocyanate according to JENTZSCH's method (1963), and finally, the labelled sera were absorbed with acetone-extracted rabbit liver and brain powder (COONS et al., 1955).

In the present investigations spleen, liver, kidney, brain, peritoneal exudate of experimentally or naturally infected animals and fetal membranes of died or aborted animals were used. Organs were ground in saline in a mortar. From each organ suspension 2 thin smears were prepared on a slide, the smears were dried at room temperature and fixed above a flame.

One drop (about 0.06 ml) of the conjugate diluted to the titre value was put in each fixed smear and the slides were placed in a wet chamber in a 37 °C incubator for 25 minutes. After the period required for the interaction had passed the preparations were washed several times in PBS of pH 7.4 for 19 minutes, then rinsed in distilled water and dried at room temperature without blotting.

To ascertain the specificity of the method the following controls were used:

Inhibition of specific fluorescence with unlabelled homologous antibodies. A remarkable, frequently full, inhibition of the specific fluorescence was observed, if prior to staining of the slides the conjugate was mixed with an equal amount of unlabelled antiserum. The test was performed as follows: 0.3 ml of the working dilution of each conjugate was placed in two tubes, than 0.3 ml unlabelled toxoplasma antiserum was added to the one, and 0.3 ml unlabelled normal rabbit serum to the other tube. On each slide the left smear was stained with the mixture containing the antiserum, the right smear with that containing the normal serum. The slide was put in a wet chamber of 37 °C for 30 minutes, then rinsed and dried as described above. In the right smear the intensity of the specific fluorescence was not influenced by the normal serum (4+), whereas it was inhibited in the left smear. Occasionally, toxoplasmas fluoresced even at the site inhibited, but the intensity of the fluorescence was markedly reduced as compared with that of the control smear.

The specificity of the reaction was controlled routinely with each preparation made from animals suspected of being infected with toxoplasma. First, the uninhibited site was examined, and if no specific fluorescence was observed the preparation was considered negative. If in the smear at the uninhibited site specifically fluorescent formations were observed the outline and size of which were comparable with toxoplasmas (see Fig. 1), while at the inhibited site specific fluorescence was lacking or markedly reduced in intensity, the preparation was considered positive. No difference in intensity of the fluorescence indicated nonspecific reaction and the examination had to be repeated.

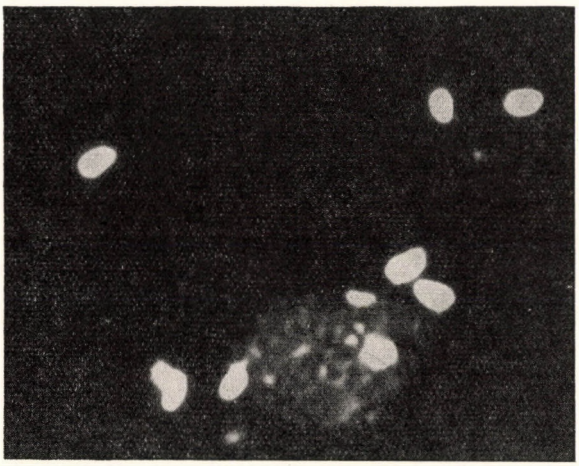

Fig. 1. Parasites fluoresce specifically in a smear prepared from the spleen homogenate of a rabbit died of toxoplasmosis. Magnification approx. ×1200

Adsorption of the labelled antibodies with homologous antigen. This method of specificity control is convincing, but for routine work less suitable. Of the working dilution of conjugate 0.3 ml was placed in each of two tubes. To one of the tubes 0.3 ml homogenate of a toxoplasma-infected mouse spleen, to the other 0.3 ml homogenate of a normal mouse spleen was added. The tubes were then placed in an incubator of 37 °C for 2 hours. During the period of interaction full or partial absorption of the fluorescent antibodies took place in the mixture containing the homologous antigen. The serum thus absorbed failed to react with the toxoplasma antigen when put on positive preparation. Specific fluorescence of unchanged intensity was observed only in the smear stained with the mixture that contained the homogenized normal mouse spleen. This test for specificity was carried out with each newly prepared conjugate.

Furthermore, the specificity of the method was evidenced by the fact that after staining toxoplasmas with heterologous labelled antibodies (leptospira, brucella, rabies conjugates) or normal sera labelled with fluorescein-iso-

thiocyanate (rabbit, swine, cattle or sheep serum) specific fluorescence was not observed in any of the cases.

Immunofluorescence was studied by using the Soviet ML – II fluorescence microscope, viewing the preparates under immersion objective. Combinations of "SZhS 7—2", "FS 1—2" and "BS 8—2" blue filters were used and "ZhS—18" and "ZhS—19" filters served as barrier filter.

For direct demonstration of the causative agent, rapid and slow Giemsa staining methods were used. These smears were prepared from the organs as described above and fixed with methanol.

Results

In the course of a toxoplasmosis enzootic occurred in a rabbit breed recently, 24 blood samples (taken from 7 affected and 17 apparently healthy animals) were examined with the CF test. Results are shown in Table 1. Ten rabbit sera negative with the Sabin—Feldman test, obtained from the breed of our Institute served as controls.

As Table 1 shows, positive results at low titre values (1 : 10—1 : 40) occurred in some negative control samples (3 samples at 1 : 20, 2 samples at 1 : 40 titre value), presumably due to the anticomplementary action of the serum samples in spite of their inactivation at 62 °C for 30 minutes. Therefore reactions above 1 : 100 dilution were only considered positive.

Fluorescent antibody technique was compared to Giemsa staining method by examining several organs of rabbits affected with, or died of, toxoplasmosis during the enzootic.

Table 2 shows that it depended on the pathological picture which of the organs contained detectable amounts of the pathogen. We succeeded in demonstrating the pathogen with both methods in acute cases mostly in the spleen and liver, in the chronic form of the disease associated with emaciation and nervous symptoms mostly in the brain, and in cases of abortion mostly in the fetal membranes. In natural infections and deaths following natural infection toxoplasma could be demonstrated with Giemsa staining in a significantly lower rate (22%) than with the fluorescent antibody technique (85%). These rates, however, were significantly higher in the case of experimentally infected laboratory animals: 51% and 93% of the positive cases were detected by Giemsa staining and fluorescent antibody technique, respectively.

One of us (SZEMERÉDI, 1968) has already reported that 48 hours following death the causative agent could not be demonstrated with Giemsa staining. With the immunofluorescence method on the other hand toxoplasmas could be demonstrated in the organs or discharges 72 hours, or in case the sample had been kept at +4 °C for even 2 months, after death.

Table 1

Results of the CF tests

Designation of blood samples	Serum dilutions							
	1 : 10	1 : 20	1 : 40	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1000
1	+++	++	—	—	—	—	—	—
2 ill	+++	+++	++	+++	—	—	—	—
3	—	—	—	—	—	—	—	—
4	+++	+++	+++	+++	—	—	—	—
5	++	+	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—
8	+++	+++	+++	+++	+++	+++	+++	+
9	+++	+++	+++	+	—	—	—	—
10	+++	+++	+	—	—	—	—	—
11 ill	+++	+++	+++	+++	+++	++	—	—
12 ill	++	—	—	—	—	—	—	—
13	+++	+++	—	—	—	—	—	—
14	+++	+++	+++	++	—	—	—	—
15 ill	+++	+++	+++	+++	+++	+	—	—
16 ill	++	+	+	—	—	—	—	—
17 ill	+++	+++	+++	+++	—	—	—	—
18	—	—	—	—	—	—	—	—
19 ill	+++	+++	+++	+++	—	—	—	—
20	—	—	—	—	—	—	—	—
21	+++	+++	+++	+++	—	—	—	—
22	+++	+++	+++	+++	+++	++	—	—
23	+++	+	—	—	—	—	—	—
24	+++	+++	+++	++	—	—	—	—
A Control	—	—	—	—	—	—	—	—
B Control	+++	+++	++	—	—	—	—	—
C Control	++	+	—	—	—	—	—	—
D Control	—	—	—	—	—	—	—	—
E Control	—	—	—	—	—	—	—	—
F Control	++	+	—	—	—	—	—	—
G Control	+++	++	—	—	—	—	—	—
H Control	—	—	—	—	—	—	—	—
I Control	+++	++	+	—	—	—	—	—
J Control	—	—	—	—	—	—	—	—

Table 2

Results of the comparative studies between Giemsa staining and the immunofluorescent method

Species	No. of animals	Pathological changes	No.	Spleen		Liver		Kidney		Ascites		Brain stem		Cortex		Placenta	
				G	IF	G	IF	G	IF	G	IF	G	IF	G	IF	G	IF
Rabbit	18	Acute form	14	2	12	1	10	0	5	1	7	0	2	0	0	×	×
		Chronic form	2	0	1	0	0	0	0	0	1	1	1	0	1	×	×
		Abortion	2	0	0	1	1	0	0	0	1	0	0	0	0	1	2
Golden hamster*	4	Experimentally infected, showing pathological changes indicating septicaemia		1	4	1	4	0	2	2	4	1	2	0	1	×	×
Souslik**				0	1	0	1	0	0	1	1	1	1	0	0	×	×
White mouse	85			32	79	30	75	7	14	43	74	2	15	1	10	×	×

G, Giemsa staining; IF, Immunofluorescent method; × Not tested; * *Mesocricetus auratus;* ** *Citellus citellus*

Conclusions

CF test is simpler and easier to perform than the classic Sabin—Feldman test. As for the CF test, further conclusion cannot be drawn from our limited investigations, except that the present results reassure to state that the CF test can be used to gather information about the occurrence of toxoplasmosis in a breed.

However, in our opinion, without demonstrating the causative agent, the result of the CF test alone does not enable us to make an individual diagnosis. The diagnosis must always be based on the demonstration of the causative agent.

The pathological changes alone arouse suspicion of toxoplasmosis — particularly in the acute form of the disease. Histopathological examinations directed towards the demonstration of the parasites or pseudocysts in the organs showing lesions gave negative results in all of our cases (SZEMERÉDI, 1968).

With Giemsa staining the parasites could be demonstrated in an insignificant percentage of the cases.

In our diagnostical work the immunofluorescent method proved the most reliable. The method is rapid, specific and more sensitive than any other method. By using this method the expensive and tedious experimental infection can be avoided. Its diagnostical value is confirmed by the fact that *Toxoplasma gondii* can often be demonstrated when all other methods used prove unsuccessful.

SUMMARY

Authors studied the adaptability of the complement fixation (CF) test and the direct fluorescent antibody technique in diagnosing toxoplasmosis in a rabbit breed. Rabbits affected with, or died of, the toxoplasmosis after showing various forms of the disease, and experimentally infected laboratory animals were tested. CF test was found suitable as a supplementary method for investigation, however, as to the opinion of the authors, its use for making diagnosis is not justified without demonstrating the causative agent. Thus, on making diagnosis emphasis should be laid upon the demonstration of the toxoplasmas. For this purpose the rapid and specific immunofluorescent method was found the most suitable, being more sensitive than any other method.

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QUALIFICATION OF FROZEN FOODS WITH MICROBIOLOGICAL EXAMINATIONS

By

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Due to the development of the refrigeration technique in the last years, large amounts of foods are preserved by refrigeration and freezing. Original properties of foods can be retained by these methods most favourably. These methods of preservation lie on the principle that at low temperatures the growth of most of the bacteria are stopped or slowed down.

Since frozen foods and their raw materials are extremely heterogeneous, no overall paper on scoring of foods from microbiological aspects has been published until recently. The reader is referred only to some. papers concerning quick-frozen foods (ELLIOT and MICHENER, 1961; 1965; FRAZIER, 1958).

Frozen foods include half-finished and ready-made products, greens, vegetables, fruits (with or without sugar), products prepared with cereals (meal), meat (viscera) and ready-cooked food containing meat and miscellaneous product. As for the microbiological features of these heterogeneous materials, the microorganisms present in each food product are in close correlation with the applied technology or the original properties of the raw material. Foods frozen without pre-heating (fruits), those frozen after sclading, or those half-finished, untreated by heat but combined with various additives then frozen, and finally foods frozen following heat treatment have greatly different microfloras. Our investigations aimed at throwing light upon the microbiological state of the frozen foods made in Hungary. Further, information was obtained whether these foods are exportable. Finally, we wished to contribute to the establishment of microbiological requirements of foods.

In addition, it must be emphasized that the present microbiological examinations have been connected with painstaking hygienic plant supervision, improvement of the manufacturing hygiene control of the technology (pasteurization) and explanatory work concerning hygiene among workers and consumers (TRESSLER and EVERS, 1957; ELLIOT and MICHENER, 1961; PARKER and LITCHFIELD 1962; ELLIOT and MICHENER, 1965; LOTT, 1967).

Materials

Quick-frozen foods were divided into 5 groups from the microbiological point of view, namely

(1) quick-frozen (heat-treated) ready-cooked meat; (2) quick-frozen half-finished (not treated with heat) meat products; (3) quick-frozen vegetables (scalded); (4) quick-frozen fruits; (5) other quick-frozen products.

On a total 2842 quick-frozen samples were examined.

Methods

Before being tested the samples were allowed to thaw overnight at +4 °C. In order to establish microbiological ranges, methods of investigation concerning frozen foods had to be standardized.*

Total living cell count as well as cell counts of the following microorganisms were determined:

proteolytic microorganisms; coliforms; alkali-producing microorganisms; yeasts; moulds; Lactobacilli; Clostridia; haemolyzing microorganisms; *Micrococcus pyogenes var. aureus* and psychrotolerant microorganisms. Special care was taken on the isolation of Salmonella.

Cell counts are related to 1 g of sample.

Based on the large number of the samples, geometric mean values and the simple positive dispersion values ($+1 \times s$), by applying the Gauss curve, were estimated.

Results

Results relating to the five groups of the quick-frozen products are presented in Figs 1 to 5.

Micrococcus pyogenes var. aureus, salmonellae and psychrotolerant germs, having been isolated but rarely, are not included in the figures. *Micrococcus pyogenes var. aureus* was isolated from 1 quick-frozen half-finished sliced swine liver for frying in fat, 9 quick-frozen half-finished sliced swine livers, 3 quick-frozen half-finished porks for frying in fat and from 1 quick-frozen mashed and sweetened chestnut with cream, as a total of 14 cases. Germ counts concerning *Micrococcus pyogenes var. aureus* were between 100 and 1000 in the samples, except in 3 cases when these numbers were exceeded (1400, 2000 and 40,000 per g). Some of the isolated strains were phage-typed in the National Institute of Public Health, Budapest. Dr. H. Milch is acknowledged for the typing. The strains belonged to 3 phage types, *viz.*, RTD 6/54 +, RTD (6) 42 E/54 + and RTD 42 E (47/54) 81 III.

In 13 of the samples Salmonella was found. From quick-frozen sliced swine livers 5 strains of *S. abony*, and 3 strains of *S. anatum*, from quick-frozen raspberry creams 2 strains of *S. cholerae-suis var. Kunzendorf*, and from quick-frozen mixed fruit creams 1 strain of *S. typhi-suis var. Voldagsen* were isolated. The fruit creams had been contaminated during processing. A Salmonella strain was isolated from a half-finished swine liver sample for frying in fat and another from a quick-frozen half-finished sliced ox liver. These strains were not identified.

Indices concerning the psychrotolerant germ counts are touched in the conclusion.

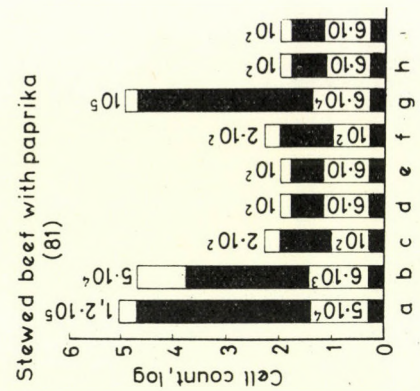
* For lack of space culture media and details of the methods used in our investigations are not described.

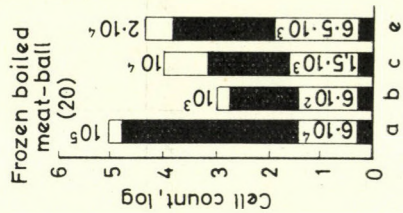
Table 1

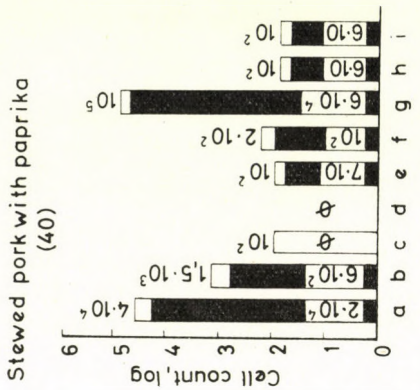
Qualification of frozen foods by microbiological tests

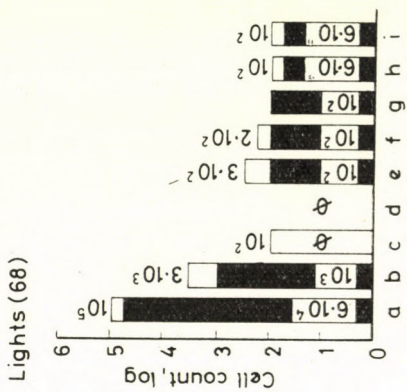
	Total living cell count per gram	Coliform cell count per gram	Yeast cell count per gram	Mold cell count per gram	Lactobacillus cell count per gram	Clostridium cell count per gram
1. Heated quick-frozen meat products ready for cooking Excellent	100,000 PM: 10,000	<1	<1000	<1000	<100,000	<100
Tolerated cell count after incubation at 21 °C (acceptable)	300,000 PM: < 100,000	<100	<10,000			
2. Unheated (raw) quick-frozen meat products Excellent	5,000,000 PM: < 250,000	100	10,000	<1000	NT	<100
Tolerated germ count (acceptable)	10,000,000 PM: 500,000	1000	30,000			
3. Scalded quick-frozen vegetables Excellent	100,000 PM: 10,000	<1	<1000	<1000	NT	<100
Tolerated germ count after incubation at 21 °C (acceptable)	500,000 PM: < 100,000	<100	<10,000			
4. Quick frozen (raw) fruits Excellent	100,000 PM: 10,000	100	Raw: <1000 Sugared: <10,000 Cream: <100,000	Raw: <1000 Sugared: <100,000 Cream: <100,000	NT	<100
Tolerated germ count (acceptable)	500,000 PM: 100,000	1000	Raw: <10,000 Sugared: <100,000 Cream: <1,000,000			

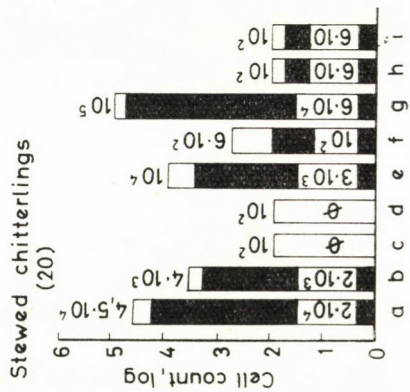
NT, not tested; PM, proteolytic germ.


Stewed beef with paprika
(81)
Cell count, log
0 1 2 3 4 5 6
a b c d e f g h i
5·10⁴ 1,2·10⁵
6·10³ 5·10⁴
10² 2·10²
6·10 10²
6·10 10²
6·10 10²
10² 2·10²
6·10⁴ 10⁵
6·10 10²
6·10 10²


Frozen boiled
meat-ball
(20)
Cell count, log
0 1 2 3 4 5 6
a b c e
6·10⁴ 10⁵
6·10² 10³
1,5·10³ 10⁴
6·5·10³ 2·10⁴


Stewed pork with paprika
(40)
Cell count, log
0 1 2 3 4 5 6
a b c d e f g h i
2·10⁴ 4·10⁴
6·10² 1,5·10³
θ 10²
7·10 10²
10² 2·10²
6·10⁴ 10⁵
6·10 10²
6·10 10²
6·10 10²


Lights (68)
Cell count, log
0 1 2 3 4 5 6
a b c d e f g h i
6·10⁴ 10⁵
10³ 3·10³
θ 10²
10² 3·10²
10² 2·10²
10² 10²
6·10 10²
6·10 10²
6·10 10²


Stewed chitterlings
(20)
Cell count, log
0 1 2 3 4 5 6
a b c d e f g h i
2·10⁴ 4,5·10⁴
2·10³ 4·10³
θ 10²
θ 10²
3·10³ 10⁴
10² 6·10²
6·10⁴ 10⁵
6·10 10²
6·10 10²

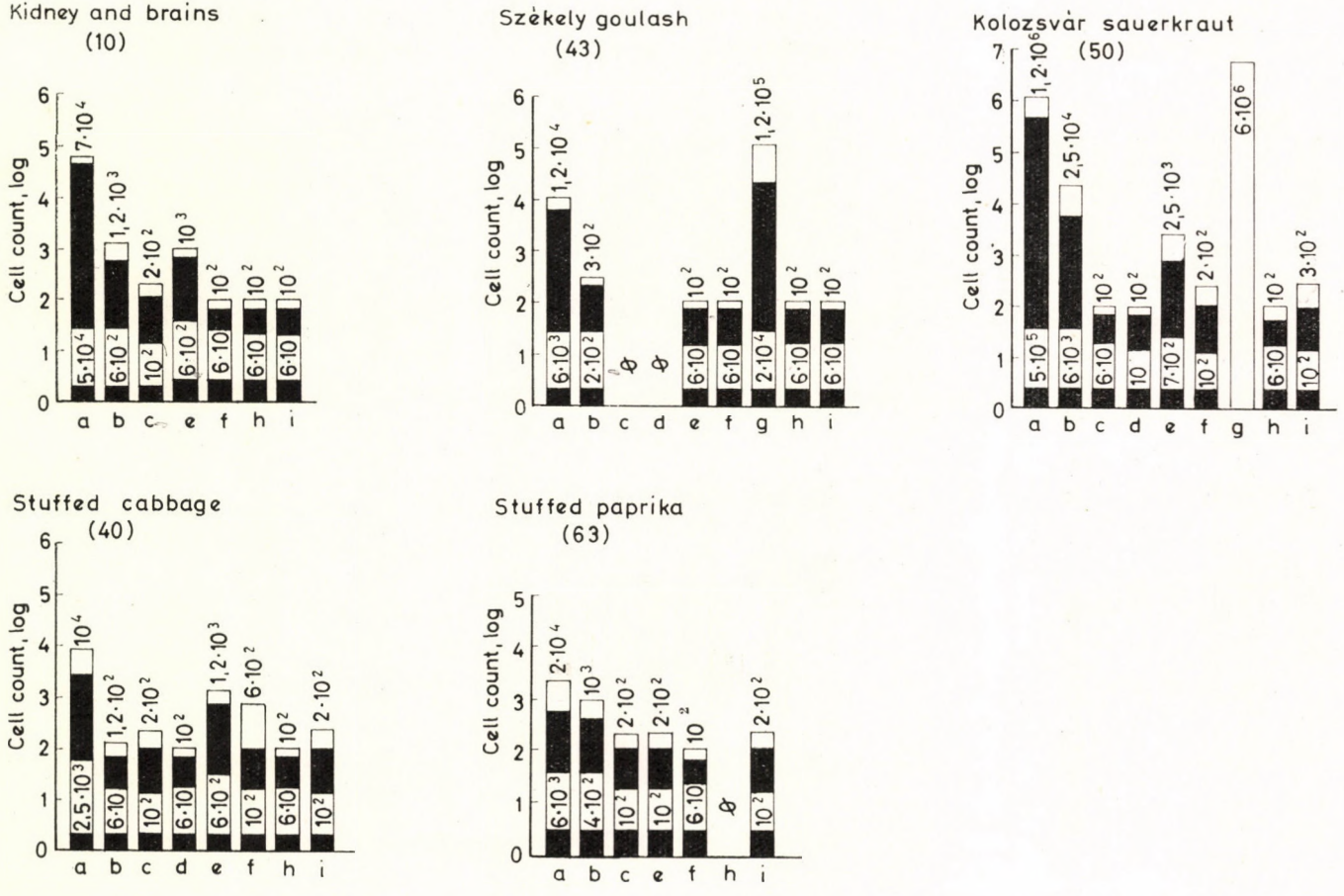
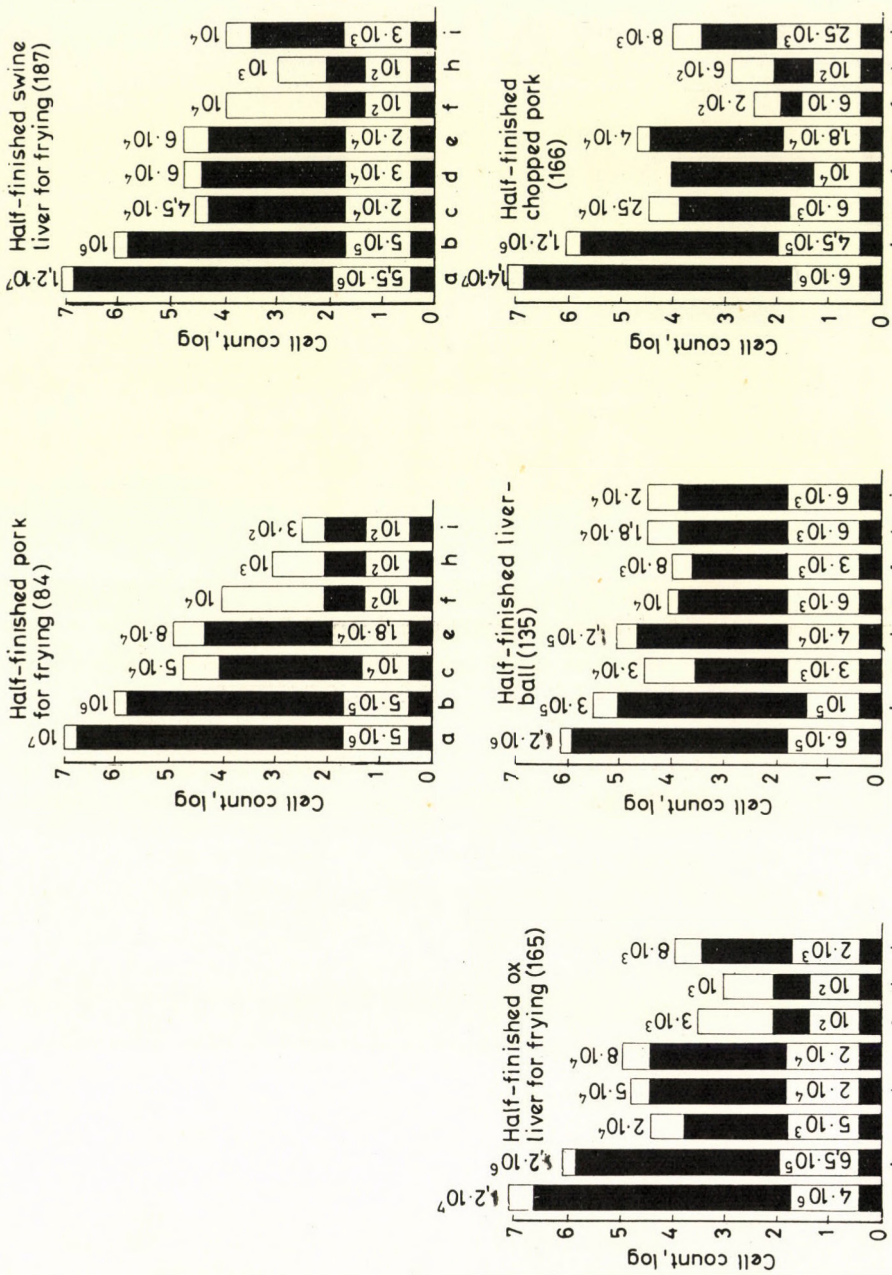


Fig. 1. Quick-frozen (pre-heated) meat products


Half-finished swine liver for frying (187)
Half-finished chopped pork (166)
Half-finished pork for frying (84)
Half-finished liver-ball (135)
Half-finished ox liver for frying (165)
Cell count, log
a b c d e f h i

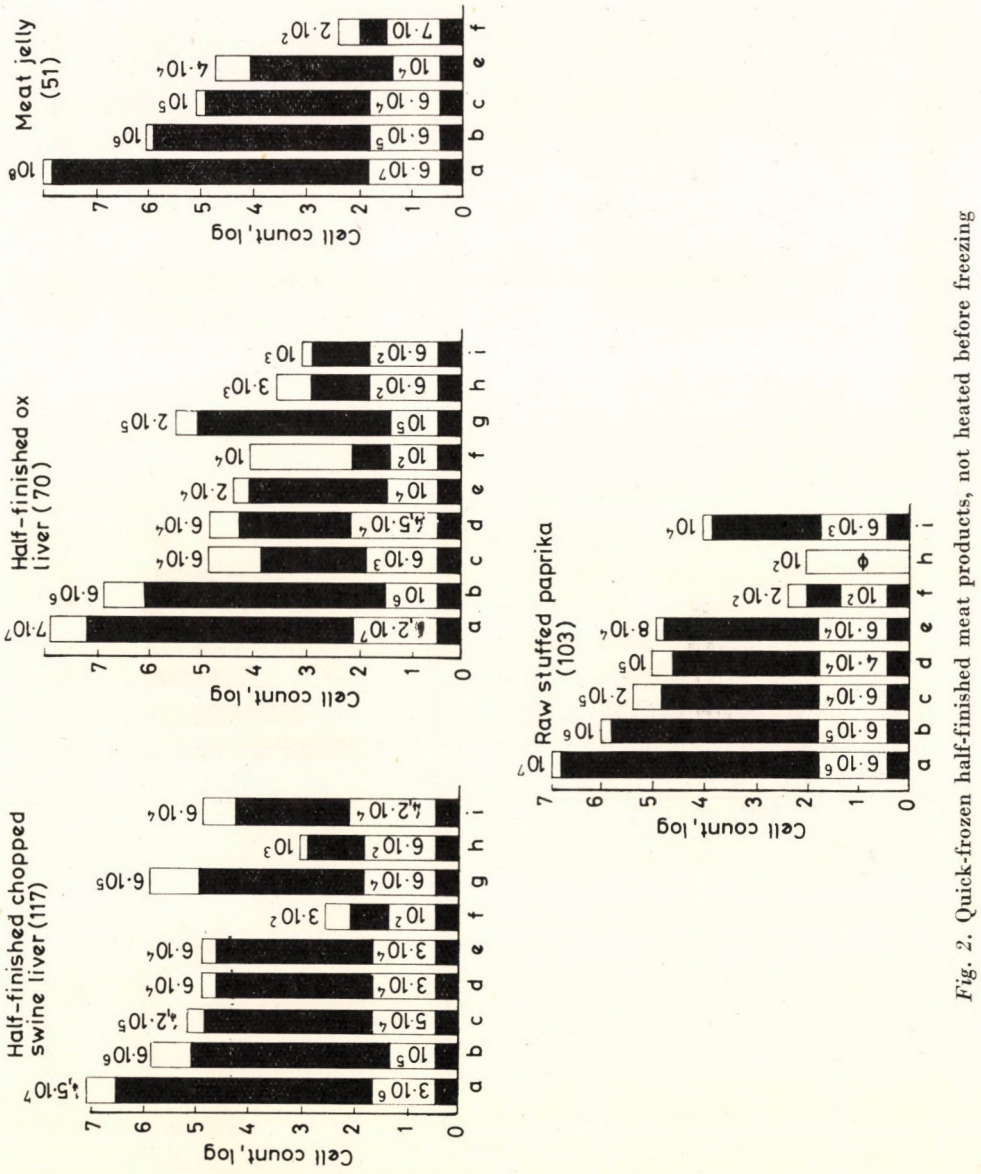


Fig. 2. Quick-frozen half-finished meat products, not heated before freezing

Spinach cream (205)
Cell count, log
a b c d e f g h i

Green peas (130)
Cell count, log
a b c e f g h i

French beans (117)
Cell count, log
a b c e f h i

Pumpkin (91)
Cell count, log
a b c e f g h i

Garden sorrel cream (37)
Cell count, log
a b c d e f g h i

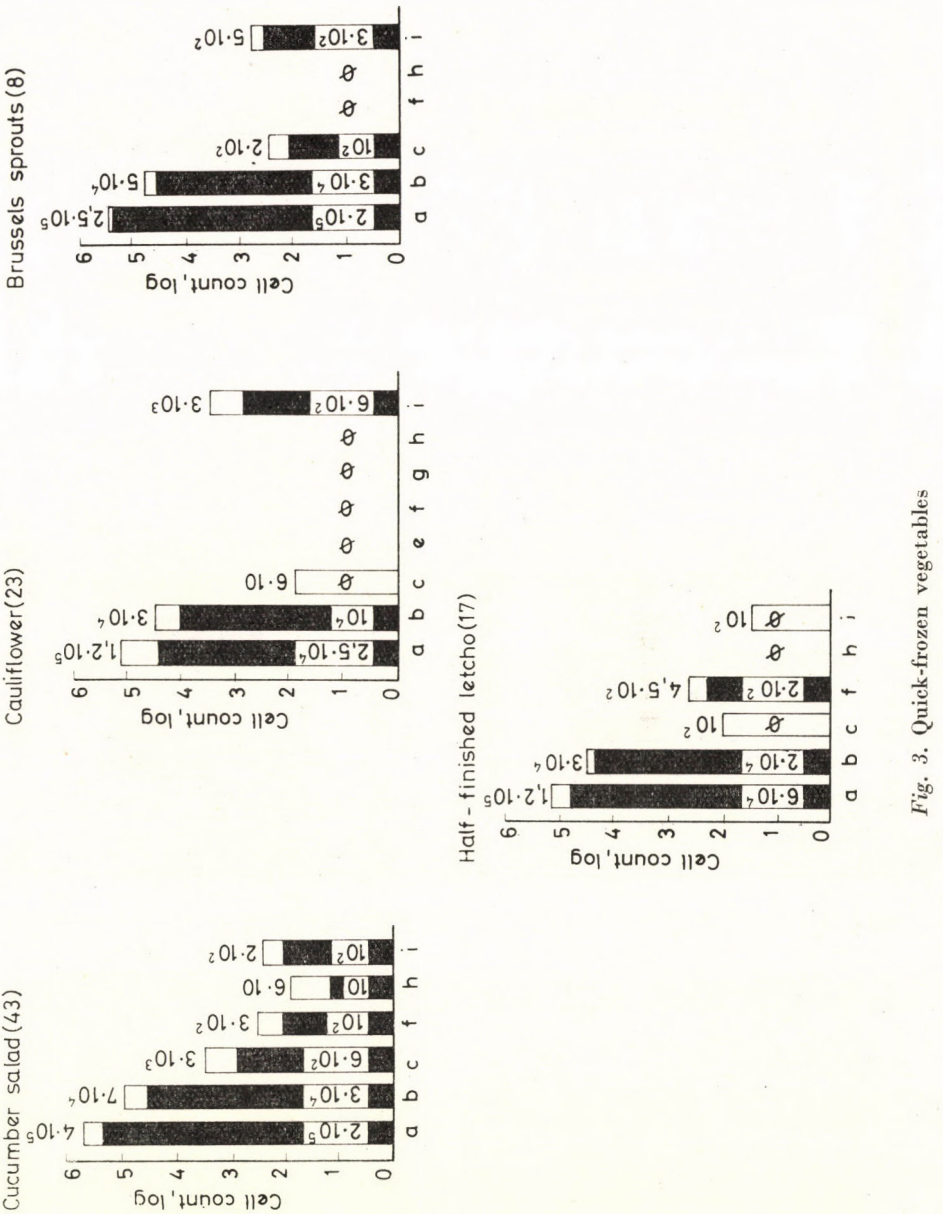


Fig. 3. Quick-frozen vegetables

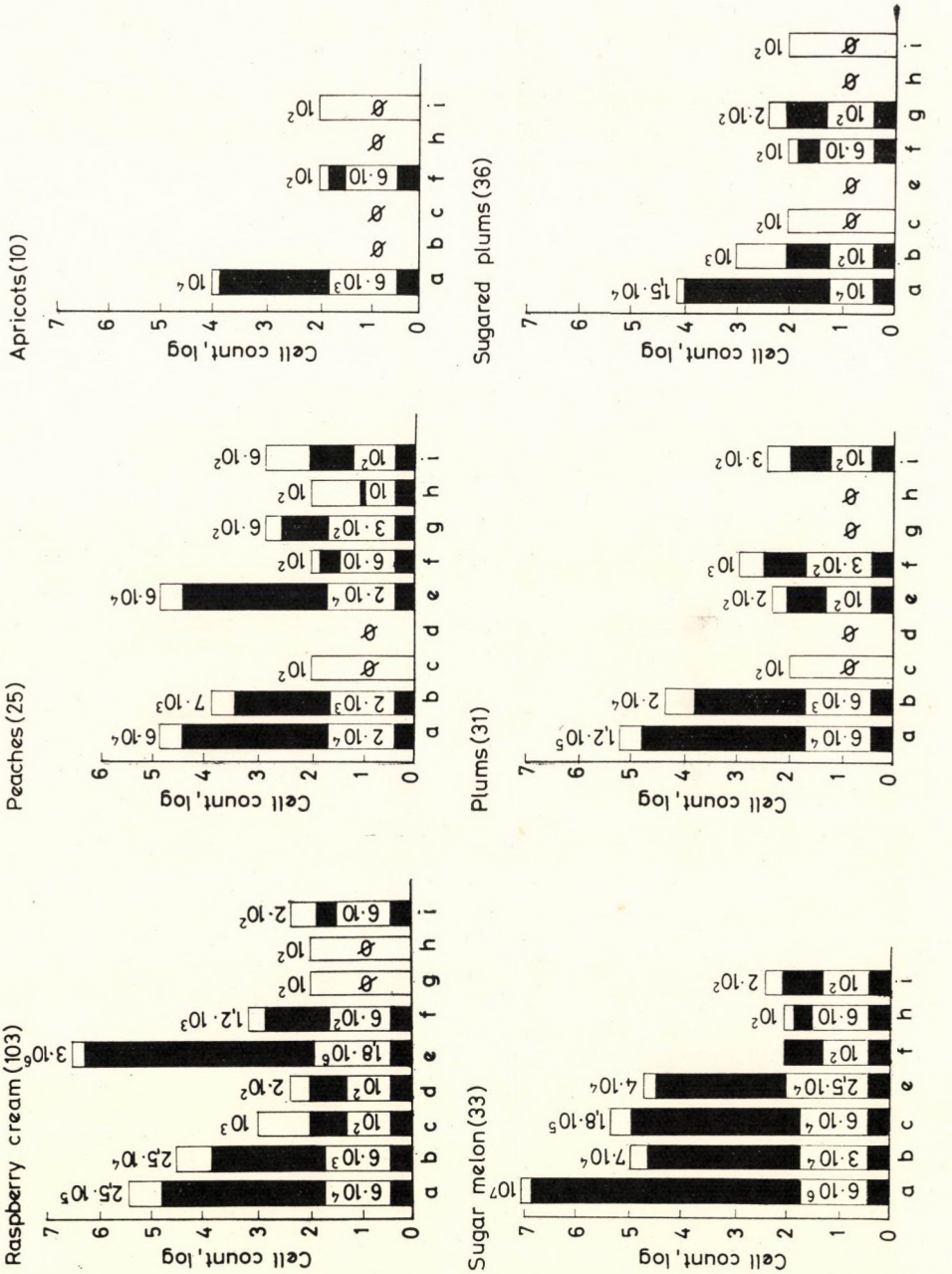
Sugared strawberries (54)
Cell count, log
0 1 2 3 4 5 6
6·10³ 1,5·10⁴
2·10³ 4,5·10³
6·10 10²
6·10 10²
6·10³ 2,5·10⁴
2·10³ 4·10³
Ø
10 10²
6·10 10²
a b c d e f g h i

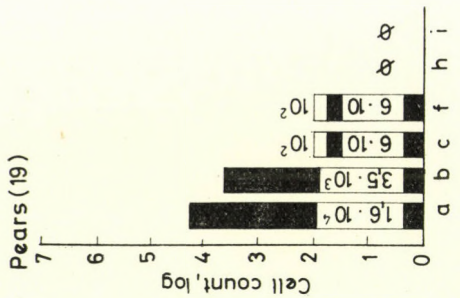
Strawberry cream (34)
Cell count, log
0 1 2 3 4 5 6 7
2·10⁴ 4·10⁴
2·10³ 3·10³
5·10 10²
10² 3·10²
10⁶ 3·10⁶
1,5·10⁵ 6·10⁵
10⁵ 5·10⁵
Ø
3·10² 6·10²
a b c d e f g h i

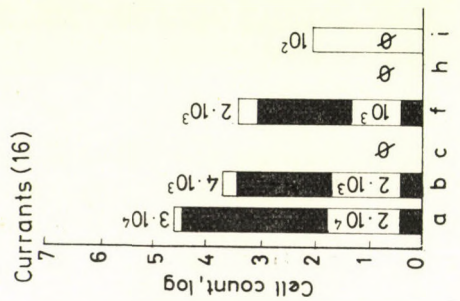
Raspberries (70)
Cell count, log
0 1 2 3 4 5 6
2·10⁴ 4·10⁴
10² 10³
Ø
10² 2·10²
10² 4·10²
Ø
Ø
Ø 10²
a b c e f g h i

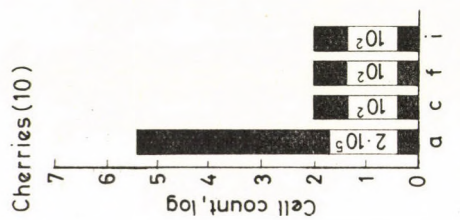
Sugared raspberries (45)
Cell count, log
0 1 2 3 4 5 6
6·10³ 4·10⁴
1,6·10³ 6·10
Ø
10² 2
6·10⁴ 10⁵
6·10³ 6·10⁴
10 10²
5·10 10²
a b c d e f h i

Bulk raspberries (20)
Cell count, log
0 1 2 3 4 5 6 7
7·10³ 1,5·10⁴
1,5·10³ 3·10³
Ø
Ø
3·10⁵ 1,8·10⁶
6·10⁴ 2·10⁵
6·10 10²
10
2·10² 2·10²
a b c d e f g h i


Apricots (10)
Sugared plums (36)
Peaches (25)
Plums (31)
Raspberry cream (103)
Sugar melon (33)
Cell count, log
a b c d e f g h i







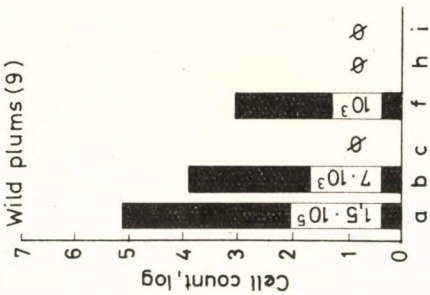


Fig. 4. Quick-frozen fruits

Conclusions

1) Foods frozen as raw (mainly fruits) possess the normal microflora. Freezing is employed to preserve fresh fruits and vegetables in order to inhibit growth of putrefacient plant-pathogenic and saprophytic microorganisms. The microflora of this group consists mainly of yeasts and moulds, the low pH value being unfavourable for the reproduction of bacteria (CAMERON, 1936; DESROSIER, 1959; ELLIOT and MICHENER, 1965). To this natural

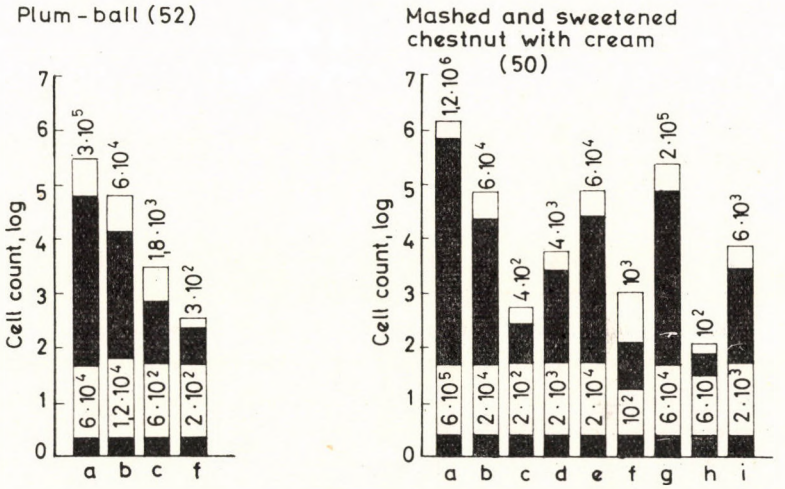


Fig. 5. Other quick frozen products. *a*, living cell count; *b*, proteolytic cell count; *c*, coliform cell count; *d*, alkali-producing cell count; *e*, yeast cell count; *f*, mould cell count; *g*, Lactobacillus count, *h*, Clostridium count; *i*, haemolyzing cell count
■ = geometric mean
□ = dispersion (+ 1 × s)

microflora a microflora adapted to the processing plant ("plant-specific microflora") and contamination caused by the processing workers (knife, working dress, utensils) are added. These foods are consumed raw.

2) Residual microflora of the foods frozen after scalding (vegetables) is qualified harmful. To this microflora the plant-specific microflora and the contamination caused by the processing workers are added. Scalding reduces microbial population to a great extent, reducing, at the same time, the normal resistance to spoilage, thus allowing rapid growth of bacteria if recontamination occurs prior to freezing.

3) Half-finished, unheated frozen foods supplemented with additives (principally meat products) possess the normal microflora completed with the microflora of the additives as well as with that of the plant and that deriving from the processing workers. On the fresh muscles of warm-blooded animals mainly mesophilic microflora can be found, which changes toward the

psychrophilic and psychrotolerant microorganisms during refrigeration. Cold-blooded animals, first of all fish, have mainly psychrophilic and psychrotolerant organisms in their original microfloras, which do not change during preservation at low temperatures. Foods ranked in this group are consumed following heating.

4) Residual microflora of meat-containing and miscellaneous heated (boiled) and subsequently frozen foods is considered harmful. This microflora is associated with the plant-specific flora and with the contamination deriving from the processing workers. These foods are heated or boiled before being consumed.

5) Among the microbiological findings total cell count, coliform cell count, yeast and mould cell counts, Lactobacillus and Clostridium cell counts can be generally used for scoring.

6) Microbiological requirements might briefly be summarized as follows:

a) Frozen foods must be free of known pathogenic and toxin-producing microorganisms.

b) Enzyme activity of the normal and residual microflora must not alter the organoleptic properties of the food.

c) Coliform cell count for heated products (ready-for-cooking foods, scalded vegetables) should be lower than 1 yeast and mould cell count lower than 1000. Coliform and yeast cell counts may be tolerated up to 100 and 10,000 respectively.

d) Coliform cell count for unheated products (raw fruits, half-finished meat products) should not exceed 100. Cell counts between 100 and 1000 may be accepted.

e) Cell count of the haemolyzing *Micrococcus pyogenes var. aureus* must not exceed 1000.

Cell count of saprophytic clostridia must not exceed 100.

g) Mould cell count must not exceed 1000 in frozen raw products (fruits). In fruits and creams sweetened with sugar cell counts up to 100,000 may be accepted.

h) Yeast count must not exceed 10,000 and 1000 in 1 g of frozen raw meat products and raw fruits, respectively, but quick-frozen raw meat products and quick-frozen raw fruits with 30,000 and 10,000 yeast cells per gram, respectively, are acceptable. Yeast cell count as high as 10,000 and 100,000 per gram fruit sweetened with sugar and creams respectively, must be accepted.

i) Enterococcus cell count in the heated quick-frozen foods must not exceed 1000.

j) Total count of living germs in the heated quick-frozen foods (ready for cooking) and vegetables (scalded) must not exceed 100,000 of which the proteolytic bacteria must not exceed 10,000 per gram if the plates are in-

cubated at 32 °C. If plates are incubated at 21 °C, total cell count of the psychrotolerant germs must not exceed 300,000—500,000; among these the cell count of the proteolytic germs must not exceed 100,000.

k) Cell counts of the living mesophilic and psychrotolerant germs in unheated quick-frozen foods (quick-frozen meat products) must not exceed 5,000,000 at 32 °C and 21 °C. Among these proteolytic germs must not exceed 250,000 per gram. Nevertheless, at present a total cell count of 10,000,000 and a proteolytic cell count up to 500,000 may be tolerated.

l) Total and proteolytic cell count raw fruits should not exceed 100,000 and 10,000, respectively, but total cell counts up to 500,000 and proteolytic cell counts up to 100,000 may be tolerated (except in muscat melon, where 5,000,000 cells per gram may be tolerated).

m) Lactobacillus cell count in heated quick-frozen meat products ready for cooking must not exceed 100,000.

The limits of unobjectionable and tolerable cell counts are summarized in Table 1.

7) We have intended to present a basis for officials supervising the service in the refrigerating plants and the trade in order to control each lot more effectively. Based on the cell count values elaborated by us stocks and production lots may be qualified from aspects of public consumption and export quality, provided sampling is correct and statistically perfect.

8) For marketing of quick-frozen foods continuous refrigeration is of utmost importance, because improper thawing may involve spoilage. Even continuous refrigeration, which is the only method in preventing growth of the pathogenic microorganisms, does not afford full protection, at most retards spoilage. Appearance of spoilage is related to the initial germ count and the storage temperature.

9) To prevent spoilage, an uninterrupted hygienic handling is needed. (LOTT, 1967). Quick-frozen foods should be kept in a state poor in germs from the raw material until the final product. To meet this requirement, permanent cleaning and disinfection are needed in order to prevent the cold-adapted so-called plant-specific, psychrophilic and psychrotolerant microflora from developing, and, last but not least, pathogenic microorganisms from spreading in the plant.

10) Deratisation and desinsection as a part of the plant hygiene, clean baths, lavatories, toilets for the workers, water of drinking-water quality, obtaining raw material in a hygienic way, permanent and continuous cleaning and disinfection of the fittings, proper sewage disposal, and, last but not least, the hygienic state of the workers substantially influence the microflora of the food products. It is the task of the sanitary service to control these factors permanently. Keeping of the plant-hygienic requirements gives aid to the microbiological qualification based upon the cell count ranges. Micro-

biological examinations throw light on the plant-hygienic circumstances. If these conditions cannot be established, microbiological feature of the products may show large fluctuations, and a permanent hygienic level cannot be established.

11) Growth of microorganisms in frozen products can occur when, due to improper handling, they are kept above −12 °C for a long period of time (ELLIOT and MICHENER, 1965).

12) As a consequence of the enzymatic action of bacteria, frozen foods can show changes apprehensible to senses prior to freezing or when thawed at 0 °C even if the cell count remains low. These enzymes can be formed below the temperature optimal for the reproduction of the enzyme-producing microorganisms and are active below the minimal temperature required for their reproduction. To prevent high cell counts in foods prior to freezing, freezing must be performed quickly. As a rule, putrification is indicated by a cell count of 10^7—10^8/ g or 1 cm^3. As for the qualification, organoleptic investigation must always be carried out.

13) Spoilage caused by psychrophilic and psychrotolerant microorganisms can be prevented by keeping the surface of the foods dry, reducing water activity and securing a low pH.

SUMMARY

2842 quick-frozen foods ready for marketing were investigated from microbiological aspects. Prior to the microbiological examination frozen foods were devided into 5 groups according to the germ-reducing technology. The groups were: quick-frozen (heated) meat products, half-finished (unheated) meat products, scalded vegetables, fruits and other foods unclassifiable in any of these groups. Along with organoleptic inspection total living cell count, proteolytic germ count, coliform cell count, alkali-forming germ count, yeast and mould cell count, Lactobacillus cell count, Clostridium cell count, haemolyzing germ count, *Micrococcus pyogenes var. aureus* cell count and psychrotolerant germ count were determined, and each sample was examined for Salmonella. In respect of each of the features investigated, logarithmic mean values and the simple positive dispersion values were estimated. Results are illustrated in Figs 1 to 5 and in Table 1. It was demonstrated that total living cell count, coliform germ count, yeast cell count, mould cell count, Lactobacillus cell count and Clostridium cell count can be used most favourably in the microbiological qualification of these foods.

Depending on whether a food had been heated prior to freezing or not, microbiological requirements are different. Coliform cell count, enterococcus cell count and total living cell count including proteolytic, psychrotolerant, and Lactobacillus cell counts are lower in the foods heated prior to freezing than in those that had not been heated. In respect of the common pathogenic and toxin-producing microorganisms, haemolyzing *Micrococcus pyogenes var. aureus*, saprophytic or facultative pathogenic clostridia no differentiation must be made between pre-heated and unheated foods. The continuous refrigeration and hygienic chain as well as securing circumstances of the hygienic production are inseparable from preventing quick-frozen foods from spoilage. Due to the extracellular enzymatic activity, frozen foods can be spoiled even at low cell count if enzymes could exert their adverse effect prior to freezing or at thawing. Spoilage caused by the psychrophilic germs can be prevented by securing dry surface and low pH values of the foods. If cell count has increased to 10^7—10^8/ g or 1 cm^3, the frozen food must be qualified as spoiled.

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ANTIBIOTIC CONTENT OF EGGS LAID BY HENS FED ERRA CONTINUOUSLY

By

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Increased production and lowering price of the antibiotics made possible their use in fields other than therapy. Recently antibiotics have also been used as plant-protecting agents and, in preserving food of animal and plant origin, food industry also utilizes their bacteriostatic action. For about one and a half decade antibiotics have been used in the feeding of livestock, too, in order to increase yield of animal products. Recently, antibiotics have been incorporated in the so-called preventive premixes and "medicated feedstuff" all over the world.

On using antibiotics for therapeutic purposes resistant bacterium strains have emerged, and following their inefficient use the normal intestinal flora has been imbalanced. A variety of their use has involved new disadvantages as compared to the economic profit. Now antibiotics are brought into the human organism not only as therapeutics but also in nonmedical ways. It might be disadvantageous for the public health if antibiotics brought in the animal body for various purposes (therapy, preservation, feed supplementation) reached bacteriostatic levels in the meat or organs, thus leading to false negative bacteriological results (CLARENBURG, KAMPELMACHER, 1957; KISSLING, 1960). The same may be valid for the milk obtained for several days following antibiotic treatment of cows with mastitis. The antibiotic present in the milk may interfere even with cheese production, thus causing heavy economic losses (FRANK, 1964).

Consuming antibiotic-containing foods might result in adverse effect in man. Allergic symptoms may arise (WELCH, 1959; MEARA, 1958; KUSCHINSKY, 1960; FEHÉR and GÓZONY, 1961), important symbiotic bacterium strains in the mouth and intestines may be suppressed and overgrown by harmful bacteria, and strains resistant to antibiotics may develop (ZINZIUS, 1954).

Owing to the important public health consequences, numerous investigators have dealt with the problem. The conclusion has been drawn that unlike large therapeutic doses or preservative concentrations, antibiotics fed at a low level in the ration for a longer period of time does not reach in the meat and organs a level harmful for the consumer or interfering with the result of the bacteriological examination. While antibiotic content of the animal tissues has been examined by several authors (KÄFERSTEIN, 1964), that of the eggs has poorly been studied (SCHÖNHERR, 1957).

We had plenty of reasons to study antibiotic content of eggs laid by antibiotic-fed hens.

1. While meat and organs are usually consumed after heating (roasting or cooking) which destroys possible residual antibiotics, egg (whole egg, egg white and yolk) is frequently consumed unboiled even by babies.

2. It is known that laying hen accumulates in the yolk not only essential nutritives but also concentrated antibodies produced by her own organism. Thus chicks are not only nourished but also provided with passive immunity for the first days after hatching. Thus, yolk acts like the colostrum in mammals. From the 7th day on colouring substances eaten by the hen also appear in the yolk. It seemed reasonable to consider that a significant amount of the antibiotic circulating in the blood and present in the body fluids will be deposited in the yolk.

3. Since experiments reported in the literature had been carried out with antibiotic other than the oxytetracycline (OTC), the antibiotic incorporated in the Hungarian preparations Erra, it seemed justified to initiate investigations in order to get more information on the home-made product.

The aims of our studies were:

1. to select the methods most suitable for the detection of the antibiotic in the egg white and yolk;

2. to show whether in the eggs (white and yolk) laid by hens kept on antibiotic-supplemented feed the antibiotic may reach a level that sensitizes the consumers of unboiled eggs, causes allergic symptoms or any other adverse effect (influence on the intestinal flora, development of resistant strains) or makes bacteriological tests unreliable.

Materials and methods

Two hundred and ninety-three eggs were examined. The eggs were laid by 15 hens weighing 2 kg each in a 90-day period, and distributed in 3 groups. Six hens were fed a ration supplemented with Erra–2 at a level of 150 g per 100 kg feed (30 mg OTC per kg feed), another 6 were given 10 times the prescribed amount of Erra–2, *i.e.*, 1.5 kg Erra–2 in 100 kg feed (300 mg OTC per kg feed). The hens were allowed to eat *ad libitum* throughout the experiment. Three hens were fed the plain ration and served as control.

Several methods suggested in the literature to extract antibiotic content from tissues were used.

The method of the Austrian KISSLING (1959) seemed very promising. He suggests to perform extraction with hydrochloric acid—acetone. Organs ground with this solvent are stored overnight in refrigerator (in his opinion long extraction is more perfect). Following centrifugation of the suspended tissue water-clear extracts are obtained. Since hydrochloric acid—acetone of approximately pH 1.0 itself has strong inhibitory action on the test organism, the pH of the extract must be adjusted to a value more than 2.5. According to KISSLING the inhibiting action of the hydrochloric acid—acetone mixture disappears at pH 2.5.

OTTE (1960) and BAUER and STEIGLER (1961) extract the antibiotic with phosphate buffer solution of pH 4.5. Citrate buffer of pH 4.5 is also used for the same purpose.

On checking OTC-containing feed supplements in our institute, a mixture of oxalic acid and glycine buffer of pH 2.0 is used to extract the antibiotic (MIZSEY and SZABÓ, 1955).

To obtain perfect extraction and water-clear organ extracts, KISSLING's method was attempted primarily. The inhibitory action of the hydrochloric acid—acetone mixture was studied at different pH values. *B. subtilis* was used as test organism throughout the experiment. At the original pH value (0.8), at pH 2.5 and between pH 4.5 and 5.0 a huge, a moderate and no zone of inhibition, respectively, was observed due to the solvent. Based on this experience pH values of the organ extracts were adjusted to 4.5. In addition to the hydrochloric acid—acetone solution, saline of pH 4.5 was parallelly used for extraction. Hydrochloric acid—acetone extracts yielded considerably wider zones of inhibition than those obtained by extraction with the saline of the same pH. To resolve our doubts both acetone and saline extracts were autoclaved at 14-pound pressure for 2 hours in order to ascertain if the inhibiting agent would really be antibiotic. After autoclaving, saline extracts yielded no inhibition while inhibitory action of acetone extracts was retained, and in case of some organ extracts even wider zones were observed than before.

Further control investigation was carried out by extracting horse meat used for preparing culture medium with hydrochloric acid—acetone and saline at pH 0.8 and at pH adjusted to 5.0. None of the saline extracts inhibited the growth of *B. subtilis*, while acetone extracts of pH 0.8 and 5.0 resulted in a zone of about 30 mm and 15 mm, respectively, in diameter. The latter inhibition zone turned more pronounced (19 to 20 mm width) if the extracts were autoclaved before testing. Autoclaved saline extracts yielded negative results.

Consequently, KISSLING's method had to be omitted.

Comparative studies were performed to extract OTC with the other three methods mentioned above.

As test organism *B. subtilis* ATCC 6633 applied according to the so-called sensitized procedure was used throughout (ILLÉS, 1962).

First of all it was ascertained that phosphate and citrate buffer of pH 4.5 as well as oxalic acid + glycine buffer alone would not inhibit the growth of *B. subtilis* even in sensitized standard plates. To compare the reliability, sensitivity and results of the methods, OTC solutions were thoroughly mixed with egg white or yolk in a mortar for about 10 minutes to make mixtures of antibiotic concentrations 1 μg, 10 μg and 100 μg per g. Then each mixture was shaken with an equal amount of the solvent for 1 hour to extract the active substance. Undoubtedly OTC-free egg white and yolk was shaken with

the solvents as control. The yolk was added directly to the standard plate, while egg white was filtered through gauze before adding. Samples with minimal concentrations of OTC were tested as undiluted, those with the medium and maximal concentrations of OTC were tested as undiluted and in 1 : 10 and 1 : 100 dilution, respectively.

Results

Results are summarized in Table 1.

Table 1

Recoverability of the OTC from egg yolk and white by various solvents

Added amount of OTC μg/g		Solvents used for extraction		
		Oxalic acid + pH 2 glycine buffer	pH 5 phosphate buffer	pH 5 citrate buffer
1	yolk	1.3	1.0	—
	white	1.4	1.2	0.4
10	yolk	8.0	8.0	8.4
	white	8.8	9.9	9.8
100	yolk	90.7	89.0	98.0
	white	93.7	116.0	83.0
0 Control	yolk	—	—	—
	white	0.8	0.9	0.8

It is shown that OTC was recovered from the mixtures containing 10 or 100 μg OTC per g with all the three methods, with an accuracy remaining within the usual limits of error. Citrate buffer proved unsatisfactory for the detection of the minimal concentration. Unidentified growth-inhibiting factor in a concentration of approximately 1 μg per g was extracted with all the 3 methods from the egg white undoubtedly free of OTC. In the following experiments extraction with oxalic acid + glycine buffer and phosphate buffer of pH 4.5 was used parallelly.

As to the evaluation, inhibition in growth of *B. subtilis* could be measured with our method at as low a concentration as 0.2 μg OTC per g. At lower OTC concentrations, however, reduction in growth could be perceived only.

Discussion

All the eggs tested, including the control ones, contained a factor in a concentration of 1—1.5 μg per g egg white that had inhibited the growth of *B. subtilis*. Our observation agrees well with that of PELCZYNSKA and SLU-

ZEWSKI (1966), who also demonstrated in the egg white a substance of antibiotic action and suggested it to be a normal antibiotic. We attribute the bacteriostatic action of the egg white to its lysozyme content. Besides this normal bacteriostatic factor, in the egg white or yolk laid either by controls or by hens fed normal amount or 10 times the amount of Erra–2, demonstration of OTC failed. Thus, antibiotic demonstrable with the methods used by us was not deposited in the eggs of hens continuously fed a ration supplemented with antibiotic. Consuming of eggs of hens fed with Erra–2, for nutritive, or Erra–6 for preventive purposes is harmless even if the eggs are eaten unboiled.

SUMMARY

While on feeding ration supplemented with antibiotic the antibiotic residues in the meat or other tissues are inactivated at the temperature of roasting or cooking, those present in the unboiled egg may cause sensitization, allergic symptoms, adverse change of the intestinal flora and development of antibiotic-resistant strains in the consumer.

Oxytetracycline (OTC) content of eggs, marketed in Hungary, having laid by hens kept on a ration supplemented with OTC-containing Erra was investigated. Following critical evaluation of several methods, oxalic acid + glycine buffer mixture and phosphate buffer of pH 4.5 were used parallelly for extraction. As test organism *B. subtilis* was applied in the "sensitized" procedure.

Experiments were carried out on 293 eggs laid by 15 hens. Six hens were fed the usual amount of Erra, *i.e.*, 150 g Erra–2 in 100 kg ration (30 mg OTC per kg ration), another 6 received 10 times this amount. Three hens kept on the plain ration served as control.

All eggs tested, including those laid by the controls, contained a bacteriostatic factor, presumably lysozyme, in a rate of 1 to 1.5 μg per g egg white. Besides this normal inhibitor, no OTC could be demonstrated in the eggs even in those laid by hens fed 10 times the usual amount of Erra–2 (egg white and yolk were tested separately).

It is concluded that widely practiced supplementation of feedstuff with antibiotic involves no danger to the health of man consuming unboiled egg or yolk.

The present experiments have confirmed that supplementation of the ration with the prescribed amount of antibiotic increases the egg yield. An increase of this amount failed to further increase in egg yield.

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EGG ALBUMEN PROTEINS IN FOUR BREEDS OF POULTRY

By

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By means of starch-gel electrophoresis the albumen proteins of the hen egg can be separated into four distinct groups. These groups form four regions: ovalbumins, region II, region III and conalbumins (LUSH, 1961; LUSH, 1964; WILLIAMS, 1962a,b; OGDEN et al., 1962; STRATIL, 1966). It has been proved that all of these groups represent a two allele—three genotype system.

WILLIAMS (1962a,b) presumed that the egg white conalbumins and the serum transferrins are controlled by the same locus. This was proved by OGDEN et al. (1962) and by STRATIL (1966).

In the present study the egg white proteins of hens were determined in four different breeds (Hungarian Yellow, Sussex, White Leghorn, Rhode Island Red). As the genotypes of cocks cannot be determined directly, the main purpose of our investigation was to decide how the egg white polymorphism of mother and progenies can be used for the determination of the sire's genotype.

Material and methods

Fresh eggs were collected from 60 Hungarian Yellow, 85 Sussex, 61 White Leghorn and 28 Rhode Island Red hens. Most of these hens belonged to families with known parentage (Hungarian Yellow, 6 mothers and 54 daughters; Sussex, 13 mothers and 26 daughters; Rhode Island Red, 4 mothers and 24 daughters; White Leghorn, 9 mothers and 24 daughters). Egg white samples were stored at —16 °C.

The buffer system I described by STRATIL (1966) was used to determine the fractions controlled by the conalbumin locus and loci III and II. Another method from STRATIL (1967) was used for ovalbumin typing.

Results

Table 1 shows the results summarized according to the breeds and the egg white regions.

Table 1

Systems	Sussex		Hungarian Yellow		White Leghorn		Rhode Island Red	
	No.	%	No.	%	No.	%	No.	%
Conalbumins								
A	—	—	—	—	—	—	—	—
AB	6	7.05	—	—	—	—	—	—
B	79	92.94	60	100.00	61	100.00	28	100.00
Region III								
A	1	1.18	—	—	—	—	—	—
AB	8	9.41	4	6.66	—	—	3	10.74
B	76	89.41	56	93.33	61	100.00	25	89.28
Region II								
A	60	70.58	55	91.66	60	98.36	11	39.28
AB	22	25.88	5	8.33	1	1.63	13	46.42
B	3	3.52	—	—	—	—	4	14.28
Ovalbumins								
A	47	55.29	52	86.66	45	73.77	10	35.71
AB	32	37.64	8	13.33	12	19.67	15	53.56
B	6	7.05	—	—	4	6.55	3	10.71

Polymorphism at the conalbumin locus was observed in the Sussex breed only. From the 85 samples 6 (7.05%) were heterozygote type AB. No homozygote type AA was found. This finding is in agreement with that of STRATIL (1966).

No polymorphism was in White Leghorns at region III (BB = 100%). The heterogeneity of this region was the most expressed in the Sussex breed, where type AA was also found.

In respect of region II all the tested breeds showed polymorphism but the homozygote BB type occurred in the Sussex and Rhode Island Red breeds only (3.52 and 14.28%, respectively).

Ovalbumin polymorphism was observed in all the four breeds studied, but no homozygote type BB was found in the Hungarian Yellow breed.

In the families available, the genotypes of the cocks were determined at each locus. It was found on the basis of segregation among the offsprings that the expected and observed occurrence of each genotype was in agreement with the Mendelian rules.

Tables 2, 3 and 4 contain examples for determination of the genotypes in cocks.

Table 2

Determination of the genotype of the Hungarian Yellow cock No. 2436 for all the four loci

Progenies	Conalbumin	Region III	Region II	Ovalbumin
532	B	B	A	AB
542	B	B	A	A
545	B	B	A	AB
550	B	B	A	A
615	B	B	AB	AB
699	B	B	A	A
737	B	B	A	A
766	B	B	AB	AB
781	B	B	AB	AB
Dam No. 73	B	B	A	A
Sire No. 2436	B	B	AB	AB

Table 3

Determination of the genotype of the Hungarian Yellow cock No. 4456 for all the four loci

Progenies	Conalbumin	Region III	Region II	Ovalbumin
147	B	B	A	AB
179	B	B	A	A
188	B	AB	A	A
678	B	AB	AB	AB
687	B	AB	A	A
704	B	AB	A	A
805	B	B	AB	AB
Dam No. 224	B	B	A	A
Sire No. 4456	B	AB	AB	AB

Discussion

The present studies, in agreement with data published by others, prove that egg white polymorphism — besides the blood group systems determined by red cell antigens — can well be used in the research and practical work concerning chickens. In this species besides the 13 blood group systems further 4 well-distinguishable systems are available for typing of individuals. For this purpose, on the basis of our investigations, the polymorphism of regions II and III and chiefly the ovalbumin system seem to provide useful data.

Table 4

Determination of the genotype of the Sussex cock No. 7222 for all the four loci

Progenies	Conalbumin	Region III	Region II	Ovalbumin
1803	B	B	AB	AB
29851	B	B	A	B
59446	B	B	AB	AB
59488	B	B	B	A
Dam No. 11462	B	B	AB	AB
Sire No. 7222	B	B	AB	AB

SUMMARY

Fresh eggs were collected from 60 Hungarian Yellow, 85 Sussex, 61 White Leghorn, and 28 Rhode Island Red hens and the genotypes of 6 Hungarian Yellow, 13 Sussex, 9 White Leghorn, and 4 Rhode Island Red families were determined by starch-gel electrophoresis. On the basis of the mothers' and daughters' genotypes authors determined the sire's genotypes for all the four egg white loci.

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HISTOLOGICAL DEMONSTRATION OF GLYCOGEN DURING THE AGEING OF MEAT

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The post-mortem condition of meat is generally characterized by its pH-value, which is essentially determined by the lactic acid deriving from the decomposition of glycogen. Further to this, other carbohydrate-decomposing and proteolytic enzyme systems play a role in determining the post-mortem biochemical status of meat.

The basic relations in the changes of meat pH are known chiefly from the studies of Lawrie (1955), Wismer-Pedersen and Briskey (1961), and Bendall (1963). Changes of pH in cooled, stored and cooked meats have also been investigated (Kauffman and Carpenter, 1964) and some connected enzymatic processes have been clarified by histochemical methods (Bodwell et al., 1965).

Considering the literary data relevant to the present topic, three publications seem to be of special interest.

Wartenberg and Pytasz (1963) determined the glycogen and reducing sugar contents in various types of muscle from several species. As to the glycogen content, besides the species differences, there was a notable variation with the muscle type in one and the same animal. The longissimus dorsi, minor psoas, femoral biceps and supraspinal muscles were very rich, whereas the abdominal rectus muscle was very poor in glycogen.

Examining swine muscle, Millo and Schilling (1964) have found that white and red muscles differed markedly in their glycolytic and ATPase activities. While the white muscles (longissimus dorsi) assumed rigor mortis in the first hour after slaughter, for the red muscles it took 24 hours. Investigation of the morphological state of the muscle fibres revealed that also the proteolytic processes taking place during the ripening of meat differed with the two types of muscle.

Beecher et al. (1968) differentiated white and red components in the porcine semitendinosus muscle and, examining them separately, found their glycogen contents identical. But, on further comparison, the white muscle contained more lactic acid, while the red muscle more myoglobin, sodium, zinc, and iron, but less phosphorus, fat and myoplasmic nitrogen.

In the present study glycogen was demonstrated histochemically in order to assess its distribution in the muscle tissue.

Material and method

Specimens from the biceps brachii muscles of rabbits and pigs were examined. Muscle samples from rabbits were removed and fixed immediately after exsanguination, whereas those from pigs were secured only a few hours after death. The specimens were fixed in Sanomiya solution, embedded in

paraffin, cut into 3—4 μ sections and stained with PAS (KISZELY and BARKA, 1958). To evidence the specificity of the glycogen reaction, PAS staining was repeated after digestion with saliva. Negative Schiff reaction, performed without periodic acid, also attested the presence of glycogen. Further rabbit muscle specimens were secured after the carcase was stored at +6 °C for 72 hours. Processing was made as described above. At both sampling times, besides staining with PAS, glycogen was determined, using the GOOD and SOMOGYI method as described by HEGEDÜS and BÁLINT (1955). Cross sections were prepared and the photos were taken at 160-fold magnification.

Results

On staining with PAS, the cross sections of the fresh rabbit muscle fibres showed a homogeneous dark red colouring (Fig. 1). It is known that the bulk of the muscle tissue's glycogen content is located in segment "A" of the fibre. With uniform distribution of glycogen throughout the fibre, the colouring of the cross section appeared to be homogeneous. Omitting the periodic acid treatment, no oxidation of the specific groups took place, thus no positive Schiff reaction developed. In such cases the section showed an undefinable greyish-brown or greyish-yellow colour (Fig. 2). Staining saliva-digested material with PAS, the muscle fibres assumed a pale pinkish shade and the interstitial tissue showed a positive reaction (Figs 3 and 6). The pinkish hue of the fibres may be indicative either of unaccomplished saliva-digestion or, more likely, of the presence of PAS-positive neutral mucopolysaccharides and/or mucoproteins as suggested by the positive reaction of the interstitial tissue.

The rabbit muscle specimens stored 72 hours after exsanguination showed a very typical PAS reaction. Some fibres showed a dark red colouring, others a pale granular staining and again others assumed a very pale yellowish-pink shade (Fig. 4).

The variation of the fibres' colour shades, indicating a variation of their glycogen contents, permitted the assumption that the process of ripening does not take place simultaneously in all muscle fibres involved. Observation of fibre morphology during ripening also showed that in the different muscle types the rigor mortis takes different periods of time to develop, and even fibres in rigor exhibit a wide range of contraction types. The initial difference in the biochemical circumstances appears to persist in the state of relaxation. In that state, the fibre morphology is uniform, in spite of the biochemical differences. It is, nevertheless, obvious that the phase shifts are retained throughout the proteolytic processes, manifesting again by morphological differences when the muscle assumes a stringy consistency.

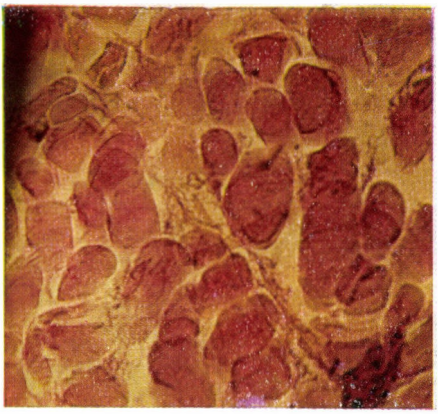

Fig. 1. PAS reaction of rabbit muscle

Fig. 2. Rabbit muscle, Schiff reaction without periodic acid

Fig. 3. Rabbit muscle, PAS reaction after digestion with saliva

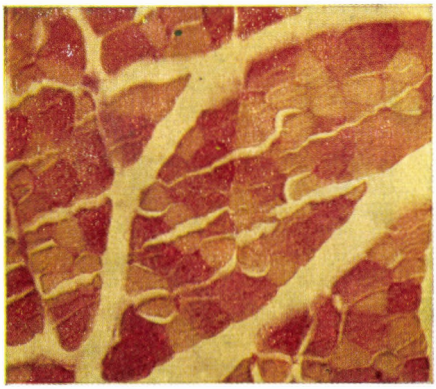

Fig. 4. PAS reaction of rabbit muscle

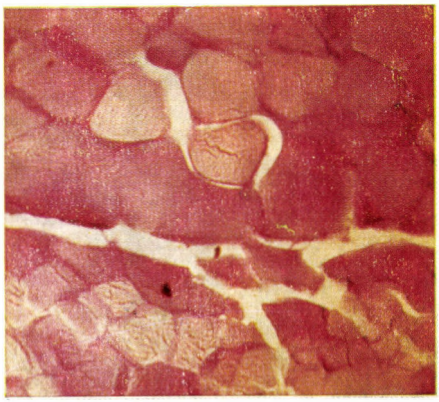

Fig. 5. PAS reaction of pig muscle

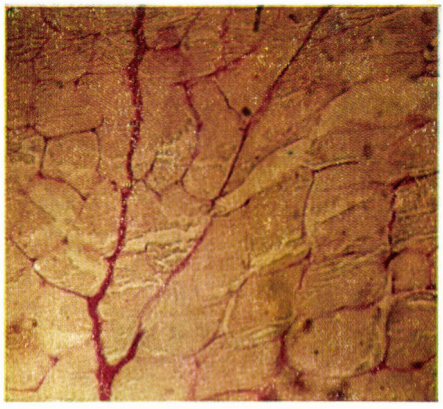

Fig. 6. Pig muscle, PAS reaction after digestion with saliva

The chemically determined glycogen content of the rabbit muscle was 29.8 mg per 100 g in samples secured immediately post mortem and 14.8 mg per 100 g in those stored over 72 hours. These concentrations represent averages for the entire muscle, while the histochemical examination enabled the assessment of the glycogen content and the corresponding biochemical state for each individual fibre in a bundle.

Fig. 5 presents a section of a pig muscle specimen secured several hours post mortem. The deep colouring obtained in the PAS reaction is characteristic of the behaviour of the individual fibres.

The question may arise whether the differences seen between the muscle fibres presented in the Figures are not simply due to the inherent dissimilarities of white and red muscles.

It is known that white and red muscle types have dissimilar glycogen contents. Accordingly, the fibres of mixed muscles assume different colour shades in the PAS reaction. The same applies to fresh muscle. The immediate samples of rabbit muscles exhibited a homogeneous colour, indicating a uniform glycogen content in the fibres. Varying shades of PAS staining were seen only in the 72-hour ripened sample. This has been attributed to the biochemical differences related to the unequal contractility of the individual fibres which they seem to retain during the further process of ripening. A morphological differentiation of the red and white muscle types can be based exclusively on KRÜGER's classification (1952).

Acknowledgement. The author wishes to express his thanks to DR. B. TÓTH for his help in the glycogen determinations.

SUMMARY

The glycogen contents of rabbit and pig muscles were determined histochemically by the PAS reaction. Fibres with uniform glycogen content assumed a homogeneous deep red colour on staining with PAS. During the ripening process the quantitative differences between the glycogen contents of the individual fibres manifested themselves by assuming varying colour shades in the PAS reaction. The histochemical approach enables the assessment of the glycogen content and of the corresponding biochemical state of each individual fibre in a bundle of muscle.

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EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DEN REGULIERUNGSMECHANISMUS DES SÄURE—BASEN-GLEICHGEWICHTS BEI SCHAFEN

Von

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(Eingegangen am 17. Juli 1968)

Es ist bekannt, daß sich der pH-Wert des Vormageninhaltes bei Futteraufnahme verändert. Besonders bedeutend ist diese Änderung nach Aufnahme großer Mengen von an leichtverdaulichen Kohlenhydraten reichem Futter. Wir konnten nachweisen, daß sich in solchen Fällen im Organismus eine kompensierte und in schweren Fällen sogar eine tödliche inkompensierte, metabolische Azidose entwickeln kann (JUHÁSZ und SZEGEDI, 1965, 1968a, b; SZEGEDI und JUHÁSZ, 1968). LEWIS (1960) beobachtete bei Schafen die Entwicklung von Alkalose nach der Aufnahme einer großen Menge von Harnstoff. ANNISON und Mitarb. (1959) stellten bei Schafen zu Beginn der Weideperiode oft Azidose fest. Auch diese Beispiele deuten dahin, daß die Prüfung des Regulationmechanismus des Säure—Basen-Gleichgewichts — nach Futteraufnahme mit Rücksicht auf die mikrobiologischen Verdauungsprozesse in den Vormägen — von besonderer Bedeutung ist.

Das Säure—Basen-Gleichgewicht des Organismus wird durch Puffersysteme und eine vitale Regulation gesichert. Zu letzterer wird die Atmung und die Nierenfunktion gerechnet. Bei eigenen Untersuchungen zur Klärung der Ätiologie der Pansenazidose konnte festgestellt werden, daß sich die Menge und Zusammensetzung des während des Syndroms gebildeten Speichels bedeutend verändern (JUHÁSZ und SZEGEDI, 1965, 1968b). Auf Grund der erzielten Ergebnisse vermuteten wir, daß die qualitativen und quantitativen Verhältnisse des Speichels nicht nur durch bereits bekannte, sondern auch durch zusätzliche Faktoren reguliert werden. Es wurde vermutet, daß möglicherweise auch der Speichel in der Regulation des Säure—Basen-Gleichgewichts eine Rolle spielen kann (KUTAS, 1966).

Bei Wiederkäuern ist der in großen Mengen sezernierte, stark alkalische Speichel von lebenswichtiger Bedeutung, da er den normalen Ablauf der Vormagenverdauung sichert. Es ist aber nicht vollständig bekannt, was für einen Einfluß die Menge und Zusammensetzung des Speichels auf den Wasser- und Mineralstoffhaushalt sowie auf das Säure—Basen-Gleichgewicht des Organismus ausüben. Unsere Kenntnisse sind auch insoweit mangelhaft, was für qualitative und quantitative Zusammenhänge zwischen dem Ablauf der Vormagenverdauung, Menge der Pansenflüssigkeit und der Speichel- bzw. Harnabsonderung bestehen. Zur Klärung dieser Fragen führten wir Versuche bei Schafen durch, um festzustellen was für Änderungen in bestimmten Blutparametern sowie in der Menge und Zusammensetzung des Speichels und des Harns nach Verabfolgung von Milchsäure und Karbamid im Pansen zu vermerken sind.

Methode

Unsere Versuche wurden bei 24—45 kg schweren, weiblichen Schafen der ungarischen Kammwoll-Merinorasse vorgenommen, die mit einer Jarret-

schen Pansenfistel versehen waren. In die Leitung der linken Parotisdrüse sowie in die Harnblase wurden Kunststoffkatheter eingeführt. Der Speichel (unter Paraffinöl) und der Harn wurden jede Stunde gesammelt. Das Mengenverhältnis ihrer Ausscheidung wurde mittels eines elektrischen Tropfenschreiber-Apparats, gemeinsam mit der Atemfrequenz und den Pansenbewegungen auf einen Russkymographion registriert.

Jede Stunde wurden Proben von Pansenflüssigkeit und Blut entnommen. Zur chemischen Analyse wurde Blut von der V. jugularis, zur Feststellung der Parameter des Säure—Basen-Gleichgewichts aber Kapillarblut von der Ohrmuschelhaut verwendet. Bei der Pansenflüssigkeit, dem Blut, Speichel und Harn wurden pH-Wert, Milchsäure- (DISCHE und LÁSZLÓ, 1962), bzw. Ammoniak- (JUHÁSZ und SZEGEDI, 1958) und Karbamid- (JUHÁSZ, 1962), sowie Na- und K-Ionkonzentration (Flammenfotometer; Zeiss, Jena) bestimmt. Die Bestimmung der Parameter des Säure—Basen-Gleichgewichts beim Kapillarblut erfolgte mittels eines nach dem Prinzip des Radiometers funktionierenden Astrup Mikro-Tonometers und Mikro-Elektrode-Unit Geräts. Mit Hilfe des verwendeten Verfahrens wurden folgende Blutwerte festgestellt: aktueller pH-Wert, Alkalireserve (Alk. Res.), pCO_2-Wert (mm Hg), Basenüberfluß (B.Ü.) und Basenpuffer (B.P.). Der Basenüberfluß des Blutes bedeutet den Überfluß der gebundenen Säuren bzw. Alkalien in m.Äqu/Liter-Werten ausgedrückt. Ein positiver Wert deutet auf Basen-, ein negativer aber auf Säurenüberfluß. Der Basenpufferwert bedeutet das Verhältnis der Anione mit Pufferwirkung zu den gesamten Kationen des Blutes in m. Äqu./Liter-Werten ausgedrückt (SIGGAARD-ANDERSEN und ENGEL, 1960, 1962).

Den Tieren wurde in Dauerinfusion eine verhältnismäßig konzentrierte Milchsäurelösung intraruminal verabfolgt, um den Wasserhaushalt durch größere Wasserzufuhr nicht zu stören; Harnstoff wurde — nach Kraftfutteraufnahme — über die Fistel direkt in den Pansen verbracht.

Ergebnisse

Wir möchten zwei von unseren Versuchen (insgesamt 12) demonstrieren. In dem einen Versuch (Körpergewicht; KGW 24 kg) infundierten wir während 11 Stunden (St.) insgesamt 144 g Milchsäure in einer 20%igen Lösung intraruminal (i.r.); im zweiten (KGW 32 kg) verbrachten wir in den Pansen 0.6 g je kg KGW Harnstoff. Aus vorangehenden eigenen Untersuchungen (JUHÁSZ und SZEGEDI, 1965, 1968a) stellte sich heraus, daß einerseits die verabfolgte Milchsäuredosis bei Schafen eine schwere aber nicht tödliche inkompensierte metabolische Azidose verursacht, andererseits, falls der pH-Wert der Pansenflüssigkeit sauerer ist (z.B. pH 5,5), vertragen die Tiere sonst toxische Karbamidmengen ohne Schädigung.

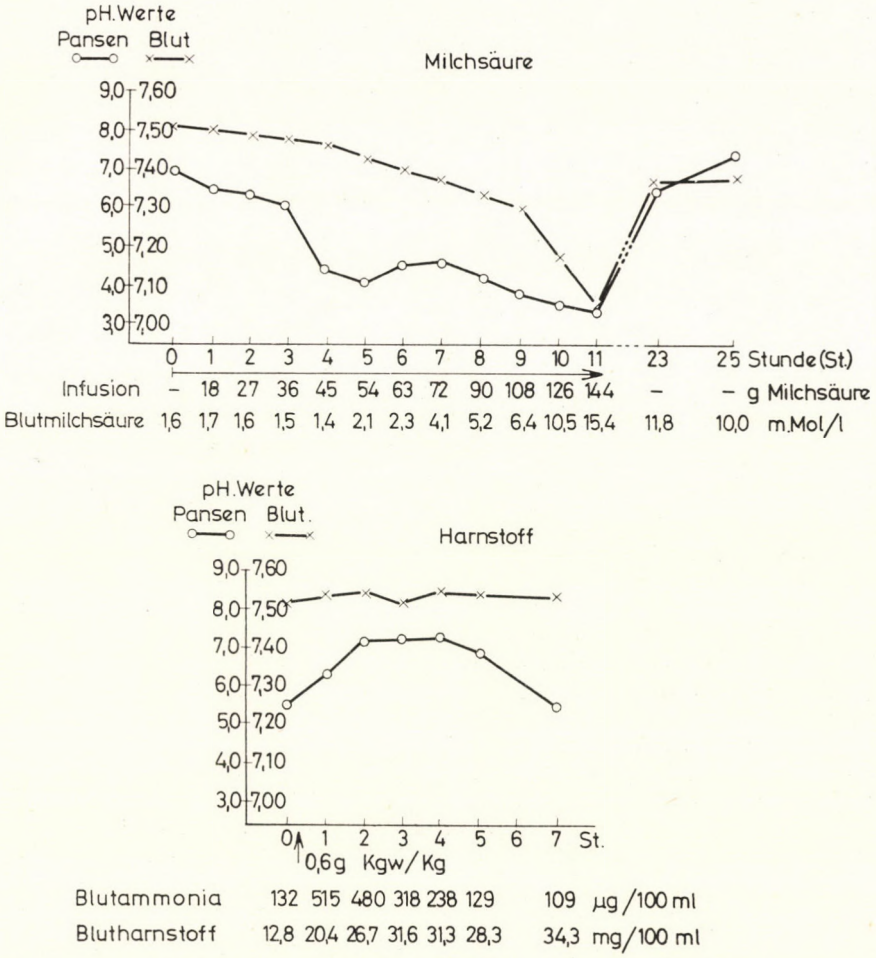

Abb. 1. Änderungen im pH-Wert der Pansenflüssigkeit und des Blutes sowie in der Konzentration der Milchsäure bzw. des Ammoniaks und Karbamids des Blutes nach intraruminaler (i.r.) Dauerinfusion von Milchsäure (während 11 St. 144 g in einer 20%igen Lösung) (obere Kurve) und nach der Verabfolgung von 0,6 g/kg KGW Karbamid (untere Kurve)

Auf der Abbildung 1 sind Änderungen in dem pH-Wert des Panseninhalts und Blutes, sowie in der Konzentration des Blutes an Milchsäure bzw. Ammoniak und Karbamid angeführt. Bei der Infusion von Milchsäure fällt der pH-Wert des Panseninhalts plötzlich ab (von pH 6,95 in der 4. Stunde bis pH 4,0) und darauf folgt eine graduelle Verminderung der Blut-pH-Werte. Nach Einstellen der Infusion (11. Stunde) normalisierten sich die Werte bis zur 25. Stunde. Die Milchsäurekonzentration des Blutes beginnt nur in der 7. Stunde bedeutend anzusteigen. Wie wir dies in vorangehenden Untersuchungen beweisen konnten (Juhász und Szegedi, 1965; Szegedi und Juhász,

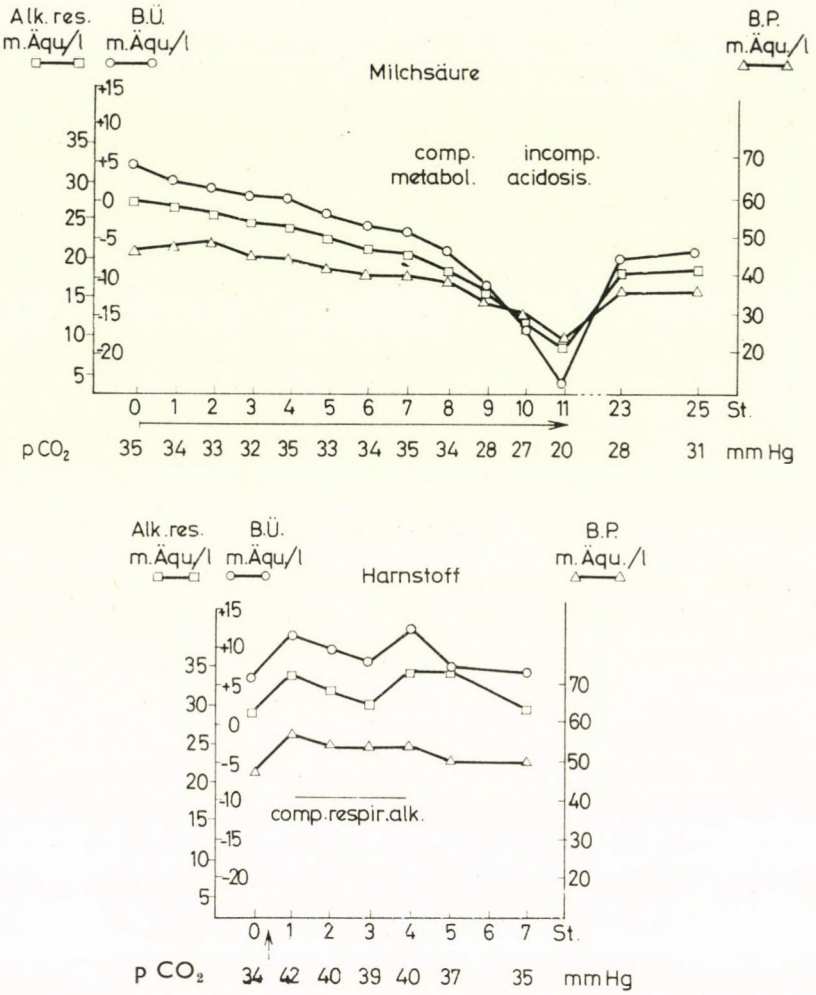

Abb. 2. Änderungen der Alkalireserve (Alk. Res.), im Basenüberfluß (B.Ü.), Basenpuffer (B.P.) sowie im pCO_2-Gehalt des Blutes nach Milchsäureinfusion (obere Kurve) und nach der Verabfolgung von Karbamid (untere Kurve)

1968), geschieht dies infolge der steigenden Transformation in der Leber und raschen Oxydation in den Gewebezellen der im Pansen resorbierten Milchsäure.

Nach der Verabfolgung von Harnstoff verschiebt sich der pH-Wert der Pansenflüssigkeit in alkalischer Richtung (von pH 5,50, in der 2. Stunde bis pH 6,90). Der pH-Wert des Blutes zeigt eine nur unbedeutende Änderung; seine Ammoniakkonzentration aber steigt bereits in den ersten Stunden bedeutend, und sein Karbamidgehalt stufenweise an.

Auf Abbildung 2 sind Angaben über das Säure—Basen-Gleichgewicht des Blutes angeführt. Bei Milchsäureinfusion zeigten die geprüften Parameter

einen langsamen Abfall und es entwickelte sich in der 7.—9. Stunde eine kompensierte und demnach eine inkompensierte metabolische Azidose, die sich nach Abbrechen der Infusion (in der 11. Stunde) bis zur 25. Stunde ausglich.

Bei Karbamidverabreichung steigen alle geprüften Parameter an. Ihre Analyse zeigt, daß bereits in der 1.—2. St. eine kompensierte respiratorische Alkalose aufgetreten ist. Es soll bemerkt werden, daß sogar nach Verabfolgung größerer Karbamidmengen keine inkompensierte Alkalose beobachtet wurde. Dies unterstützt unsere frühere Vermutung, daß bei Karbamidvergiftung nicht die Verschiebung des Säure—Basen-Gleichgewichts, sondern die toxische Wirkung des gebildeten Ammoniaks zum Tode der Tiere führt (JUHÁSZ, 1962). Die respiratorische Alkalose erfolgt wegen der Reizwirkung des Blut-Ammoniaks auf die Atmungszentrale (LEWIS, 1960). Bei der Verabfolgung großer Milchsäuremengen aber gehen die Tiere wegen der starken Verschiebung in Säurerichtung des Säure—Basen-Gleichgewichts ein (JUHÁSZ und SZEGEDI, 1965, 1968a).

In der Abbildung 3 wurden Änderungen der pH-Werte des Speichels und des Harns, der sekretorischen Aktivität der Speicheldrüsen (linker Parotisdrüse). bzw. der Nieren, sowie der mit dem Speichel und Harn je Stunde ausgeschiedenen Mengen von Na- und K-Ionen bei dem Versuch mit Milchsäure bzw. Karbamid angegeben. Außerdem ist auch die Gestaltung der Na- und K-Ionkonzentration des Blutes ersichtlich.

Bei Milchsäureinfusion wird der Speichel von der 2. St. an alkalischer (von pH 8,30 in der 4. St. bis 8,60) und es steigerte sich seine je Minute ausgeschiedene Menge; demgegenüber sank der pH-Wert und die Menge des ausgeschiedenen Harns bereits zu Beginn der Infusion. Sein pH-Wert blieb nachher bis zur 8.—9. Stunde auf ständiger Höhe, die Menge des Harns aber verminderte sich auch weiterhin. Die erwähnten Änderungen der Speichel- und Harnwerte setzten ein, sobald der pH-Wert der Pansenflüssigkeit auf einen Wert von etwa 6,0 gesunken ist. In der 7. St. des Versuchs bei kompensierter Azidose verminderte sich etwas der pH-Wert und die Menge des Speichels, der Harn wurde noch saurer, seine Menge aber stieg an. Es ist interessant, daß im Falle von inkompensierter Azidose, bei einem Blut-pH-Wert von 7,17 der pH des Speichels noch immer stark alkalisch (pH 8,35) war (Abb. 4).

Bei Harnstoffverabfolgung zeigte der pH-Wert des Speichels (8,53) keine Änderung, seine Menge aber verminderte sich bereits in der ersten St. Der Harn wird alkalischer und seine pro St. abgesonderte Menge stieg stufenweise, in der 3. Stunde aber bedeutend an. Nach der Spaltung des Harnstoffs im Pansen (ab 4. St.) stieg die Menge des abgesonderten Speichels plötzlich bis auf den Ausgangswert an. Auf den Anstieg der Speichelproduktion folgte die Abnahme der Harnausscheidung.

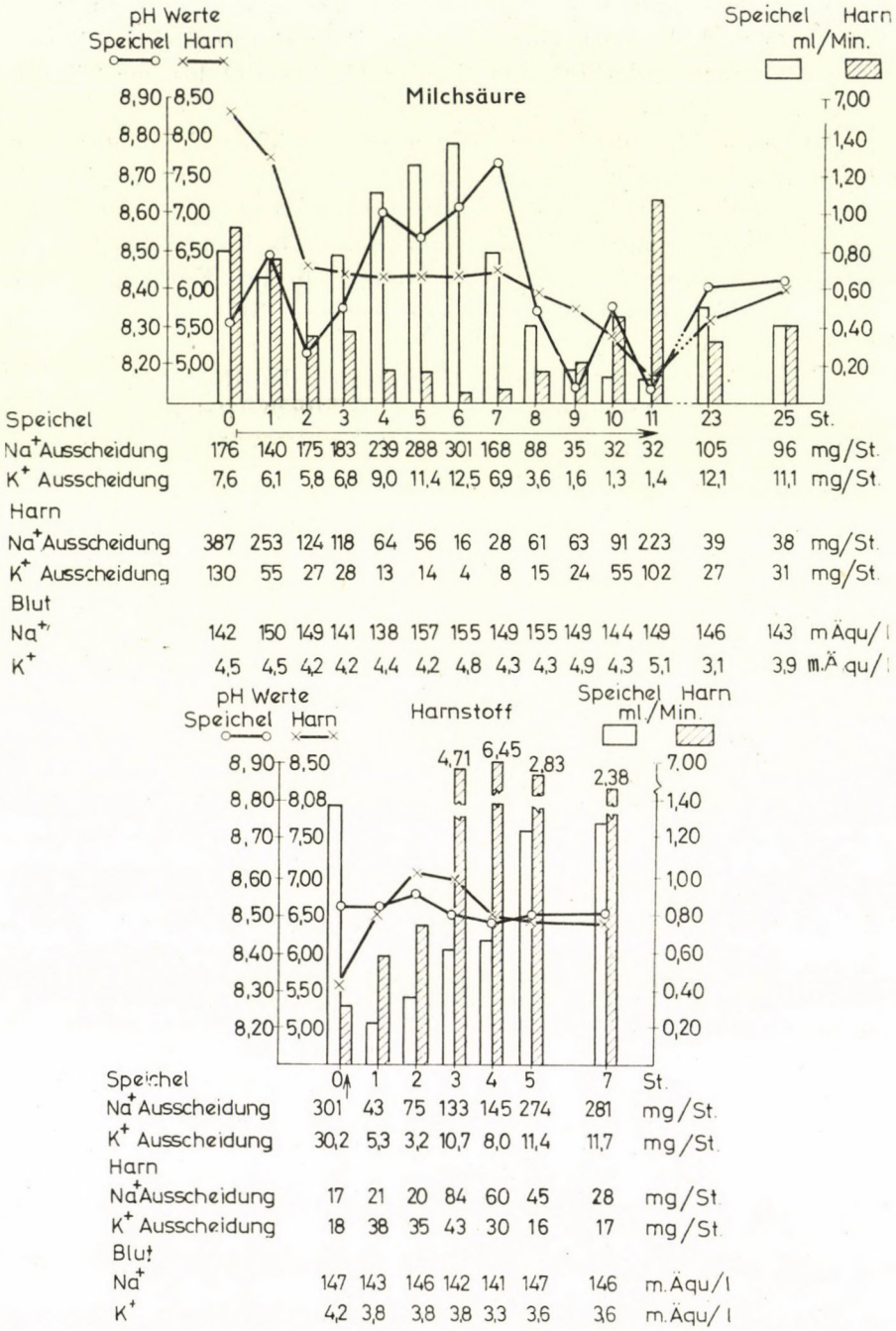

Abb. 3. **Änderungen im pH-Wert, im Ausmaß der Ausscheidung (ml/Min.), in der Menge und Konzentration des Na^+ und K^+ im Speichel und Blut nach i.r. Dauerinfusion von Milchsäure (obere Kurve) und nach Verabfolgung von Karbamid (untere Kurve)**

Bei Milchsäureinfusion stieg die Menge der mit dem Speichel abgesonderten Na- und K-Ionen nach übergänglicher Verminderung stark an, später aber konnte bei der kompensierten oder inkompensierten Azidose eine starke Verminderung der Speichelausscheidung beobachtet werden. Der Quotient der Konzentration der Na- und K-Ionen (Na^+/K^+) im Speichel zeigte bis zur 11. Stunde des Versuchs keine bedeutende Änderung, er lag zwischen 36 und 45, durchschnittlich bei 41. In der 23. und 25. Stunde aber fiel dieser Wert auf 15. Die im Harn ausgeschiedene Menge dieser Elemente zeigte eine entgegengesetzte Tendenz. Im Harn lag der K^+/Na^+ Quotient bis zur 9. Stunde bei 0,1—0,2, von diesem Zeitpunkt stieg er an und erreichte in der 25. Stunde einen Wert von 0,8. Während des Versuchs konnte keine Änderung in der Konzentration an Na^+ und K^+ des Blutes vermerkt werden.

Nach Verabfolgung des Karbamids zeigte die Menge des mit dem Speichel abgesonderten Na- und K-Ions einen plötzlichen Abfall, später aber stieg sie stufenweise an. Im Speichel gestaltete sich der Wert des Na^+/K^+ Quotienten wie folgt: 0. St. 17; 1. St. 14; 2. St. 24; 4. St. 31; 5. St. 41 und 6. St. 38. Die mit dem Harn ausgeschiedene Menge dieser Ionen stieg bis zur 3. Stunde an, später aber verminderte sie sich. Im Harn ergab der K^+/Na^+ Quotient folgende Resultate: 0. St. 1,5; 1. St. 1,0; 2. St. 1,0; 4. St. 0,3; 5. St. 0,2 und 6. St. 0,3. Die Na- und K-Ionenkonzentration des Blutes zeigte auch in diesem Fall keine Änderungen.

Vier Stunden vor der Verabfolgung von Karbamid erhielt das Tier ein leicht verdauliches, an Kohlenhydraten reiches Futter. Deswegen war der pH-Wert der Pansenflüssigkeit bei Versuchsbeginn 5,50 (Abb. 1). Bei der Bewertung der bisherigen Ergebnisse unserer Versuche kann festgestellt werden, daß sich zu Beginn der zwei Versuche in den quantitativen und qualitativen Verhältnissen der Speichel- und Harnabsonderung bedeutende Unterschiede ergaben. Diese Unterschiede können wahrscheinlich mit Abweichungen bei den pH-Werten der Pansenflüssigkeiten erklärt werden. In dem Milchsäureversuch wurde das Tier 12 Stunden früher gefüttert. Der pH-Wert seiner Pansenflüssigkeit war 6,95, der des Speichels 8,30, jener des Harns aber 8,35. Vor der Verabfolgung des Karbamids war die Pansenflüssigkeit des Tieres stark sauer, der pH-Wert des Speichels betrug 8,52, jener des Harns aber 5,55. Vor dem Milchsäureversuch war die Menge des abgesonderten Parotisspeichels 0,81 ml/Min., die des Harns aber 0,92 ml/Min. Vor der Verabfolgung des Karbamids wurden Werte von 1,38 bzw. 0,31 ml/Min. erhalten. Es zeigten sich auch in der je Stunde ausgeschiedenen Menge von Na^+ und K^+ bedeutende Unterschiede (Abb. 3). Abschließend kann festgestellt werden, daß die Parameter, die zu Beginn des Karbamidversuchs beobachtet wurden, in großen Zügen jenen entsprechen, die in der 3.—5. Stunde nach der Milchsäureinfusion beobachtet werden konnten. Auf Grund dieser Folgerungen kann vermutet werden, daß Ergebnisse, die nach der Milchsäureinfusion beobachtet wurden auch für Verhältnisse nach Futteraufnahme gültig sein können.

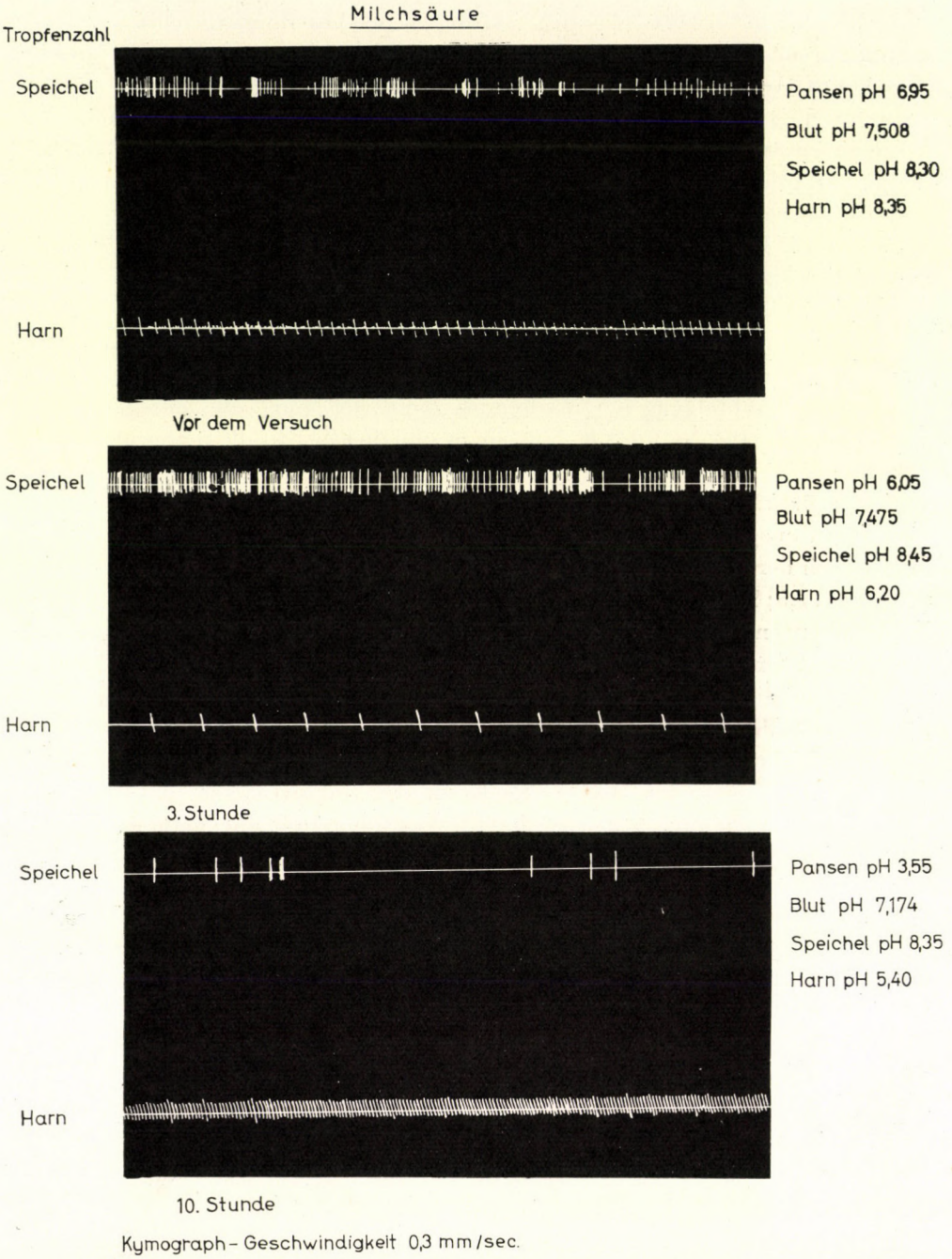

Abb. 4. Änderungen in der Speichel- und Harn-Ausscheidung nach i.r. Infusion von Milchsäure, mit einem Tropfenschreiber registriert. Oben: vor dem Versuch; Mitte: in der 3. Stunde des Versuchs; unten: in der 10. Stunde des Experiments. Zu Beginn des Versuchs lag der pH-Wert der Pansenflüssigkeit bei 6,95, der des Speichels bei 8,30

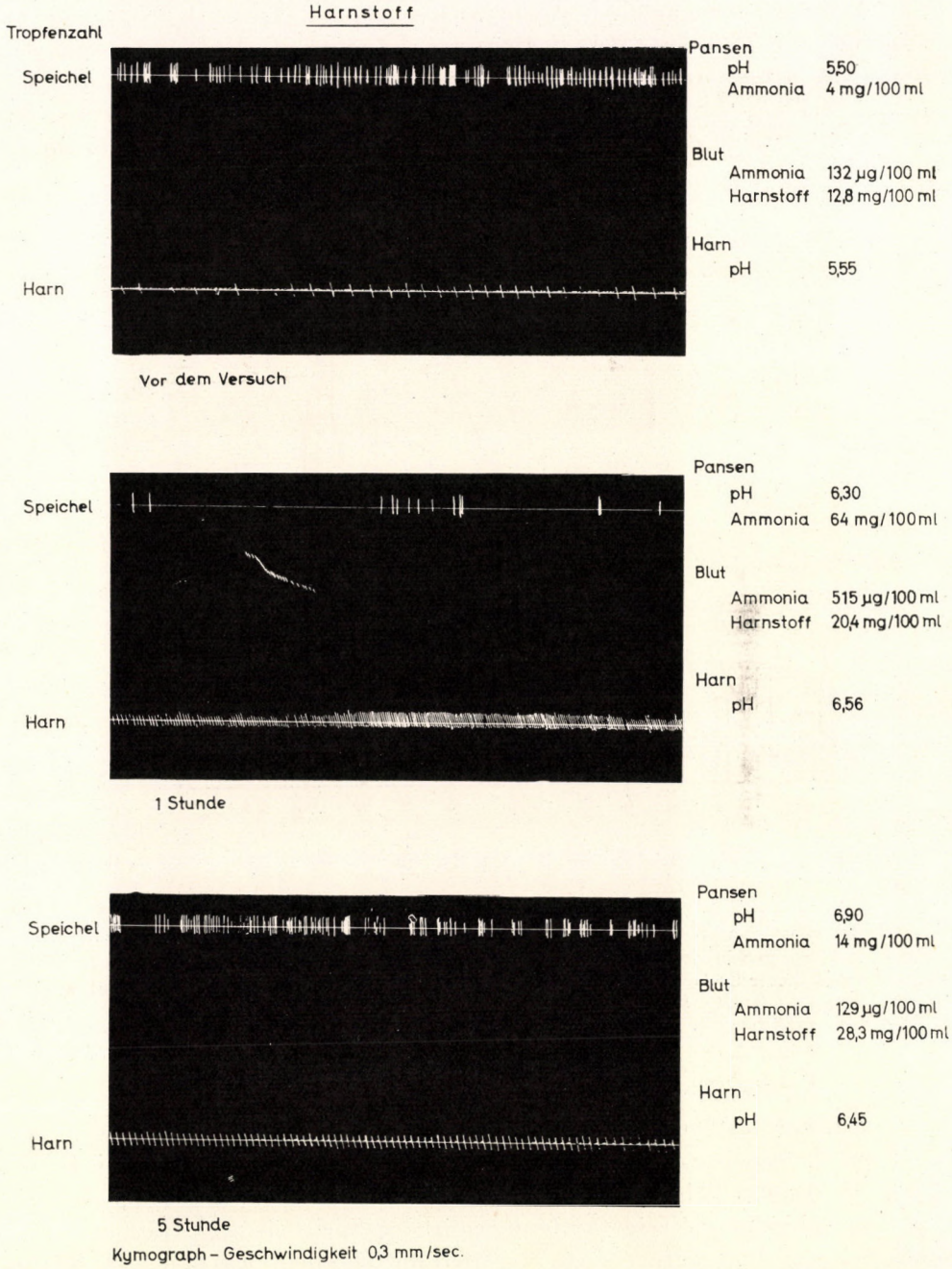

Abb. 5. Änderungen in der Speichel- und Harnabsonderung nach Verabfolgung von Karbamid, mittels eines Tropfenschreibers registriert. Oben: vor der Verabfolgung des Materials; Mitte: in der 1. Stunde; unten: in der 5. Stunde des Versuchs
Der pH-Wert der Pansenflüssigkeit lag zu Beginn des Versuchs bei 5,50, der des Speichels bei 8,53

Vorangehend erwähnten wir die Änderungen der Harn-pH-Werte (Abb. 3) während des Versuchs. Zu Beginn des Experiments kann der plötzliche Abfall des pH-Wertes mit einer abnehmenden Menge der Alkalibikarbonate erklärt werden, worauf die verminderte Ausscheidung der Alkalien deutet. Gleichzeitig steigt die Ammoniakkonzentration des Harns in bedeutendem

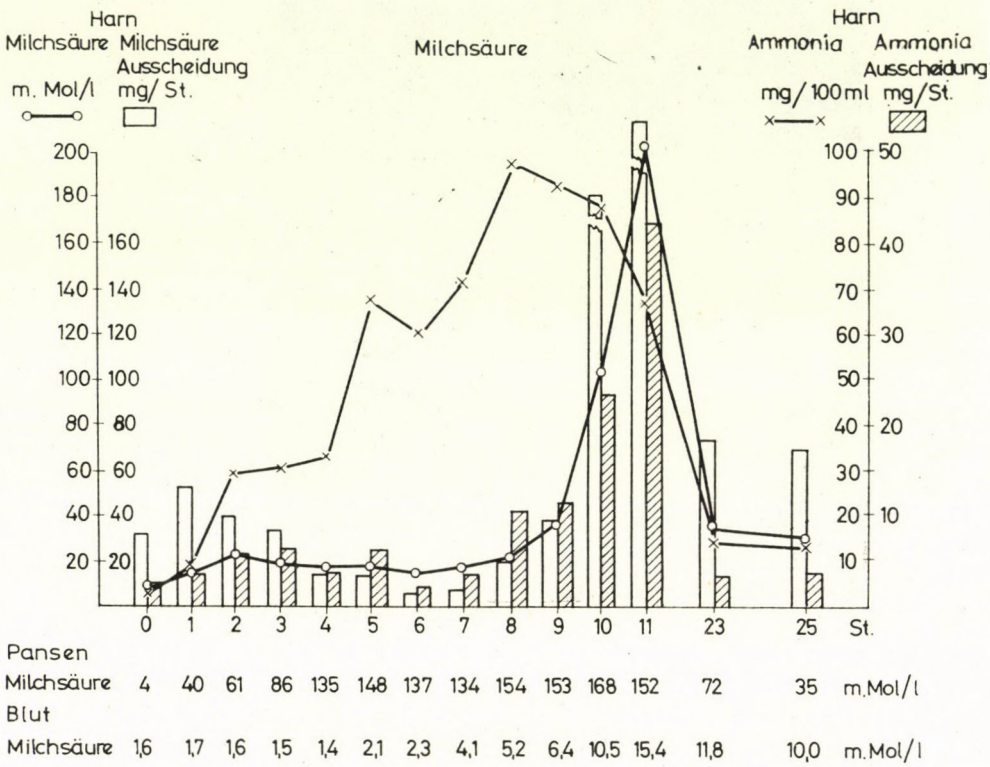

Abb. 6. Änderungen in der Milchsäure- und Ammoniakkonzentration und in der ausgeschiedenen Menge dieser Stoffe, sowie Gestaltung des Milchsäuregehaltes der Pansenflüssigkeit und des Blutes nach i.r. Dauerinfusion von Milchsäure

Maße an. Auf dieselbe Periode fällt der Anstieg des pH-Wertes und der Alkalikonzentration des Speichels. Eine weitere Verminderung des Harn-pH-Wertes nach der 9. Stunde erfolgt wegen der in gesteigertem Maße ausgeschiedenen freien Milchsäure (Abb. 6).

Die Abbildung 4 zeigt Änderungen in der Speichel- und Harnausscheidung bei dem Milchsäureversuch, registriert mittels eines elektrischen Tropfenschreibers. Das obere Kymogramm zeigt die Verhältnisse bei Versuchsbeginn, das mittlere, jene der 3. Stunde; die Speichelabsonderung ist erhöht, die Harnausscheidung dagegen vermindert. Das untere Kymogramm gibt

die Werte der 10. Stunde (bei inkompensierter Azidose) an; die Speichelproduktion ist vermindert, die Harnabsonderung aber stieg bedeutend an.

Bei dem Harnstoffversuch (Abb. 5) zeigt das obere Kymogramm das Anfangsstadium. Das mittlere, eine Stunde nach Verabfolgung des Karbamids; die Tropfenzahl des Speichels sinkt, jene des Harns steigt an. Das untere Kymogramm registriert dieselben Werte in der 5. Versuchsstunde; die Speichelproduktion steigt an, jene des Harns vermindert sich, sie bleibt aber wegen der Harnstoff-Ausscheidung noch immer über den Ausgangswerten.

Es sollen nun bei beiden Versuchen die Konzentration der Milchsäure bzw. des Ammoniaks und Karbamids in der Pansenflüssigkeit und im Blut sowie ihre im Speichel und Harn ausgeschiedene Menge verfolgt werden.

In Abbildung 6 kann beobachtet werden, daß infolge der i.r. Dauerinfusion, der Milchsäuregehalt der Pansenflüssigkeit selbstverständlich stufenweise angestiegen ist. Die Milchsäurekonzentration des Blutes aber stieg nur in der 7. Stunde in bedeutendem Maße an. Zu dieser Zeit entwickelte sich eine kompensierte metabolische Azidose (Abb. 2). Unsere vorangehenden Untersuchungen zeigten, daß die Milchsäure bereits in den Vormägen resorbiert wird; die Leber ist aber fähig, große Mengen von Milchsäure (zu Glykogen und Glukose) aufzuarbeiten, deswegen steigt die Konzentration an Milchsäure des Blutes nur in jenem Fall an, falls die resorbierte Menge die Transformierungsfähigkeit der Leber überschreitet (Szegedi und Juhász, 1968). Die Speicheldrüsen schieden sogar bei äußerst hohen Blutkonzentrationen keine bedeutenden Milchsäuremengen aus, deswegen wurden diese nicht angegeben. Im Harn zeigten die Konzentration und die Menge der Milchsäure bis zur 7. Stunde eine eher abnehmende Tendenz, nachher stiegen sie plötzlich an. Die Milchsäurekonzentration und Ausscheidung im Harn verfolgt aber den Anstieg der Milchsäurekonzentration im Blut und stimmt mit der Entwicklung der kompensierten Azidose überein. Bei der inkompensierten Azidose (Abb. 2) stieg sie weiter an. Die gesteigerte Milchsäureausscheidung fordert die Exkretion großer Alkalimengen im Harn, wie dies aus Abbildung 3 entnommen werden kann. Zur Verhinderung des übermäßigen Alkaliverlustes steigt die Harn-Ammoniakkonzentration an. Auf der Abb. 6 ist ersichtlich, daß während des Versuchs die pro Stunde ausgeschiedene Ammoniakmenge nur in jenem Fall bedeutend angestiegen ist, in dem die Menge des ausgeschiedenen Harns ebenfalls bedeutend anstieg, d.h. bei kompensierter oder inkompensierter Azidose.

Abbildung 7 veranschaulicht die Versuchsergebnisse mit Harnstoff. Während des Versuchs wurde das Karbamid im Pansen innerhalb von 3—4 Stunden zersetzt. Die Ammoniakkonzentration der Pansenflüssigkeit erreichte in der 3. Stunde den Maximalwert (86 mg/100 ml). Es ist aber interessant, daß die Ammoniakkonzentration des Blutes bereits in der ersten Stunde den Gipfelpunkt erreichte (etwa 500 μg/100 ml). Nachher verminderte sie

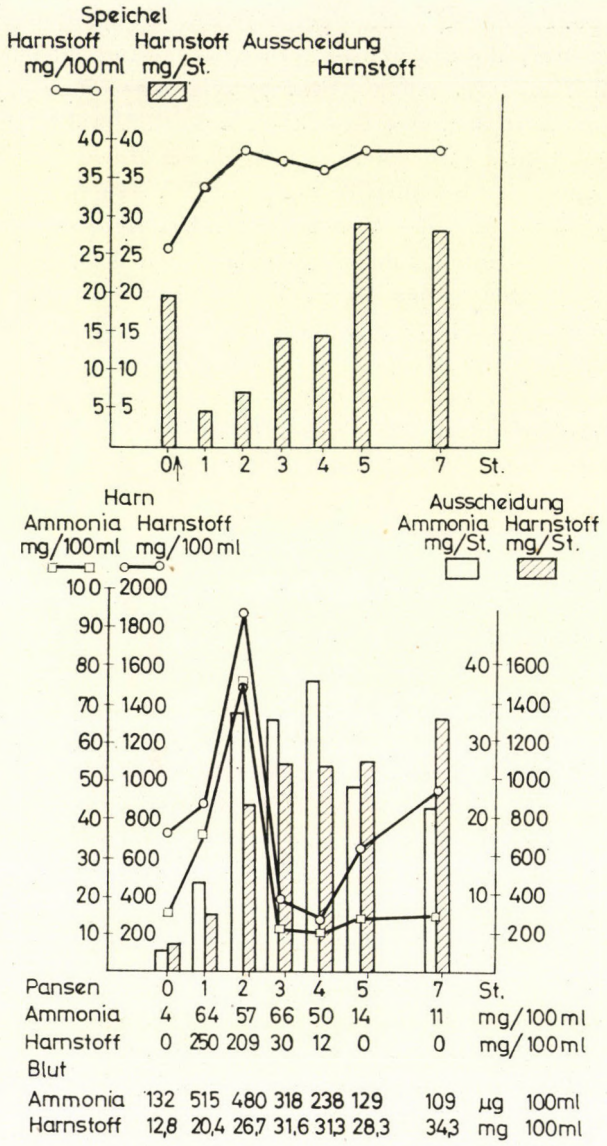

Abb. 7. Änderungen in der Konzentration und in der mit dem Speichel ausgeschiedenen Menge von Karbamid (obere Kurve) sowie in der Konzentration und in der mit dem Harn ausgeschiedenen Menge von Ammoniak und Karbamid; Gestaltung des Ammoniak- und Karbamidgehaltes der Pansenflüssigkeit und des Blutes nach Verabfolgung von Karbamid (untere Kurve)

sich und erreichte in der 5. Stunde den Ausgangswert. Der Karbamidgehalt des Blutes stieg bis zur 7. Stunde stufenweise an. In früheren Versuchen bewiesen wir, daß die Leber große Mengen von Ammoniak detoxizieren kann (über Karbamid-Bildung); sobald aber die Resorption des gebildeten Ammo-

niaks den Schwellenwert der Leber überschreitet, steigt die Ammoniakkonzentration im Blut plötzlich an (JUHÁSZ, 1962).

Ammoniak wird auch im Speichel ausgeschieden, infolge der äußerst geringen Mikrogramm-Mengen (z. B. der höchste Wert in 3. St.: 30 μg/St.), dieser Ausscheidungsweg kann aber vernachlässigt werden. Die obere Kurve der Abbildung 7 zeigt die Karbamidkonzentration im Speichel sowie seine pro Stunde ausgeschiedene Menge. Es kann festgestellt werden, daß zwar ihre Konzentration zur Zeit des Beginns des Versuchs etwas ansteigt, ist die Gesamtmenge des ausgeschiedenen Karbamids infolge der Abnahme der Speichelproduktion bedeutend vermindert (von 20 mg/St. bis 5 mg/St.). Dieser Zustand dauert so lange, bis im Pansen Harnstoff vorhanden ist (4. St.); nachher steigert sich auch die Karbamidausscheidung parallel mit dem Anstieg der Speichelproduktion. Im unteren Teil der Abbildung ist ersichtlich, daß bereits zu Beginn des Versuchs die Ammoniak- und Karbamidkonzentration des Harns parallel — nur in einem verschiedenen Größenverhältnis — plötzlich ansteigt, in der 3. St. aber wieder abfällt. Nachher zeigt die Ammoniakkonzentration keine weitere Änderung, die des Karbamids aber steigt wieder an. Die mit dem Harn pro Stunde ausgeschiedene Ammoniak- und Karbamidmenge steigt in der 2. Stunde plötzlich an, von der 4. Stunde ab vermindert sich die Ammoniakmenge, die des Karbamids aber steigt weiter an und verfolgt auf diese Weise die Blutkonzentration beider Substanzen.

Diskussion

Bei der Bewertung unserer Versuchsergebnisse kann festgestellt werden, daß die in den beiden Versuchen beobachteten Änderungen in großen Zügen entgegengesetzten Charakters sind. Die Milchsäureinfusion verursacht letzten Endes eine Azidose, obwohl das aus dem Karbamid freigesetzte toxische Ammoniak im Organismus alkalisch wirkt. Bei Milchsäureverabfolgung kann nur eine einfache Störung des Säure—Basen-Gleichgewichts beobachtet werden (metabolische Azidose), bei Harnstoffaufnahme aber muß sich der Organismus bei kompensierter respiratorischer Alkalose in erster Reihe gegen die toxische Wirkung des Ammoniaks währen. Der zeitliche Ablauf der beiden Vorgänge sowie das Größenverhältnis der bei diesen teilnehmenden Verbindungen sind aber bedeutend verschieden (Milchsäure mg, Ammoniak μg).

Der Ausgangspunkt der durch die beiden Verbindungen hervorgerufenen bedeutenden Änderungen kann auf Grund unserer Versuche zuerst in den Vormägen angegeben werden, da die Reaktionen einerseits zu einem Zeitpunkt erscheinen als im Blut nock keine wesentlichen Änderungen festgestellt werden können (z.B. bei Milchsäure) und anderseits verhältnismäßig rasch ablaufen (bei Harnstoff). Wie auf Grund unserer Versuche aus den Änderungen

in dem Mengenverhältnis und der Qualität des Speichels und des Harns hervorgeht, verursachen diese Änderungen im Wasser- und Mineralsalzhaushalt des Organismus bestimmte Probleme. Der Wasserhaushalt des Wiederkäuerorganismus im Ruhestadium und darauffolgend nach Verabfolgung der erwähnten Verbindungen wird auf folgender Weise schematisch veranschaulicht. In der Abbildung 8 ist ersichtlich, daß die zur Tätigkeit der Speicheldrüsen

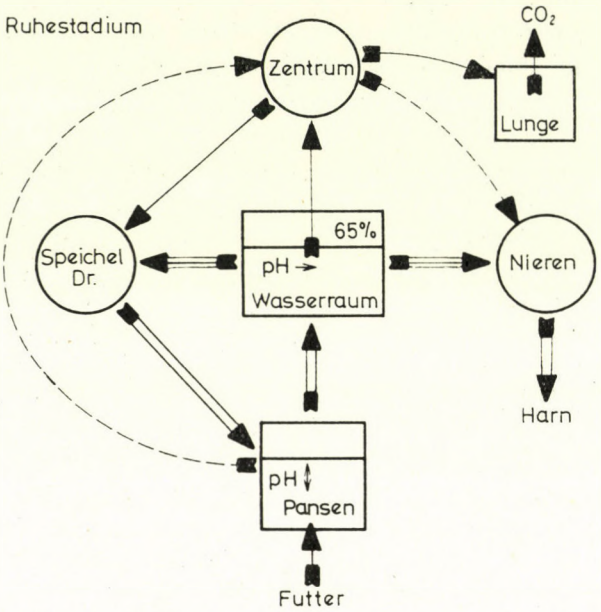

Abb. 8. Schema der Wasserzirkulation sowie der angenommenen neuralen und humoralen Regulation im Wiederkäuerorganismus unter normalen Umständen. Dr. = Drüse

und Nieren benötigte Menge von Flüssigkeit und Mineralsalzen letzten Endes vom Wasserraum des Organismus stammt; der Wasserraum, die Speichelsekretion und die Pansenflüssigkeit bilden in Hinsicht des Wasserhaushaltes einen Zyklus. Aus dem Organismus wird Flüssigkeit besonders über die Nieren mit dem Harn ausgeschieden. Diese Menge wird normalerweise durch die im Futter und Trinkwasser aufgenommene Flüssigkeitsmenge ersetzt. Bei Verabfolgung in den Pansen einer größeren Menge von Milchsäure oder Karbamid, wird das Gleichgewicht des angegebenen Umlaufes gestört. Bei der Auslösung der Änderungen kann dem humoralen Weg eine sehr untergeordnete Bedeutung beigemessen werden.

Es ist bekannt, daß sich in der Wand der Vormägen für mechanische und taktile Reize empfindliche Rezeptoren (Mechanorezeptoren) befinden (Ash und Kay, 1957). Nach einigen Ansichten ist die Bedeutung der chemischen Reize für die Speichelabsonderung gegenüber den mechanischen

Stimulatoren beim Wiederkäuer weitgehend untergeordnet (KOLB, 1967). Nach Angaben neuerer Arbeiten soll jedoch beim Schaf beim Anblick und Geruch von Futter eine beträchtliche psychische Sekretion ausgelöst werden, die zu einer Steigerung der Ruhesekretion führen kann (DENTON, 1961). Auf Grund unserer Versuche kann vermutet werden, daß außer Mechanorezeptoren auch für chemische Reize empfindliche Chemorezeptoren im Pansenepithel vorhanden sind, die für Änderungen des pH-Wertes und der chemischen Zusammensetzung der Pansenflüssigkeit empfindlich sind, und auf reflektorischem Wege die Menge und Zusammensetzung des Speichels und Harns beeinflussen. Falls nämlich z.B. die Milchsäurekonzentration der Pansenflüssigkeit ansteigt und ihr pH bedeutend sinkt, steigt die Menge (Hypersekretion) und der pH-Wert des sezernierten Speichels an, die des Harns aber vermindert sich (Oligurie) (Abb. 9, I.). Falls dieser Zustand längere Zeit hindurch andauert, entsteht im Organismus eine bestimmte Störung im Wasser- und Mineralsalzhaushalt. Es wurde bereits erwähnt, daß infolge der gesteigerten Milchsäureproduktion oder -Infusion, die Milchsäurekonzentration (über 150 mMol/l; 1400 mg/100 ml; Abb. 6) und die osmotische Konzentration der Pansenflüssigkeit ansteigt. Dies hindert die Flüssigkeitsresorption vom Pansen, gleichzeitig aber gelangt durch gesteigerte Speichelproduktion mehr Flüssigkeit in die Vormägen, der Panseninhalt wird allmählich verdünnt, im Wasserraum des Organismus vermindert sich der Flüssigkeitsvorrat (Hämokonzentration). Dies wird durch den Anstieg des Hämatokritwertes angedeutet (JUHÁSZ und SZEGEDI, 1965; 1968a).

Für unsere Ergebnisse zeugen jene Resultate, in welchen unterschiedliche Konzentrationen von Fettsäurelösungen, die auf einen pH-Wert von etwa 4,0 gepuffert waren, in Mengen von 2—4 Liter in den Pansen eingeführt wurden und die Speichelmenge stieg auf das 2—4fache des Ausgangswertes an. Aber bei der Pufferung auf einen den Verhältnissen im Pansen ähnlichen pH-Wert (6,0) entfalten die Fettsäuren keine Wirkung, so daß sie während der Verdauung an der Auslösung und Erhaltung der Speichelsekretion vermutlich nicht beteiligt sind (BODA und Mitarb., 1965; YARNS und Mitarb., 1965). Diese letzte Feststellung ist nach unserem Experiment — man kann sagen, unter physiologischen Verhältnissen — widerlegbar. EMERY und Mitarbeiter (1960) demonstrierten in Versuchen an Kühen, daß bei Steigerung der Heuration der Tiere der pH-Wert des Speichels signifikant anstieg. Die Aufnahme von Kraftfutter aber steigerte den Na^+- und K^+-Gehalt des Speichels. BOCEVA (1967) stellte fest, daß bei Silagefütterung von Färsen der pH-Wert der Pansenflüssigkeit auf etwa 6,0 fiel und parallel die Speichelabsonderung anstieg und der Speichel alkalischer wurde. Falls die Tiere ein Futterkonzentrat erhielten, wurde mehr Speichel erzeugt als bei der Verabfolgung von Maissilage oder Heu. BLAIR-WEST und Mitarb. (1965) stellten fest, daß bei Wiederkäuern der Speichel in der Regulierung des Alkalihaushaltes eine bedeutende Rolle

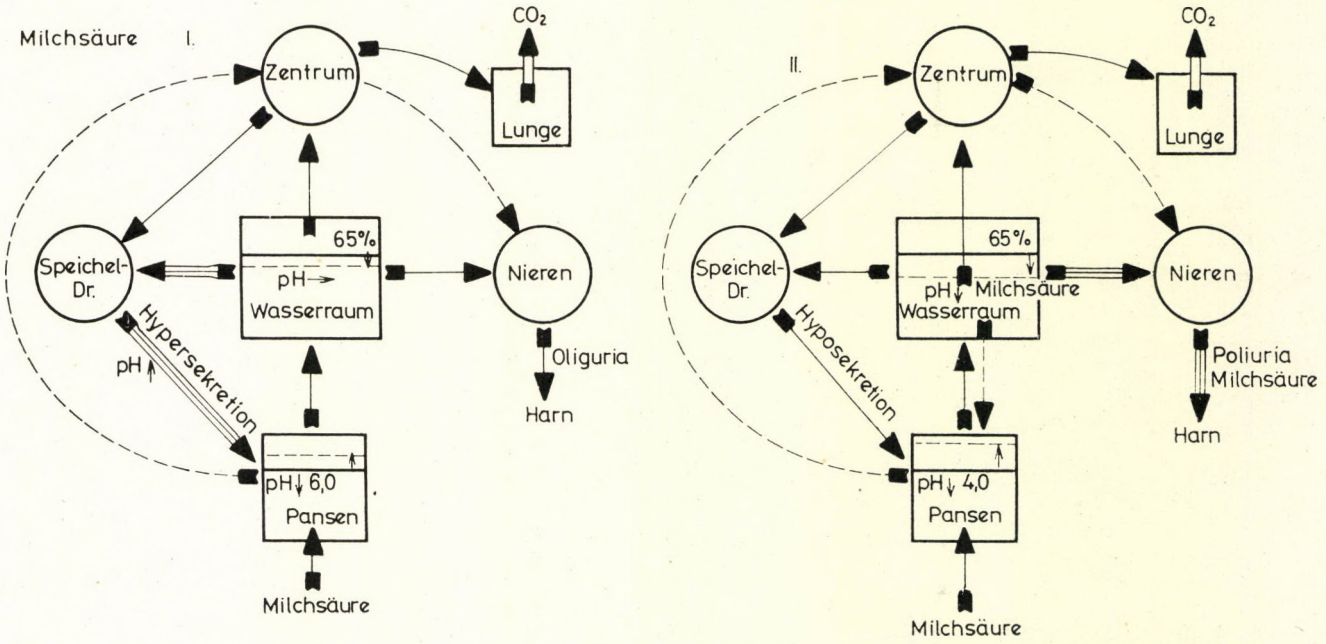

Abb. 9. Gestaltung der Wasserzirkulation des Organismus bei i.r. Dauerinfusion von Milchsäure. I.: 4.—6. Stunde des Versuchs; II.: bei inkompensierter Azidose in der 10.—11. Stunde (→ keine Änderung, ↓ Verminderung, ↑ Anstieg)

spielt. Bei einer Na^+-armen Fütterung vermindert sich der Na^+-Gehalt des Speichels, des Harns und des Kotes. Die Alkaliausscheidung und der Alkaligehalt des Speichels werden durch Aldosteron reguliert. Sie stellten fest, daß Wiederkäuer den Alkaligehalt des Speichels auf hormonalem Wege verändern können und dadurch die Aufrechterhaltung eines normalen Elektrolyt-Haushaltes des Organismus sichern.

Falls in die Vormägen weitere Milchsäuremengen verabreicht werden, beginnt der Anstieg der Milchsäurekonzentration im Wasserraum infolge der Milchsäureresorption und es entsteht eine kompensierte Azidose (Abb. 9, II.). Gleichzeitig gelangt im Organismus die Milchsäureausscheidung in den Vordergrund, und die Menge des ausgeschiedenen Harns steigt an (Polyurie). Dies aber kann nur durch eine Verminderung der Speichelproduktion (Hyposekretion) erfolgen. In diesen Fällen steigt die osmotische Konzentration der Pansenflüssigkeit infolge der Steigerung der Milchsäurekonzentration weiter an. Demzufolge wird die Resorption aus den Vormägen verschlechtert, es kann sogar eine Rediffusion aus dem Wasserraum in den Pansen erfolgen. Im Pansen häuft sich immer mehr Wasser an, demzufolge wird sein Inhalt verdünnt. Infolge des Anstiegs der Milchsäurekonzentration des Blutes (Laktazidämie) versucht der Organismus von der Milchsäure frei zu werden, deswegen wird mehr Harn ausgeschieden (Polyurie). Diese Umstände führen zu einer graduellen Verminderung des Wasser- und Elektrolytgehaltes des Organismus. Die in bedeutendem Maße verminderte Speichelproduktion (Hyposekretion) verlangsamt bis zu einem gewissen Grad die Entwicklung dieses Zustandes. Der Vorgang wird in der inkompensierten Azidose nur weiter gesteigert und endet in gänzlichem Erschöpfen der Kompensierungsfähigkeit des Organismus (Juhász und Szegedi, 1965; 1968a).

Die erste Hälfte des angezeigten Prozesses kann als physiologische Reflextätigkeit aufgefaßt werden, die nach jeder Futteraufnahme, infolge der Steigerung der Mikrobenaktivität des Pansens zustandekommen kann. Durch Erzeugung einer gesteigerten Menge stärker alkalischen Speichels bemüht sich der Organismus die übermäßige Säuerung des Panseninhaltes zu verhindern. Die zweite Hälfte aber kann als deutlich pathophysiologischen Charakters betrachtet werden und erfolgt letzten Endes wegen der gänzlichen Erschöpfung der Puffersysteme, der vitalen Reaktionen und der Transformierungsfähigkeit der Leber.

Falls der pH-Wert und der Ammoniakgehalt der Pansenflüssigkeit bei der Harnstoffverabfolgung ansteigen (Abb. 10, I.) wird eine entgegengesetzte Wirkung auf reflektorischem und humoralem Wege hervorgerufen. Die Konzentration des gebildeten Ammoniaks in der Pansenflüssigkeit ist aber bedeutend geringer (Maximalwert 86 mg/100 ml) als die der Milchsäure in dem vorangehenden Versuch (Abb. 6 und 7). Auf diese Weise bleibt der osmotische Druck der Pansenflüssigkeit auf konstanter Höhe. Ammoniak wird aus dem Pansen

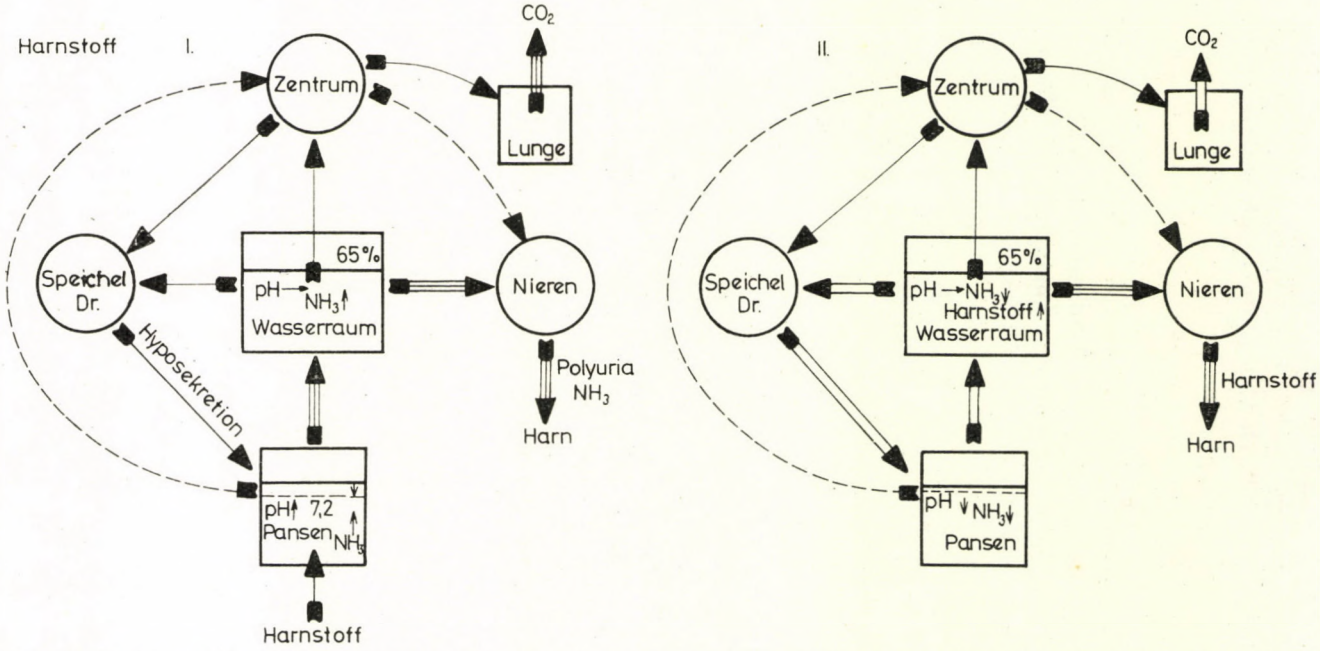

Abb. 10. Gestaltung der Wasserzirkulation des Organismus nach der Verabfolgung von Karbamid. I.: in der 3.—4. Stunde nach der Verabfolgung; II.: nach der 7. Stunde

rasch resorbiert und die Ammoniakkonzentration des Blutes steigt bereits am Anfang des Versuchs auf einen verhältnismäßig hohen Wert (Abb. 1). Ammoniak kann aus dem Organismus schnell nur mit dem Harn ausgeschieden werden. Die gesteigerte Harnausscheidung (Polyurie) führt zu einer verminderten Speichelproduktion (Hyposekretion). Nach unserer Auffassung erfolgt die Reaktion nach der Verabfolgung von Karbamid infolge der hohen Ammoniakkonzentration auf neuralem Reflex- und humoralem Wege (Abb. 1 und 7). Infolge der ansteigenden Flüssigkeitsresorption vom Pansen und der verminderten Speichelsekretion verringert sich allmählich die Menge des Wassers im Pansen, aber infolge der Harnausscheidung verändern sich das Volumen und die Zusammensetzung des Wasserraums nicht. Falls sich die Ammoniakresorption aus dem Pansen vermindert (Abb. 10, II.), kann als Ergebnis der Leber- und Nierentätigkeit auch eine Verminderung der Ammoniakkonzentration im Blut festgestellt werden. Nachfolgend gelangt die Entfernung des aus dem Ammoniak in der Leber gebildeten Harnstoffs in den Vordergrund (Abb. 7). Dieser Prozeß geht langsamer vor sich als ersterer, deswegen vermindert sich die Menge des Harns stufenweise und die Speichelerzeugung wird langsam normal. Es soll wiederholt betont werden, daß solange in der Pansenflüssigkeit Karbamid vorhanden ist, wird mit dem Speichel bedeutend weniger Harnstoff ausgeschieden als normalerweise (Abb. 7). Falls in die Vormägen größere Karbamidmengen gelangen, kann der Organismus den aus diesem freigesetzten Ammoniak nicht detoxizieren und ausscheiden. Deswegen entwickelt sich im Blut eine toxische Ammoniakkonzentration (mehr als 1200 μg/100 ml), die zum Tode des Tieres führt (Juhász, 1962).

ZUSAMMENFASSUNG

Auf Grund der durchgeführten Untersuchungen kann festgestellt werden, daß die Menge und Konzentration des Speichels bei Schafen nicht nur durch die früher bekannten Faktoren (Konsistenz, Trockensubstanz des Futters, Kauen, Schlucken, Wiederkauen, Fülle des Pansens, Teilchengröße des Panseninhaltes usw.) reguliert werden, sondern auch durch die Änderungen im pH-Wert und der chemischen Zusammensetzung der Pansenflüssigkeit. Es kann angenommen werden, daß sich in der Wand der Vormägen für chemische Reize empfindliche Rezeptoren (Chemorezeptoren) befinden, deren Reizung auf reflektorischem Wege die Menge, Zusammensetzung und den pH-Wert des Speichels und Harns beeinflußt. Solange bei dem Panseninhalt und dem Wasserraum von der Norm abweichende Verhältnisse vorwiegen, stehen die Mengenverhältnisse der Speichel- und Harnausscheidung miteinander in einem verkehrten Verhältnis. Aus den Ergebnissen unserer Versuche geht nämlich hervor, daß bei der Verminderung des pH-Wertes der Pansenflüssigkeit die Menge des erzeugten Speichels ansteigt und der Speichel alkalischer wird. Gleichzeitig vermindert sich die Menge des abgesonderten Harns und er wird saurer. Die Menge des mit dem Speichel ausgeschiedenen Na^+ und K^+ steigt an, jene des Harns aber sinkt in bedeutendem Maße. Sogar bei dem Anstieg der Milchsäurekonzentration des Blutes kann dieser Stoff im Speichel nicht nachgewiesen werden, mit dem Harn aber wird gleichzeitig viel Milchsäure und Ammoniak ausgeschieden. Bei inkompensierter Azidose bleibt der Speichel auch weiterhin alkalisch.

Es kann weiters festgestellt werden, daß bei einer stärker sauren Reaktion der Pansenflüssigkeit die Tiere eine größere, sonst toxische Karbamidmenge ohne jegliche Schädigung vertragen. Bei der Verabfolgung einer größeren Karbamiddosis verändern sich Menge und

Zusammensetzung des erzeugten Speichels. Die Karbamidkonzentration des Speichels steigt zwar an, die pro Stunde ausgeschiedene Menge von Karbamid sowie von Na^+ und K^+ aber vermindert sich bedeutend. Gleichzeitig aber steigt die Menge des ausgeschiedenen Harns sowie die des mit diesem ausgeschiedenen Ammoniaks, Karbamids sowie Na^+ und K^+ an. Die Zusammensetzung und Mengenverhältnisse des ausgeschiedenen Speichels und Harns zeigen also bedeutende Veränderungen, die mit den qualitativen und quantitativen Verhältnissen der im Pansen vor sich gehenden chemischen Prozesse eng verbunden sind. Auf diese Weise spielt der Speichel neben dem Harn bei Schafen eine ausschlaggebende Rolle im Wasser- und Alkalistoffwechsel sowie in der Regulierung des Säure—Basen-Gleichgewichts des Organismus.

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EVALUATION BY BLOOD VOLUME CHANGES OF SOME PREPARATIONS USED IN TRANSFUSION THERAPY

By

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In earlier studies on bleeding and blood substitution, the compounds applied in transfusion therapy have been characterized by numerical survival data, re-normalization of the general condition, fluctuation of blood pressure, bleeding volume index (BVI) and lethal rebleeding volume (LRV) (LAMI, 1962; LAMI and TAMÁS, 1963a, b; TAMÁS, LAMI and PETHES, 1964, 1965, 1966a, b; TAMÁS, 1966).

One of the most important requirements put with blood and blood plasma preparations, particularly with plasma substitutes, is the securing of a constant blood volume (BOHMANNSSON et al., 1946, 1948; GROPPER et al., 1952b; STERBA et al., 1957; etc.).

In the present paper studies on the influence of certain compounds to be specified below on blood volume are reported.

BLOOM (1951) has found that after the infusion of 500—1000 ml of dextran to humans from whom 500—800 ml blood was taken previously, the smaller dose increased the plasma volume by 10—15%, while the larger one by 15—20%. According to BOLLMAN et al. (1951), rabbits from which 20 ml/kg blood was drawn and immediately substituted either with 0.87% saline or a dextran preparation of 24,000 average molecular weight, showed no notable difference in plasma volume after 24 hours as compared to untreated animals. If, however, substitution was made with a dextran preparation of 120,000 average molecular weight, postinfusion plasma volume exceeded the joint amount of preinfusion volume and substitute. In the same experiments, polyvinylpyrrholidone compounds, gelatin and acacia were considerably less active than the above preparations, as confirmed also by experiments on dogs. GROPPER et al. (1952a) have reported that out of the substitutes infused to dogs after bleeding, during the first three hours dextran produced the largest expansion of plasma volume as compared to homologous plasma, and saline; the effect of oxypolygelatin (OPG) was the only comparable to that of dextran. HAMMARSTEN and HELLER (1952) and HAMMARSTEN et al. (1953) have taken 1000 ml blood from healthy humans and substituted it with an equal volume of dextran solution; as a result, plasma volume rose by 1020 ml in the 1st and the excess volume was still 860 ml in the 24th post-infusion hour. In unbled convalescents 1000 ml of a dextran preparation expanded the blood volume by 776 ml in the 1st hour and the excess volume fell to 534 ml by the 6th post-infusion hour. In contrast, on infusion of 500 ml dextran preparation to humans HYDE et al. (1952) found maximum expansion of plasma volume (+1350 ml) in the 5th post-infusion hour; with 500 ml infusion doses each of 6% gelatin solution, homologous plasma and hypertonic serum albumin, maximum expansion (+1118, +1173 and +1342 ml) was reached within 75 minutes, 45 minutes and 8 hours, respectively. MEYER et al. (1952) have reported that in humans, the average increase of plasma volume produced by the infusion of 500 ml dextran solution was 1880 ml, reached in the period between 15 minutes and 6 hours after infusion. The same authors found no increase in the plasma volume following the infusion of 500 ml saline. In dogs, following bleeding NARAT et al. (1952) have found a greater expansion of plasma volume with polyvinylpyrrholidone than with dextran preparations. Substituting the blood loss with dextran or OPG, RAISZ and STINSON (1952) observed in dogs that these preparations increased plasma volume as for the lost volume. In BOLLMAN's experiments (1953) the blood volume of rabbits which had been reduced to 56.9 ml/kg, rose by 40—45% in 2 minutes after the infusion of dextran solution, but 1 hour later the increase was only 12%; the blood volume reduced to 54.9 ml/kg increased by only 10—20% in 5 minutes after the infusion of saline. In unbled dogs GODWEY et al. (1964) have reported good results with acacia, GODWEY and YOUNG (1954) with dextran. In humans,

500 ml saline produced a plasma expansion in 30 minutes which corresponded quantitatively to the amount of infusion, but by the 3rd hour the preinfusion level was reassumed; with 500 ml Periston (polyvinylpyrrholidone preparation) a more marked expansion of plasma volume took place, reaching a maximum in 1 hour and reassuming the initial level in 3 and a half hours, after some fluctuation. In these experiments, the best results were obtained with Macrodex (a preparation containing 6% dextran), of which 500 ml produced a plasma volume expansion by round 2000 ml, while the same dose of a 10% dextran preparation expanded plasma volume by 3000 ml. Under the effect of dextran, the plasma volume fell but slightly below the peak value by the 4th postinfusion hour (GRÜNING and ROTH, 1954). Following the infusion of dextran and polyvinylpyrrholidone preparations, HEIM and OSTEN (1954) found a persistence of the elevated plasma volume over about 6 hours. HUNZINGER et al. (1954) suggest that in respect of the expansion and stabilization of plasma volume homologous plasma is most efficient, dextran and polyvinylpyrrholidone are nearly equivalent and saline is the least effective. On infusion of various dextran preparations into healthy dogs in doses corresponding to nearly one quarter (23.2%) of the calculated plasma volume, SEMPLE (1954a, b) observed an expansion of plasma volume up to 119.1% within 15 minutes; one day later the plasma volume was 100.6% Similar doses of saline were less effective. Of particular interest are WASSERMAN and MAYERSON's data (1954), who performed their experiments with renally excretable (subrenal threshold) dextran of 10,600 average molecular weight and renally unexcretable (suprenal threshold) dextran preparations of 125,000, 158,000 and 208,000 average molecular weights, respectively. In previously bled dogs the three suprenal preparations produced a considerable expansion of plasma volume which exceeded the injected quantity remarkably. Nevertheless, the alteration of plasma volume differed markedly in dogs bled and not bled, as in the latter infusion of 40 ml/kg dextran never produced as great an expansion as in the former. Above authors have further made the therapeutically important observation that in dogs given an equal volume of dextran to substitute the withdrawn blood, a second infusion failed to produce a notable expansion of plasma volume. They believe that the initial (pre-infusion) plasma volume is primarily the decisive factor for the difference between the expansion of plasma volume in bled and unbled dogs and for the lack of producing a notable rise following a second infusion. Administering to humans 1000 ml of saline or a 6% dextran preparation by drop-infusion for 3 hours, YOUNG et al. (1954) observed an increase, by 1024 ml, exclusively with dextran. NORLANDER et al. (1955) report that in patients given dextran postoperatively, the rise of plasma volume correlated with the amount of the infusion, whereas JENKINS et al. (1956) state that under similar conditions, 1000 ml of polyvinylpyrrholidone increased the plasma volume in 2 hours by only 320 to 886 ml, and 500 ml polyvinylpyrrholidone only by 398 to 696 ml. Slightly better results were obtained when the patients were not restricted in drinking.

Experimental

Based on the above data, influence of saline, Plasmodex*, heparinized dog blood and plasma infusion on the plasma volume of dogs has been studied after a massive loss of blood.

Materials and methods

The experiments were performed on 23 dogs of both sexes and various races, 2—4 years of age, 7—23 kg in weight, well-developed and clinically healthy. For methodological reasons, to be explained below, only 18 animals

* Active substance content, 6% dextran, dissolved in 0.9% saline; average molecular weight 75,000. Made available ready for use by HUMAN Institute for Serobacteriological Production and Research, Budapest.

were included in the evaluation. Prior to the experiment the animals fasted for 12 hours but received drinking water *ad libitum*.

Prior to withdrawal of blood and infusion, a mixture of Hibernal and Depridol was given intramuscularly. During the experiments the narcotized animals were fixed on a warmed table to prevent any change of blood volume resulting from hypothermia. For infusion and repeated blood sampling the femoral and jugular veins were exposed surgically (TAMÁS, 1963) and 0.5—1.0 ml of heparin was injected to prevent coagulation. To ensure rapid and undisturbed blood sampling and to eliminate the dead space liable to interfere with the measurement of blood volume, a T-shaped cannula was inserted into the jugular vein.

The dogs were narcotized and fixed in lying position on their backs. From one of the common carotid arteries 30 ml/kg blood was drawn quickly at an average rate of 70 ml/minute (LAMI and TAMÁS, 1963a, b; TAMÁS, 1963, 1966). According to DUKES (1955) this amount of blood covers approximately one half of the circulating volume, while according to WINTROBE (1956) somewhat less. Anyway, withdrawal of 30 ml/kg blood implies a notable loss of blood, as in our earlier studies (TAMÁS et al., 1964, 1965; TAMÁS, 1966) drawing of 32.4 ml/kg blood was followed by a fall of blood pressure to one fifth of the initial value.

Blood was substituted by infusion of freshly prepared saline, Plasmodex or heparinized dog blood or plasma. The infusions were warmed to body temperature (37 °C) and infused into the jugular vein in 10—15 minutes; 10 minutes following blood withdrawal.

Donors were selected and bled, heparinized blood and plasma were prepared according to the methods applied in the Surgical Clinic (TAMÁS, 1963; 1966). Blood and plasma preparations were made individually, *i.e.*, each recipient received blood or plasma from a given donor.

Plasma volume was determined prior to bleeding and after infusion by radioisotope method, using ^{131}I-labelled dog albumin. In the second assay, the blood sample withdrawn prior to the administration of labelled serum albumin served as background. An amount of 10—15 μCi of ^{131}I-labelled dog albumin was given in 1—2 ml volume. Subsequently, blood samples were taken 10, 20, 30, 45 and 60 minutes afterwards (HUGGINS et al., 1960) and used to assay the ^{131}I-activity of the plasma and to determine the haematocrit value after centrifugation at 5000 r.p.m. for 30 minutes. After the usual corrections (LAJTHA, 1961), the plasma volume was calculated. The volume theoretically measurable at the administration of the isotope was assessed by extrapolating the individual readings to zero on semi-logarithmic paper. To eliminate the error resulting from the adsorbing capacity of the glass jars, the stock solution was diluted in a 1% albumin solution (CHASE and RABINOWITZ, 1967). The radioactivity of the plasma and reference standard samples was determined

by means of a well-type NaI (Tl) crystal and a gamma NK-108 semi-automatic scaler. All ^{131}I-labelled albumin materials were checked for purity prior to the experiment. Measurement error did not exceed 5%.

It should be mentioned here that in preliminary experiments performed on 5 dogs, regularly higher values were obtained for the normal plasma volume with the isotope than with the dye method (JUHÁSZ and PETHES, 1952). After the elimination of the possible sources of error, the 20—30% higher values were tentatively attributed to partial phagocytosis of the ^{131}I-labelled human serum albumin by the RES. This supposition was confirmed by the finding that substitution of dog albumin for human albumin had completely eliminated this error.

All further experiments on the remaining 18 dogs were therefore performed with ^{131}I-labelled dog albumin. The preparation was not older than 1 week, and it had been checked for purity and was free of inorganic ^{131}I.

Results

During and after the withdrawal of 30 ml/kg blood, and subsequently during infusion, the behaviour and general condition of each experimental animal was the same as observed in our previous experiments (LAMI, 1962; LAMI and TAMÁS, 1963a, b; TAMÁS et al., 1964, 1965, 1966a, b; TAMÁS, 1966).

Though the present studies were not aimed at collecting data on the physiological blood volume of the canine species, it may be noted that it was assessed as 86.9 ± 17.9 ml/kg, that is 8.7% of the body weight. This value is within the physiological limits (1/10 and 1/15 of the body weight) given in one of the relevant textbooks (MAREK and MÓCSY, 1960).

The numerical values pertaining to the influence of the applied plasma expanders on blood volume are shown in Table 1.

In three of the five experimental animals (Nos 12, 19 and 20) treated with Plasmodex, the posthaemorrhagic blood volume considerably surpassed the prehaemorrhagic level, *i.e.* the expansion exceeded the volume of infusion. The effect of Plasmodex on the other two animals (Nos 7 and 14) was also notable, as in both cases the effectivity index was of roughly the same order as the group average obtained for the animals treated with heparinized plasma.

The results obtained with heparinized plasma may also be considered fairly good, as one (No. 10) of the four dogs showed the same response as Nos 12, 19 and 20 of the Plasmodex-treated group. With the remaining three animals, the average postinfusion blood volume was by only 12.2% lower than the initial value.

The individual readings made in the group treated with heparinized blood were hardly lower than the group average of the dogs treated with Plasmodex.

Table 1

Changes produced by expanders in the blood volume of dogs drawn 30 ml/kg blood

Experimental animals		Plasma expander	V_1 (ml)	V_2 (ml)	V_1 (ml/kg)	V_2 (ml/kg)	E.I.	V_1	V_2
Serial No.	Body wt (kg)							(in per cent of body weight)	
1.	13		1362	961	104.7	73.9	70.5	10.5	7.0
2.	9		885	844	98.3	93.8	95.4	9.8	9.4
3.	14	Saline	1402	1277	100.1	91.2	91.1	10.0	9.1
6.	20		1124	951	56.2	47.5	84.6	5.6	4.7
22.	11		831	592	75.5	53.8	71.2	7.5	5.4
23.	17		1356	624	79.8	36.7	46.0	8.0	3.7
Average:	14		1160	874.8	85.8	66.2	76.5	8.6	6.5
21.	12		974	782	81.2	65.2	80.3	8.1	6.5
4.	17	Heparinized	1275	1156	75.0	68.0	90.7	7.5	6.8
5.	19	blood	1444	1330	76.0	70.0	92.1	7.6	7.0
Average:	16		1231	1089.3	77.4	67.7	87.7	7.7	6.8
10.	14		1706	1866	121.8	133.3	109.4	12.2	13.3
15.	13	Heparinized	1181	1075	91.1	82.7	90.8	9.1	8.3
16.	10	plasma	892	723	89.2	72.3	81.1	8.9	7.2
18.	7		576	527	82.3	75.3	91.5	8.2	7.5
Average:	11		1088.7	1047.7	96.1	90.9	93.2	9.6	9.1
7.	20		1122	1029	56.1	51.4	91.7	5.6	5.1
12.	10		858	890	85.8	89.0	103.7	8.6	8.9
14.	17	Plasmodex	2063	1940	121.3	114.1	94.0	12.1	11.4
19.	23		1867	2081	81.2	90.5	111.5	8.1	9.0
20.	17		1514	2220	89.0	130.6	146.6	8.9	13.0
Average:	17.4		1484.8	1632	86.7	95.1	109.5	8.7	9.5

Explanation: V_1, initial blood volume; V_2, postinfusion blood volume; E.I., (effectivity index) $= \frac{V_2 \cdot 100}{V_1}$.

The least favourable results were obtained in the saline-treated group, where the average effectivity index was 76.5%.

Fig. 1 clearly indicates that as calculated from the formula $\frac{V_2 \cdot 100}{V_1}$ where V_2 is the postinfusion, while V_1 the prehaemorrhagic blood volume expressed in ml, the effectivity indices were 109.5% for Plasmodex, 93.2% for heparinized plasma, 87.7% for heparinized blood and as low as 76.5% for saline.

Discussion

In all groups where blood withdrawal was followed by substitution therapy, an increase of the blood volume was observed; in the Plasmodex-treated group the postinfusion volume even exceeded the initial value. These observations are supportive of the findings made on human clinical material and in animal experiments by BOLLMAN et al (1951); HAMMARSTEN and HELLER (1952); HAMMARSTEN et al. (1953); HYDE et al. (1952); MEYER et al. (1952); GRÜNING and ROTH (1954); SEMPLE (1954a, b); WASSERMAN and MAYERSON

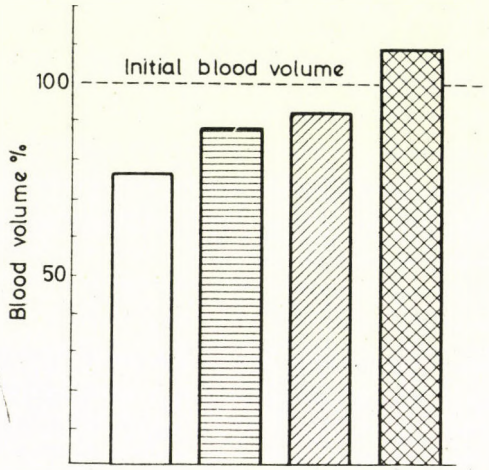

Fig. 1. Changes of the blood volumes of dogs drawn each 30 ml/kg blood, on subsequent infusion of plasma expanders
□ Saline; ≡ Heparinized blood; /// Heparinized plasma; ▩ Plasmodex

(1954) and YOUNG et al. (1954). These results are quite feasible in the case of dextran-containing Plasmodex, as according to HAMMARSTEN et al. (1953) and KØSTER et al. (1957), 1.0 g dextran can bind 21.2 ml and 18.8 ml of water, respectively. Considering that in its 6% solution only 16.7 ml water is bound by 1.0 g of dextran, the full binding capacity, presumably, develops within the organism. The fluid flowing in from the interstitial space becomes added to the volume of Plasmodex, whereby a practically biphasic increase of the circulating blood volume is taking place. This property of dextran can be utilized also for the re-normalization of blood pressure (LAMI, 1963, 1967).

Less favourable results than with Plasmodex were obtained with heparinized plasma and blood; the latter two preparations differed also in respect of the effectivity index. The 93.2% efficiency of heparinized plasma as compared to 87.7% found with heparinized blood is explicable by the fact that the former can carry more albumin, responsible for intravasal colloidal pressure, into the circulation.

Data recorded during a 90-minute postinfusion observation period indicate that the saline's effect must not be disregarded either, as its effectivity index reached 76.5%. Though on the infusion of saline the water is rapidly lost by diffusion into the extracellular space, this process is too slow to prevent life-saving action of saline immediately after infusion.

Comparison of the results of all our studies on bleeding and blood substitution has revealed a fairly good correlation of the results obtained with diverse experimental aims.

Following the withdrawal of 40 ml/kg blood, of the animals given heparinized or citrated blood or plasma or plasma expanders (Intradex, Periston-N) none, of those given saline 33%, while of the untreated controls 60% died during a 2-week period of observation (LAMI, 1962; LAMI and TAMÁS, 1963a, b; TAMÁS, 1966). Further to the more or less favourable survival rate, re-normalization of the clinical state took 2 weeks in the control group, 8 days in the saline-treated group, 4 days in the Intradex-treated group, 2 days after the application of blood and plasma preparations and only 1 day after treatment with Periston-N.

In our previous studies (TAMÁS et al., 1964, 1965; TAMÁS, 1966), normalization of blood pressure reduced to one fifth by bleeding was most efficiently produced by Plasmodex, the postinfusion pressure amounting to 90.8% of the initial value. Based on the experimental results, the following sequence of efficiency can be established: heparinized blood (83.2%), heparinized plasma (81.0%), citrated blood (73.2%), saline (72.0%), citrated plasma (63.8%) and Periston-N (43.1%).

The BVI-values of the infusion preparations (TAMÁS et al., 1964, 1965; TAMÁS, 1966) were the following: Plasmodex (98.6), heparinized blood (87.9), citrated blood (84.9) Periston-N (81.4), heparinized plasma (65.6), citrated plasma (49.9) and saline (49.1).

The sequence in respect of LRV-value (TAMÁS et al., 1966a, b; TAMÁS, 1966): control (44.7), Plasmodex (58.9), heparinized plasma (51.4), heparinized blood (48.6), Periston-N (46.0), citrated plasma (41.9), saline (40.2) and citrated blood (39.7).

The succession based on the effectivity indices established in the present study (Fig. 1): Plasmodex (109.5%), heparinized plasma (93.2%), heparinized blood (87.7%) and saline (76.5%).

Correlating all data derived from our relevant examinations (survival rate, re-normalization of the general condition, restitution of blood pressure, BVI and LRV values, and changes of blood volume), we may state that the dextran-containing plasma expanders find an excellent therapeutic use also in veterinary medicine, if the conditions of oxygen transport can be provided. Good results have been obtained also with blood and plasma preparations, though the heparinized lots proved to be somewhat superior to the citrated

lots. This is due to the toxic effect of sodium citrate (NOVÁK et al., 1963), which has been described in detail in our previous papers (TAMÁS et al., 1964, 1965; TAMÁS, 1966). The results obtained with Periston-N are equivocal. It should be mentioned that in human medicine the polyvinylpyrrholidone preparation of 12,600 average molecular weight has been used as a detoxicant rather than a plasma expander (SCHUNERT, 1950, 1951; GROPPER et al., 1952; MÖLLER, 1956).

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SUMMARY

A 30 ml/kg amount of blood was drawn rapidly and continually from one common carotid artery of 18 dogs each, and afterwards substituted by an equal volume of saline, heparinized (homologous) blood or plasma, or Plasmodex, a Hungarian dextran preparation.

Plasma volume was determined prior to bleeding and after infusion, by means of ^{131}I-labelled dog serum albumin. Blood volume was calculated from the plasma volume and the hematocrit value.

The effectivity index (postinfusion blood volume related to the initial volume as 100) assessed after a 90-minute period of observation was 109.5% for Plasmodex, 93.2% for heparinized plasma, 87.7% for heparinized blood and 76.5% for saline.

The blood volume of healthy dogs as disclosed by radioisotope assays was 86.9 ± 17.9 ml/kg.

A comprehensive evaluation is presented including the results of the authors' previous studies on bleeding and blood substitution (survival rate, normalization of the general condition, restitution of blood pressure, bleeding volume index, lethal rebleeding volume and changes of blood volume).

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PRESERVATION AND REACTIVITY OF PIG RED CELLS IN BLOOD TYPING

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Introduction

In the past years investigations have been carried out on the preservation of the red blood cells (RBC) of different farm animals. Preservation has special importance in the blood typing because the permanent use of comparative RBCs as well as their shipment in good condition is a fairly great problem.

PAPP and BRILLES (1967) reviewed the theoretical bases of this question; accordingly., one of the ACD solutions, *i.e.*, Alsever's solution completed by cyanides was found to be the best for the preservation of chicken RBCs in the practice.

The present study was initiated to find a practical method for the storage of pig RBC. The main differences in the work with pig RBC, comparing to poultry erythrocytes, are the less favourable conditions at blood collection, and the different structure of pig RBC.

Materials and Methods

In the first experiment blood was taken from 5 Large White pigs to find the optimum of the KCN or NaCN concentration in citrate and Alsever's solution. In the second experiment 50 Large White pigs were used. Blood was taken from the v. cava cranialis, without previous cleaning of the skin and using a syringe containing a small amount of sodium citrate solution (sodium chloride, 5 g and sodium citrate, 20 g to 1 litre of distilled water). The blood was immediately put into a glass tube of 16×105 mm, which contained 4 ml citrate solution. In the laboratory, RBCs were washed three times — the second washing took place in graduated tubes — and an RBC suspension of exactly 2% was prepared. In each case the final volume was 5 ml, which was kept in a glass tube of 10×100 mm.

In the first experiment, citrate and Alsever's solution were used as suspending medium. Each solution was completed with KCN or NaCN, in

dilutions varying from 1 : 1000 to 1 : 20,000. In each group five tubes remained as cyanide-free controls. Later, on the basis of results, another experiment was carried out, using only citrate solution with KCN or NaCN in dilutions from 1 : 10,000 to 1 : 25,000.

In the second experiment 50 individual RBC samples were stored in Alsever's solution completed with KCN of 1 : 2500 dilution. All the samples were tested with 10 different complete agglutinating reagents — Ac, Da, Ea, Eb, Ed, Ee, Ef, Gb, Ja, La — on the first day and on days 21, 35 and 47. Besides, the tubes were examined every day in both experiments macroscopically for the degree of haemolysis or deterioration.

Results

In citrate solutions RBCs haemolyzed in the presence of 1 : 1000 to 1 : 10,000 cyanide dilutions within a few days. The degree of haemolysis and the number of haemolyzed samples increased gradually, but in the presence of cyanide dilutions from 13,000 to 20,000 there was hardly any haemolysis by the 30th day of storage. There was no difference in effect between KCN and NaCN.

Repeating the first experiment in citrate solution completed with KCN or NaCN in dilutions from 1 : 10,000 to 1 : 25,000, KCN gave better results than NaCN and showed a more marked picture (Table 1). On the 30th day of storage the range between 1 : 17,000 and 1 : 21,000 proved to be the best. In Alsever's solution in the presence of the 1 : 1000 cyanide dilutions haemolysis started as early as on the 3rd day of storage and reached the highest degree (H^4) on the 5th day. In Alsever's solution containing cyanide in 1 : 14,000 to 1 : 20,000 dilutions haemolysis was first observed on the 12th day and from the 1 : 4000 only on the 35th day. The best preservative effect of Alsever's solution was at cyanide dilutions of 1 : 2000 and 1 : 3000 where no haemolysis took place even on the 60th day of storage. Considering the results of the whole period of storage, KCN seemed to be slightly superior to NaCN (Table 2).

All the controls, both in citrate and Alsever's solutions began to deteriorate on days 5—8 of storage.

In the second experiment when the stored RBCs were tested on the days 1, 21, 35 and 47 of storage, there was a slight or medium decrease in the degree of direct haemagglutination. After 47 days very few reactions missed. On the basis of the results, the decrease of the degree of reactions appeared to depend on the samples rather than on the reagents. Furthermore, — contrary to the chicken RBC — the 47-day-stored pig RBCs displayed much better reactions after 6 hours' incubation than after 3 hours.

Table 1

The degree of haemolysis and bacterial deterioration of pig red cells preserved in citrate solution of different potassium cyanide concentrations*

Blood sample No.	Dilutions of postassium cyanide										
	1 : 10,000	1 : 12,000	1 : 14,000	1 : 16,000	1 : 17,000	1 : 18,000	1 : 19,000	1 : 20,000	1 : 21,000	1 : 22,000	1 : 24,000
1	H^3	H^2	H^1	H^2	—	H^1	H	H	H	H	H
2	H^4	H^4	H	H	—	H	H^1	H^1	H	D^1	H^1
3	H^4	H	H^2	H	H^1	—	—	—	—	D^1	D^1
4	H^1	H^1	—	H^1	H^1	—	—	H	—	—	D^2
5	H^4	H^2	H^3	H^3	H^1	H	H	H	H	H	H

* Setting up on 8th May, 1968. Reading on 7th June, 1968
Symbols: H, H^1, H^2, H^3, H^4, degree of haemolysis; D^1, D^2, degree of deterioration

Table 2

Preservative effect of cyanides in Alsever's solutions on pig red cells*

Cell No.	Cyanide	Dilutions of cyanides																
		1 : 1000	1 : 2000	1 : 3000	1 : 4000	1 : 5000	1 : 6000	1 : 7000	1 : 8000	1 : 9000	1 : 10,000	1 : 11,000	1 : 12,000	1 : 13,000	1 : 14,000	1 : 15,000	1 : 16,000	1 : 17,000
1	KCN	H^4	—	—	H^1	—	—	D^3	D^3	D^2	D^1	D^2	D^3	D^3	D^4	D^4	D^3	D^4
	NaCN	H^4	—	—	H^3	H^3	H^3	D^3	D^3	D^3	D^4	D^4	D^4	D^4	D^3	D^4	D^4	D^4
2	KCN	H^4	—	—	H^2	H^2	D^3	D^2	D^2	D^2	D^3	D^3	D^4	D^4	D^4	D^4	D^3	D^3
	NaCN	H^4	—	—	—	—	—	H^1	H^2	D^2	D^1	D^2	D^2	D^3	D^4	D^4	D^4	D^3
3	KCN	H^4	—	—	H	H^2	H^2	H^2	D^2	D^3	D^3	D^3	D^2	D^3	D^4	D^3	D^4	D^4
	NaCN	H^4	—	—	H	H^2	H^2	H^2	D^4	D^3	D^3	D^4	D^3	D^4	D^4	D^4	D^4	D^4
4	KCN	H^4	—	—	—	—	H^2	H^1	H^3	H^2	D^2	D^3	D^3	D^3	D^3	D^4	D^3	D^4
	NaCN	H^4	—	—	H^3	H^2	H^2	D^2	D^2	D^2	D^2	D^3	D^3	D^3	D^4	D^4	D^4	D^4
5	KCN	H^4	—	—	—	H^1	—	H^2	D^2	D^2	D^4	D^3	D^3	D^4	D^4	D^3	D^4	D^4
	NaCN	H^4	—	—	H^2	H^1	H^3	D^2	D^2	D^3	D^4	D^4	D^3	D^4	D^4	D^4	D^4	D^4

*Setting up on 19th February, 1968. Reading on 20th April, 1968.
Symbols: H, H^1, H^2, H^3, H^4, degree of haemolysis; D^1, D^2, D^3, D^4, degree of deterioration

Table 3

Scores of direct saline agglutination tests with pig red cells stored in Alsever's solution completed with KCN of 1 : 2500

Reagents	Ac					Da					Ea					Eb					Ed				
Day of reading	1st	21st	35th	47th		1st	21st	35th	47th		1st	21st	35th	47th		1st	21st	35th	47th		1st	21st	35th	47th	
Incubation time	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h
Cells No.																									
1																					4	3	3	0+	1+
2	4	4	3	1+	2						4	3	2+	1	1+						4	4	3+	1+	2+
3																					4	3+	2	0+	2+
4											4	2	1	—	1						4	2+	2	—	1+
5																					4	3+	3	0+	2
6																4	3+	4	2+	3	4	3+	3+	2	3
7											4	3	1+	—	1+	4	3+	3	1	2+	4	2	1	—	1
8																					4	3+	3+	1+	2
9																					4	3+	3+	2	2
10											3+	3+	4	2+	2+										
11											4	4	1	—	—						4	4	1	+	—
12											4	4	0+	—	1+						4	4	1+	0+	2
13																4	4	4	2	3	4	4	3+	1+	2+
14											4	4	1+	—	2+	4	4	4	2	3	4	3+	1+	0+	2
15																					4	4	3+	2	2+
16																					4	4	2	0+	2+
17																					4	4	3	2	3
18	4	4	4	2+	3											4	4	4	2	3	4	4	2+	2	3
19											3+	4	1	—	1+	4	4	4	2	3	4	3+	0+	+	1+
20																					4	4	1	1	1+
21											4	4	1+	—	1	4	4	4	1	2+	4	3+	1	—	0+
22											4	4	3+	—	1+										
23											4	4	1+	—	2						4	3+	1+	—	1
24																					4	3+	3	—	2
25											4	3+	3	—	2+						4	3	1+	—	0+
26	4	4	4	2+	3	4	4	2+	—	1+											4	4	2+	0+	1
27						4	4	4	1+	3	+										4	4	3	1+	2+
28	4	4	4	2+	3						4	4	4	—	3										
29											4	4	1+	—	—	4	4	4	—	1+	4	3+	1+	—	0+
30											4	4	3	—	—						4	4	1+	+	—

Reagents	Ee					Ef					Gb					Ja					La				
Day of reading	1st	21st	35th	47th		1st	21st	35th	47th		1st	21st	35th	47th		1st	21st	35th	47th		1st	21st	35th	47th	
Incubation time	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h	3h	3h	3h	3h	6h
Cells No.																									
1	4	2	2+	—	+	4	+	1	—	—	4	1+	1	0+	1	3+	+	0+	—	0+					
2	4	3+	3+	1	2						4	2+	2+	1	2	4	3+	3+	2	2	3	2+	2+	—	2
3	4	3+	3	—	—	4	+	1	—	1	4	1+	1	—	—	4	1	0+	—	1+					
4	4	3+	3+	1	2						3+	1+	1	—	1+	4	1	1	—	2					
5	4	3+	3+	—	1+	3+	+	1	—	1+	3	0+	1	—	1+	3	1	0+	—	2					
6	4	3	3	—	—						2+	0+	1	—	1										
7	4	3+	3	0+	1+						4	1	1+	—	1	3	2	1	—	2					
8	4	3	4	1	1	4	2+	1	—	1+	4	2	2+	1	2	3+	2+	2	—	2+					
9	4	3+	4	1+	2+						4	2	2+	0+	2	4	3+	3+	1	2+					
10	4	3+	4	2+	2+						3+	1	1	0+	1+	3+	2+	2	0+	2					
11	4	4	4	1	2+						4	2	1	—	—										
12	4	4	4	1	2+						4	2+	1	—	1										
13	4	4	2+	—	—						4	2	1	—	2										
14	4	4	3	0+	2+						4	1+	1	—	1+										
15	4	4	4	1	2+						4	1+	1	0+	2	4	3+	2	1	3	3	2	0+	—	2+
16	4	4	3+	—	2+						4	1+	1	—	2	4	3	1	—	3					
17	4	4	4	1+	2+						4	2+	1+	—	2	4	3+	1	0+	3	2	1+	1	—	2
18	4	4	2+	—	2						4	1+	1	—	2	4	2+	0+	—	3					
19	3+	4	1	0+	2						4	1+	1	—	1+										
20	4	4	0+	+	—						4	1+	+	—	—	4	4	1+	—	2	3	1+	0+	—	+
21	4	4	3+	+	2						4	2	1+	+	1										
22	4	4	4	—	1+						3	2	1	—	1+	3	2+	1+	—	1+					
23	4	4	4	1	2+	4	0+	1	—	1+	4	2	1+	—	1+	4	3	1+	—	2+					
24	4	4	3+	—	2	4	+	1	—	1	4	2	1	—	1										
25	4	4	4	1	3	3	0+	1	—	1+	4	2	1+	—	1	3	1+	1	—	2+					
26	4	4	2+	—	1+						3+	0+	1	—	1										
27	4	4	3+	—	2+						3	1+	1	3	3+										
28	4	4	4	2	3						4	2	1	3	3+	4	3	1	—	3					
29	4	4	2+	—	2						4	2	1	1+	2	4	3	1	—	1+					
30	4	4	3	1	2	3+	1+	1	—	—	4	2	1	1+	2	4	3	1	—	1+	2+	1	—	—	—

Degrees of agglutination: 0+, +, 1+, 2, 2+, 3, 3+, 4

Discussion

Alsever's solution — as it was expected — proved to be a suitable medium for preservation of pig RBCs. However, it does not provide a long storage without a good antibacterial material. In earlier studies carried out with chicken RBC, cyanides proved to be the best for this purpose. It is also used with good results in the bloodgrouping of cattle (Bouw, 1964). In this work we wished to determine the optimum cyanide concentration for pig RBCs. The results suggest that the glucose in Alsever's solution which gives a good protective effect for RBCs in respect of long preservation, permits the growth of bacteria in higher cyanide dilutions. On the other hand, the glucose obviously protects the RBCs also against cyanides of higher concentrations. Therefore the cyanide concentration of 1 : 2000 and 1 : 3000 proved to be the best for the pig RBCs. This suggestion was evidenced by the fact that the cyanides in citrate solutions gave acceptable results only from 1 : 17,000 to 1 : 21,000 dilutions. However, it is surprising that the cyanides in these high dilutions also provided a relatively good preservative effect for about 4 weeks. This is very important because in our practice the citrate solution which is always used at blood collection, gives much better anticoagulant effect than Alsever's solution, and its preparation is less time-consuming. Nevertheless, after two washings with saline or citrate, the tubes containing fresh packed RBCs may be filled up with Alsever's solution completed by cyanides. In our practice, this latter method is used.

SUMMARY

Preservability of pig RBCs was examined in citrate and Alsever's solutions completed with various quantities of KCN or NaCN. Pig RBCs could be well preserved in citrate solutions containing cyanides from 1 : 17,000 to 1 : 21,000 for 4 weeks. The same cells in Alsever's solutions of 1 : 2000 and 1 : 3000 cyanide content could be preserved for 60 days in excellent condition. None of the 50 blood samples kept in Alsever's solution completed with KCN 1 : 2500, were deteriorated or haemolyzed and their reactivity with isoimmune sera in the direct haemagglutination test did not decrease considerably even by the 47th day of storage.

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TRYPSIN- AND HEAT-RESISTANCE OF SOME STRAINS OF THE HERPESVIRUS GROUP

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At the present time viruses are classified according to their nucleic acid, structure of the nucleocapsid, presence or lack of the envelope, and their size. Based on these properties most of the isolates can be grouped in one of 8 groups. In addition to the main features listed, the virions have other properties characteristic of the viruses belonging to the same group or subgroup. Such features are, *e.g.*, their behaviour towards the action of heat or trypsin. It is known that the viruses of the Herpesvirus group are sensitive to heat and trypsin.

In our investigations studying trypsin and heat resistance of some of the different strains belonging to the Herpesvirus group was intended in order to confirm or disclaim the statement that the members of the Herpesvirus group would be heat- and trypsin-labile.

The investigations have been deemed proper to carry out, for BODON et al. (1968) found exception to the rule. They observed that, unlike the virulent virus, the Aujeszky's disease virus strain of low virulence K4/61 isolated by BARTHA proved heat- and trypsin-resistant. The authors considered that heat- and trypsin-resistance is correlated with the virulence of the Aujeszky's disease virus strains.

Materials and methods

The strains were propagated in monolayers of calf testicle, rabbit kidney and cat kidney cells. The following strains were studied:

Aujeszky's disease virus

1. Ig/67 virulent strain (3rd passage)
2. K4/61 vaccine strain (141st passage)

Infectious bovine rhinotracheitis (IBR)

3. Nu/67 virulent strain (4th passage)
4. Sz2/61 laboratory strain (51st passage)
5. IBR vaccine strain (Diamond Laboratories)

Bovine herpes virus

6. Movár 33/63 strain (23rd passage)

Feline rhinotracheitis virus
7. KB/63 strain (16th passage)
Equine rhinopneumonitis virus
8. DP/65 strain (28th passage).

Excepting the IBR vaccine strain all strains were isolated in the Virological Laboratory of our Institute.

The resistance tests were carried out with freshly passaged strains. Resistance to heat was tested by adding 1 ml distilled water to 1 ml virus fluid and incubating the mixture in a 50 °C water bath for 60 minutes. Following incubation, serial tenfold dilution was inoculated into 2 cultures each. Inoculated cultures were incubated at 37 °C and read on the 3rd to 4th day.

Combined action of heat and bivalent cations was tested, too. To test this action, 1 ml infective culture fluid and 1 ml 2 *M* $MgCl_2$ solution sterilized by heating was mixed and incubated in water bath at 50 °C for 30 minutes. Then the mixture was diluted and inoculated as described above.

To test trypsin-resistance, equal amounts of infective culture fluid and 0.25% sterile trypsin solution (Difco 1 : 250) were mixed and incubated in water bath at 37 °C for 30 minutes, then serial tenfold dilutions were made and inoculated as described above.

To determine the original titre, virus fluids diluted to twice of their original volumes with distilled water were titrated in tissue cultures and served as controls.

Cytopathic effect of virus was observed under the microscope. By the 3rd to 4th day of incubation characteristic cytopathological changes appeared in the tissue cultures inoculated with the infective virus. No changes could be observed in those inoculated with inactivated virus.

Results

Results of the resistance tests are summarized in Table 1. The original virus titres ranged between 10^5 and 10^6/0.2 ml and these were reduced by trypsin or heat treatment to various extents, even in the absence of bivalent cations. It was striking that on the joint effect of heat and bivalent cations all tested strains completely lost their infectivity.

Heat treatment at 50 °C for 60 minutes reduced the original titres to various extents depending on the strain. It was revealed that the freshly isolated virulent strains were more sensitive to heat than the vaccine strains with reduced virulence and a virulent strain isolated long time ago. The reduction in titre was 4 to 5 and 1 to 2 log units, respectively. Similar results were obtained with trypsin. However, the results of the two tests did not always conform to each other.

Table 1

Changes in titre of 8 different Herpes virus strains due to the action of heat, cation and heat and trypsin

Virus strain	Titres			
	Before treatment	Following treatment with		
		Heat (at 50 °C)	Cation and heat (at 50 °C)	Trypsin
Aujeszky's virus (virulent Ig/67)	10^6	$10^{1.5}$	0	$10^{1.5}$
Aujeszky's virus (vaccine K4/61)	10^6	10^6	0	10^5
Bovine rhinotracheitis virus (virulent Nu/67)	$10^{5.5}$	$10^{1.5}$	0	0
Bovine rhinotracheitis virus (Sz2/61)	10^6	10^4	0	10^2
IBR vaccine (Diamond)	$10^{5.5}$	10^4	0	10^2
Bovine herpes virus (Movár 33/63)	10^5	10^2	0	10^4
Feline rhinotracheitis virus (KB/63)	10^5	0	0	10^2
Equine rhinopneumonitis virus (DP/65)	10^5	10^1	0	10^1

Discussion

Members of the Herpesvirus group are, in general, known to be heat- and trypsin-labile. More recently BODON et al. (1968) have observed that vaccine strain K4/61 of Aujeszky's disease virus is more resistant to heat and trypsin than the virulent strains of the same virus. In the present studies we have found that some strains of other herpesviruses possess the same properties. All of these strains are of reduced virulence. Based on our studies, small in number, it might be suggested that an increased resistance to trypsin and heat involves a reduced virulence. If this hypothesis is right, selection of innocuous strains for vaccine production against diseases (*e.g.* equine rhinopneumonitis, feline rhinotracheitis), caused by herpesviruses can be made more easily. To select innocuous strains, experimental animals had to be inoculated thus far. Based on the heat- and trypsin-stability test it will be possible to restrict the use of animal experiments to relatively resistant strains.

SUMMARY

Sensitivity to the action of heat and trypsin of 8 different strains belonging to the Herpes group was studied. The tested strains did not behave uniformly. Unlike the experiences gained so far, some of the strains resisted heat and trypsin treatment. The strains were those used for vaccine production or old laboratory strains. It is supposed that heat- and trypsin-resistance of viruses belonging to the Herpes group may indicate complete loss or decrease of their virulence. Along with the theoretical significance this fact plays a practical role, too, because selection of the strains intended to use for vaccine production could be simplified.

All the tested strains were inactivated due to the simultaneous action of heat and bivalent cations.

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BODON, L., MÉSZÁROS, J., PAPP-VID, G. and ROMVÁRY, J.: Acta vet. hung. **18** (1968), 107.

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WATERSON, A. P.: *Introduction to animal virology*. Second edition. Cambridge, University Press. 1968 (Price 35 s). 176 pages.

The book has been written chiefly for investigators working in the boundary fields of biology. The author uses the animal viruses as models to demonstrate the structure and effect on the host cell of the viruses as subcellular units, with special regard to their transforming action connected with the transfer of genetic information. As compared to the first edition, the present second edition contains numerous modifications in order to make it cope with the recent extensive progress in basic virology. The book is keeping in with the author's original intention to discuss the cellular and molecular aspects of the viruses rather than applied virology.

The first of the eight chapters deals with the history of virology from the last decade of the past century up to date. This short historical survey has been compiled so as to concentrate the reader's attention on the basic concepts underlying the recent discoveries which are opposed to the earlier macromolecular interpretation of the virus. In this light the formerly much disputed problem whether or not the virus is a living organism seems to be of less bearing. Emphasis is laid rather on the description of the decisive properties of the viruses, which are highly organized nucleoproteids, structurally more complicated than the macromolecules and are further characterized by biological specificity, heredity and genetic variability.

Chapters 2, 3 and 4 deal with the virion's structure, with virus titration and haemagglutination methods, respectively. Chapters 5, 6, 7 and 8 discuss the viral replication cycle, the cancerogenic role of viruses and the genetics and pathogenicity of animal viruses. Like in the whole small volume, the author carefully avoided details also in the individual chapters, restricting the text to the description of phenomena and events to an extent which is helpful to the non-virologist reader.

Information on the seven newest English textbooks on — chiefly animal — virology, is given in the Appendix. The bibliography comprises 355 references.

The first edition of the book, published in 1961, was based on a series of lectures read to an undergraduate audience. The substantially modified second edition discusses clearly and concisely the discoveries made in the field of virology during the past two decades, in an easily comprehensible and readable form appealing even to readers engaged in remote fields of biology.

T. SZENT-IVÁNYI

Current Veterinary Therapy. — Small Animal Practice.

Third edition. Edited by ROBERT W. KIRK. — 762 pages, with numerous figures and pictures. In cloth. Publisher: W. B. Saunders Co. Philadelphia, London, Toronto. 1968.

This is a considerably revised and enlarged edition of the book. It is completed by a new part titled Dietary Therapy. Some of the chapters were written by newly-invited authors. The work aims at offering the latest knowledge of the most up-to-date therapeutical treatments and of the diseases occurring in the veterinary practice most frequently with small animals.

The editor, Professor of Small Animal Medicine at New York State Veterinary College, Cornell University, was supported by eleven consulting-editors. The number of contributors is as many as 167. These are experts who can be counted among the best ones in their special fields. All, except several Europeans, are Americans, most of them veterinarians.

The work falls into 15 parts, each part comprising several chapters. It is complemented with an Appendix followed by a detailed, precise, practicable Index.

1. *Special Therapy.* Excellent chapters claiming very high professional merits in this part are the *Use of Antimicrobial Drugs*, *Blood Transfusion*, *Shock-*, *Fluid-*, *Radiation-*, and *Ultrasound Therapies*, and many minor chapters. A very good review of the problem of jaundice is given in the chapter *Icterus in the Dog*, though it is disputable whether this problem fits in the subject matter. It cannot be deemed very fortunate to have the characteristics of chronic and acute liver diseases included in this chapter.

2. *Dietary Therapy.* The first chapter is the Index of Nutritional Therapy giving shortly, yet very substantially and clearly, the basic principles of dietary prescriptions necessary in the course of diseases and conditions. The chapters *Hand Rearing of Newborn Puppies*, *Artificial Alimentation*, *Food-Induced Allergy*, *Prevention of Coprophagy*, *Weight Loss and Obesity* are worthy of attention. However, it is not quite clear why *hypoglycaemia*, *puerperal tetany* and *pansteatitis* are dealt with at this place, as they are essentially metabolic diseases.

3. *Physical and Chemical Injuries.* The following pages present the general principles of the diagnosis and treatment of poisonings, followed by the symptomatology and characteristics of the most frequent poisonings of mineral and plant origin. These chapters to be rated among the best parts of the book, are practicable and give a good and clear summary of the most important information on poisoning. — *Burns*, *frostbite* and *heat stroke* are treated also in this part.

4. *Diseases of the Respiratory System.* This section deals with the diseases of the respiratory system caused by bacteria, viruses, parasites, fungi, *etc.* The chapter *Nasal and Sinus Surgery* is worth to note. The chapters *Bronchial Asthma*, *Abnormal Chest Fluids Including Chylothorax and Exudative Pleurisy of Cats* are novel and interesting. This part deals with tonsillitis, so frequent and important in dogs, though the tonsils do not belong to the respiratory system.

5. *Diseases of the Cardiovascular System.* Frequent and very rare diseases can equally be found in this part. Thus the pathography of very rare developmental abnormalities, and even a very detailed description of their surgical solution can be found here as well as the digitalis and diuretic therapy in *Heart Failure*, *Heart Insufficiency* and other *Heart Diseases*. Again, it is unusual to find the chapter *Ascites* among the diseases of the cardiovascular system.

6. *Diseases of the Blood and Lymph.* This part deals with the pathography of *anaemia*, *bleeding* and *haemorrhagic diseases* and *malignant lymphoma* (lymphomatosis).

7. *Dermatologic Diseases.* A well-constructed, valuable and interesting part of the book. The complex problem is dealt with extensively and rather fully, corresponding to the significance of dermatological diseases. Among the parasitic diseases, various types of *mange*, *demodicosis* and other, less frequent dermato-parasitoses are discussed as well as damages caused by skin parasites. Detailed and high-level chapters are *Dermatophytosis*, *Cutaneous allergy*, *Flea Allergy Dermatitis* and *Otitis externa* including its surgical treatment. Less frequent but rather important skin diseases are also dealt with in this section.

8. *Diseases of the Eye.* A uniform, short, but full and well-built picture of the subject is given. After giving a review on the structure and examination of the eye, and of the vision test, a good concise discussion of the diseases of every part of the eye, as well as their treatments are presented.

9. *Diseases of Cage Birds and Exotic Pets.* The first chapters of this section discuss cage birds, their physical and laboratory examination, hospitalization and nutrition. Then the infectious, non-infectious, parasitic diseases and those demanding surgical treatment are considered. Further chapters deal with the care and feeding of monkeys, their metabolic, infectious, respiratory, surgical and obstetrical diseases. Besides these chapters, the section gives ample reading on exotic cats, mice, rats, guinea pigs, rabbits, gerbils, golden hamsters, crocodiles, turtles, snakes and lizards, kinkajous, raccoons and skunks, their keeping, nutrition and diseases.

10. *Muscoskeletal Disorders.* In this section the chapters *Hip Dysplasia* and *Canine Disc Disease* are excellent. However, besides these only *Osteochondritis* and the *Diseases of the Muscles of Mastication* can be found. Unfortunately, a number of important diseases of the organs of movement cannot be found in this section.

11. *Neurologic Disorders.* The chapter *Seizures* gives an appreciation of the differential diagnosis of seizures, summing up their origination. The part *Normal and Abnormal Behaviour*

of Dogs is very interesting, presenting a few ideas concerning the psychology of dogs. A rather lengthy chapter treats the *feline vestibular disease* and the treatment of *otitis media*. Besides these subjects, only the chapters *Intercranial and Spinal Injuries*, *Examination of the Cerebrospinal Fluid* and *Polyradiculoneuritis* are included in the section. Thus, though each chapter can be deemed valuable, on the whole a feeling of want arises on reading this rather unproportional part of the book. It is hard to understand why other frequent and important diseases of the central and peripheral nervous system have not been introduced in this section.

12. *Diseases of the Digestive System.* This comparatively short section deals with the most important diseases of the digestive system and the parasites of the intestinal tract. The chapter on the diseases of the teeth *(Dental Care)* is particularly elaborated. The chapter *Diarrhea* gives ample information about enteritis. The diseases of the pancreas are thoroughly discussed, but the liver is not mentioned.

13. *Endocrine and Metabolic Diseases.* This section deals rather amply with the diseases arising from the anomalies of endocrine glands; much less space is given to various bone diseases.

14. *Infectious Diseases.* After a short description of the most important infectious diseases and high-level chapters on the rules of disease prevention for dogs and cats, many chapters deal with the transportation and export-import prescriptions for pets.

15. *Diseases of the Urogenital System.* The first chapters of the section comprise the most important diseases of the urinary system. Consecutive chapters enumerate the problems of obstetrical help, fertility (artificial insemination) and sterility as well as various diseases of the female genital organs.

Appendix. This part contains detailed comprehensive tables of corpuscular elements, chemical constituents and physical properties of the blood, urine, cerebrospinal fluid, sperm, synovia and digestive secretion. Tables referring to physiological data of the dog, determination of the age of dogs, nutrient requirements, growth and development of various types of dogs are included. Finally units of measures, important drugs and instruments are listed.

After a short review of the book it is not easy to form an adequate opinion. Undoubtedly, it gives considerably more than promised by its title, since it deals not only with the therapy of diseases, but also with their aetiology and symptomatology, and often with their differential diagnosis, including even the laboratory-diagnostics. Most of the chapters give the practitioner full information on the subject in question, even a knowledge outgrowing the demands of the general small animal practice.

Taking any chapter at random, it is easy to see that it was written by one of the best experts of the subject. The chapters are, in overwhelming majority, pointing to the essential problem, grasping and interpreting it in an excellent style easy to follow for the practitioner — sometimes giving almost delightful reading. Some chapters excel in pointing out the interrelations; others excellently sum up all the information on the problem.

However, if the whole book, or certain parts, are considered, the impression is not so unequivocally favourable. Besides the undisputable advantages, there are certain disadvantages involved in the fact that the book was written by many authors. Some sections, sometimes not even the most important ones, are unnecessarily long; overlapping is not rare. Though this is mainly a matter of editing, the absolute lack of some extremely important subjects is definitely a weak point in the editorial work *E.g.*, Aujeszky-disease is not mentioned at all. It seems incredible that neither hepatitis, nor hepatic cirrhosis is dealt with in a separate chapter; the chapter *Icterus* does not supplement the missing chapters. It is hard to understand why meningitis, myelitis and the diseases of the peripheral nerves are not discussed. The metabolic diseases are also rather scantily dealt with. At the same time, diabetes insipidus, one of the rarest diseases in practice, is treated on five full pages; this unjustified lack of uniformity is, regretfully, characteristic of the greater part of the book. The extent and level of the treatment of certain surgical questions, *e.g.* some problems of the chest and vein-surgery, operation of otitis externa and media, the surgery of the sinuses, attain the level expected from good handbooks of surgery. At the same time, the treatment of a broken bone has no separate chapter.

The layout of the sections except those devoted to the eye and skin diseases does not show a logical line. It is rather hard to guess why the examination of the cerebrospinal fluid is treated together with the intracranial and spinal injuries; or what train of thoughts gave the layout of the whole section on the nervous system.

Similarly, the whole arrangement of the subject matter gives rise to wondering. Correctly, it begins with general problems followed by diseases of the organ-systems in consecutive chapters. But why the diseases of cage birds and exotic pets came in between the diseases of the eye and muscles, or why infectious diseases fell between the metabolic and renal diseases, is rather hard to explain.

The value of a book is given by its content and practicability. For the practising veterinarian using the book as a handbook or lexicon, the above objections are uninteresting. However, the reader will not find the answer to some questions he is interested in. And, the expert who wants to go in deeply into certain sections, or the whole of the book, will be definitely disturbed by these deficiencies.

There is no doubt that a book dealing with such an enormous subject matter can hardly, or not at all, be constructed to meet every requirement. Yet, we wish to hope that the next edition of this precious and popular manual for practising veterinarians, which comprises extremely valuable knowledge treated on a very high level, will eliminate most of the deficiencies and will give full joy to the reader.

F. KARSAI

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РЕЗЮМЕ

ИЗУЧЕНИЕ ЭНДОКРИННОЙ СИСТЕМЫ АМФИБИЙ. ПАРАЩИТОВИДНЫЕ ЖЕЛЕЗЫ RANA RIDIBUNDA

В. РИЗКАЛЛА

Rana ridibunda обладает двумя паращитовидными железами, лежащими с двух сторон продольной оси тела. Железы примыкают к внутренней стороне яремных вен и язычных артерий.

Железы овальной формы, невооруженным глазом хорошо заметны, поскольку они в длину 4 мм и в ширь 2 мм. Железы обладают углублением, напоминающим ворота, через которые афферентные сосуды внедряются в орган и эфферентные уходят от него.

Паращитовидную железу формируют эпителиальные клетки, сосредоточенные в группы или образующие хорды. Железа богато и равномерно переплетена кровеносными сосудами.

Корпус железы обычно дифференцируется на темно окрашенную периферическую зону и бледнее окрашенную центральную зону. Последняя характерна тем, что в ней чередуются темно и бледно окрашенные группы клеток. Темно окрашенные клетки — это признаки дегенерации, тогда как бледно окрашенные являются, видимо, центром регенерации паращитовидной железы.

Некоторые паращитовидные клетки выделяют ацидофильные коллоидные капельки. Этот секреторный продукт может быть носителем паращитовидного гормона.

Не удалось доказать наличия слизи в железах. Зато липоиды обнаружены в тканях желез, главным образом в клетках периферической зоны.

ВЫЯВЛЕНИЕ ОСТАТКОВ АНТИБИОТИКОВ В МЯСЕ УБОЙНЫХ ЖИВОТНЫХ

Я. ТАКАЧ и Ш. КОВАЧ

Изучалось наличие антибиотиков в мясе 244 животных, лечившихся этими препаратами до убоя. Изучение проводилось модифицированным методом Гардсайд (Gardside). Из почки вырезывались 4 блока формы кубика с размерами в $10\times10\times5$ мм. Блоки после замораживания ставились на агарные пластинки стандартного состава, на которые потом высевались суточные культуры стандартных штаммов бактерий (B. subtilis ATCC 6633, St. aureus ATCC 6538, Sarcina lutea ATCC 9341 и E. coli ATCC 9637). После размещения замороженных блоков из почек агарные пластинки ставились на срок 4—4,5 часов в холодильник температуры +4 °C, после этого же в термостат температуры 37 °C на срок 18—20 часов.

Среди 244 животных в 24,6%-ах случаев удалось доказать наличие антибиотиков. Поскольку наличие антибиотиков сильно влияет не только на результат дополнительного бактериологического исследования мяса, но повреждает и здоровье человека, потребившего такое мясо, авторы рекомендуют включить изучение наличия антибиотиков в мясе больных и потом прирезанных животных в качестве объязательного дополнительного исследования мяса при ветсанэкспертизе. Рекомендуется дальше употреблять мясо, содержащее антибиотики, только для приготовления мясных продуктов, получаемых обработкой температурой.

ЗНАЧЕНИЕ РСК И МЕТОД ИММУНОФЛУОРЕСЦЕНЦИИ В ЛАБОРАТОРНОЙ ДИАГНОСТИКЕ ТОКСОПЛАЗМОЗА

Э. МОЧАРИ и ДЬ. СЕМЕРЕДИ

На кроликах поголовья, неблагополучного по токсоплазмозу, на животных в разной степени больных и павших, дальше, искусственно зараженных лабораторных животных изучалась надежность РСК и метода прямой иммунофлуоресценции в диагносцировании токсоплазмоза. РСК авторы нашли удобной для дополнительного исследования, но ее положительный результат — согласно авторам — без прямого выявления заразного начала не имеет диагностического значения. Поэтому авторы в своей работе подчеркивают значение прямого выявления токсоплазмы. Для этой цели наиболее зарекомендовал себя очень чувствительный, быстрый и специфический метод иммунофлуоресценции.

ОЦЕНКА ЗАМОРОЖЕННЫХ СЪЕСТНЫХ ПРОДУКТОВ НА ОСНОВАНИИ МИКРОБИОЛОГИЧЕСКОГО ИССЛЕДОВАНИЯ

Я. ТАКАЧ, З. ШИМОНФИ и Э. ИМРЕХ

2842 образца из быстро замороженных, готовых к продаже съестных продуктов изучено с микробиологической точки зрения. На основании технологического процесса, понижающего количество микробов, до микробиологического исследования замороженные продукты разделили на пять групп. И то: мясные, готовые блюда, быстро замороженные (обработанные температурой); полуготовые мясные блюда (необработанные температурой); бланшированные овощи; фрукты и блюда, невходящие в эти группы. При органолептическом изучении определяли число всех живых микробов, протеолитических, колиформных, щелочьпродуцирующих микробов, число почкующихся и плесневых грибков, число клостридиев, гемолизирующих, психрофильных и микробов Micrococcus pyogenes var. aureus. Ряд исследований заканчивался изучением сальмонелл. В отношении всех изучаемых признаков исчисляли логарифмическое среднее и при помощи кривой Гауса определяли значение разового положительного рассева. Данные исследований показаны на кривых снимков 1—5 и таблице. Обнаружено, что среди микробиологических свойств для оценки микробиологического состояния продуктов наиболее удобно число всех живых микробов, колиформных и лактобациллов, почкующихся и плесневых грибков и клостридиев.

Отдельные микробиологические требования разнятся друг от друга в зависимости от того, подвергалось ли сырье (продукт) до замораживания обработке температурой, или речь идет только о замороженном продукте. Указанные микроорганизмы в первых имеются в значительно меньшем количестве, чем в последних. На этом основании однако не следует делать отличия в отношении патогенных и токсинпродуцирующих, гемолизирующих микробов Micrococcus pyogenes var. aureus, сапрофитных или условно патогенных клостридиев. Бесперебойная холодильная и гигиеническая цепь, безостаточное обеспечение гигиены производства является неотъемлемой частью недопущения порчи быстро мороженных продуктов. Благодаря действию внеклеточных энзимов и при низком микробном числе замороженный продукт может явиться испорченным, если энзимы проявили свое действие еще до заморожения или после отморожения. Порчу от психрофильных микробов можно профилактировать обеспечением сухости поверхностей продукта и низкого pH. Если число микробов достигло значения 10^7—10^8 в грамме или $см^3$, замороженный продукт нужно декларировать гнилым.

СОДЕРЖАНИЕ АНТИБИОТИКОВ В ЯЙЦАХ КОРМИВШИХСЯ ПРЕПАРАТОМ ЭРРА КУР

ЭДИТ ИЛЛЕШ и М. СЕНТ-ИВАНЬИ

Изучено содержание окситетрациклина в яйцах несушек, кормившихся долгое время подкормкой Эрра—2. После критической проверки нескольких методов экстрагирования, рекомендуемых литературой, применялись параллельно два метода экстрагиро-

РЕЗИСТЕНТНОСТЬ К ТРИПСИНУ И ТЕМПЕРАТУРЕ НЕКОТОРЫХ ШТАММОВ ИЗ ГРУППЫ ХЕРПЕТОВИРУСОВ

А. БАРТА, Ш. БЕЛАК и Й. БЕНЕДА

Изучена устойчивость 8 вирусных штаммов из группы херпес к температуре и трипсину. В исследованиях вирусные штаммы вели себя по-разному. В разрез нашим знаниям часть вирусных штаммов противостояла обработке температурой и трипсином. Это были вакцинные или старые лабораторные штаммы. Можно предполагать, что резистентность вирусов к температуре и трипсину означает потерю или понижение вирулентности. Кроме теоретического этот факт имеет и практическое значение, ибо на этом основании отбор вакцинных штаммов можно бы в значительной мере упростить.

Все изученные вирусные штаммы инактивизировались после обработки температурой и катионами.

вания: смесью буфера щавелевая кислота + гликокол и фосфатным буфером pH 4,5. В качестве тэст-организма использован B. subtilis при применении так называемого чувствительного метода.

Изучено 293 яиц от 15 несушек. Среди несушек 6 получали принятую дозу Эрра, то есть 150 г Эрра—2 к 100 кг-ам корма (30 мг окситетрациклина на 1 кг корма), 6 — десятикратную дозу Эрра—2 (300 мг ОТЦ/кг корма), 3 — оставлены контрольными и получали корм без антибиотика.

Доказано, что каждый г белка всех яиц — в том числе и от несушек, не получавших антибиотика — содержит по 1—1,5 γ вещества, тормозящего рост колоний B. subtilis. Авторы считают, что этот бактериостатический эффект вызывает имеющийся в яичном белке лизозим. Наличие ОТЦ не обнаружено даже в яйцах от несушек, получавших 10-кратную дозу Эрра—2. Этим доказано, что потребление человеком сырых яиц от несушек, подкармливавшихся долгое время антибиотик содержащим препаратом, не может быть источником повреждения здоровья.

Экспериментами одновременно лишний раз доказано, что несушки, подкармливаемые антибиотиком откладывают больше яиц, чем контрольные. Дозу антибиотика однако нецелесообразно поднять выше рекомендуемой, ибо это не сопровождается дальнейшим подъемом яйцепродукции.

КОСВЕННОЕ ОПРЕДЕЛЕНИЕ ГЕНОТИПОВ ПЛЕМЕННЫХ ПЕТУХОВ НА ОСНОВАНИИ ФЕНОТИПОВ ЯИЧНОГО БЕЛКА НЕСУШЕК И ПОТОМСТВА У КУРЫ

М. ПАПП и Л. ФЕШЮШ

Полученные от 60 несушек венгерской белой породы, от 85 несушек породы сассекс, от 61 несушки породы белый легхорн и от 28 несушек породы род айлэнд яйца изучались методом крахмаль-жель-электрофореза. Определены генотипы 6 семейств породы белый легхорн и 4 семейств породы род айлэнд. На основании материнных и дочерних генотипов авторы определили предполагаемые генотипы петухов в отношении всех четырех мест яичного белка.

ГИСТОХИМИЧЕСКОЕ ВЫЯВЛЕНИЕ ГЛИКОГЕНА ПРИ СОЗРЕВАНИИ МЯСА

Г. БИРО

Гистохимическое выявление гликогена осуществлялось в кроличьей и свиной мышце при помощи реакции PAS. При равномерном содержании гликогена на поперечном срезе получается равномерное темно-красное окрашивание. Изменения содержания гликогена между мышечными волокнами реакцией выявляются. При помощи гистохимического метода внутри мышечной связки можно следить за содержанием гликогена в мышечных волокнах и на основании этого можно судить о биохимическом состоянии последних.

ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ РЕГУЛИРУЮЩЕГО МЕХАНИЗМА РАВНОВЕСИЯ КИСЛОТА-ЩЕЛОЧЬ У ОВЕЦ

Б. ЮХАС и Б. СЕГЕДИ

Количество и концентрация слюны у овец, согласно исследованиям, кроме известных уже факторов (консистенция корма, количество сухих веществ в корме, жевание, проглатывание, отрыжка, давление на стенку рубца, размеры частиц содержимого рубца) регулируются и изменениями pH и химического состава рубцевой жидкости. Можно предполагать, что в стенке преджелудков имеются чувствительные к химическим раздра-

жителям рецепторы, раздражения которых влияют рефлекторным путем на количество, pH и состав слюны и мочи. Пока между содержимым рубца и водяным пространством имеются отклоняющиеся от нормы условия, наблюдается обратное соотношение в количестве выделяемой слюны и мочи. Данные экспериментов именно показали, что при понижении значения pH рубцевой жидкости количество выделяемой слюны повышается и она становится более щелочной, причем имеется одновременное понижение количества выделяемой мочи, которая становится более кислой. Повышается количество Na^+ и K^+, выделяемых слюной, но зато их концентрация в моче понижается. Повышение концентрации молочной кислоты в крови не ведет к появлению этого вещества в слюне, зато мочей выделяется одновременно большое количество молочной кислоты и аммиака. Даже при некомпенсированном ацидозе слюна остается щелочной.

Обнаружено дальше, что при сильной кислотности рубцевой жидкости животные переносят без вреда даже токсическую дозу карбамида. От пониженной дозы карбамида изменяются количество и состав выделяемой слизи. Концентрация карбамида в слюне повышается, но зато он, Na^+ и K^+ за час выделяются в значительно меньшем количестве. Однако одновременно повышается количество выделяемой мочи и с нею аммиака, карбамида, как и Na^+ и K^+. Таким образом состав и количественные соотношения выделяемой слюны и мочи показывают существенные изменения, которые тесно взаимосвязаны с качественными и количественными условиями химических процессов, разигрывающихся в рубце. На этом основании у овец наряду с мочей слюна тоже играет важную роль в водяном и алкалическом обмене, дальше, регулировке равновесия кислота–щелочь в организме.

ОЦЕНКА НЕКОТОРЫХ ВЕЩЕСТВ, ПРИМЕНЯЕМЫХ В ТРАНСФУЗИОННОЙ ТЕРАПИИ, НА ОСНОВАНИИ ОБЪЕМА КРОВИ

ДЬ. ПЕТЕШ, ДЬ. ЛАМИ и Л. ТАМАШ

Из общей сонной артерии от 18 собак авторы брали быстро по 30 мл/кг крови, которую потом замещали таким же количеством физиологического раствора поваренной соли, гепаризированной (гомологической) крови, плазмой и венгерским декстрановым препаратом — Плазмодекс.

До получения крови и после окончания инфузий при помощи меченого I^{131} плазменного собачьего альбумина определялся объем плазмы и, зная гематокрит, объем крови.

За время наблюдения в 1,5 часа констатировано, что показатель эффективности (показатель объема крови после инфузии в %-ном выражении к этому же показателю до получения крови) у Плазмодекс равнялся 109,5%-ам, после применения гепаризированной плазмы — 93,2%-ам, в группе, получившей гепаризированную кровь — 87,7%-ам и в группе же, где кровь заместили физиологическим раствором поваренной соли — 76,5%-ам.

Измерениями при помощи меченых атомов обнаружено, что объем крови у здоровых собак равнялся $86{,}9 \pm 17{,}9$ мл/кг (то есть 8,7%-ам живого веса).

Авторами дается новый анализ данных, полученных раньше в экспериментах по частичному обескровлению и замещению крови (выживание, нормализация общего состояния, восстановление давления крови «bleeding volume index» и показатель «lethal rebleeding volume», формирование объема крови).

ХРАНИМОСТЬ И РЕАГИРУЕМОСТЬ ЭРИТРОЦИТОВ СВИНЬИ

Л. ФЕШЮШ и М. ПАПП

Изучалась хранимость эритроцитов свиньи в цитратном растворе и растворе Алсевера с добавлением к ним разного количества цианида калия или цианида натрия. В цитратных растворах, содержащих цианиды в соотношении 1 : 17 000 — 1 : 21 000, можно хранить эритроциты четыре недели. Эти же клетки можно отлично хранить в растворе Алсевера при содержании цианидов в соотношении 1 : 2000 — 1 : 3000 — в течение 60 дней. Ни один среди 50 образцов крови, хранившийся в растворе Алсевера с содержанием цианида калия в соотношении 1 : 2500, не испортился, ни в одном не имелся гемолиз и реагируемость эритроцитов с изоиммунными сыворотками в реакции прямой гемагглютинации существенно не понизилась даже к 47-му дню хранения.

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CHANGES IN THE CONCENTRATION OF IRON IN THE LIVERS OF WHITE LEGHORN CHICKENS IN THE EARLY POST-INCUBATION PERIOD

By

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Introduction

Within the scope of an investigation of changes in the early ontogenesis we studied the iron content in chicken livers from hatching until the age of one month.

In the body iron forms a part of the haemoglobin, cytochrome C and of a number of further enzymes (Keller and Scheunert, 1952). Determination of iron in the body of chicks immediately after hatching was performed by Ewing (1951), and in young and mature chickens by Spray and Widowson (1951). Weiser et al. (1966) studied the iron content in the serum and plasma, Elvehjem and Peterson (1927) compared the iron concentration in the different organs and in red and white muscles. The fate of labeled iron in the organism after absorption of subcutaneously applied preparations was examined by Ramsay (1966), and similar problems were dealt with by Plamas (1967). The iron content in the yolk and in the albumen were determined by Elvehjem et al. (1929) and by Ewing (1951), the relations between iron in the feed ration and hatchability and laying were studied by Krieg (1965), Opichal (1965), and the iron content in the feed ration and its influence on the health of chickens were investigated by Norris (1966), Kurnick et al. (1958), and by Davis (1962).

Material and methods

Iron was determined in the livers of 147 chickens aged from 1 to 31 days, separated into different age groups as shown in Table 1. The chickens were kept under normal zootechnical conditions and were fed the K mixture for breeding of chickens. This and water were supplied *ad libitum*. The K mixture contains 1% of mineral supplements including 8% $FeSO_4 \cdot 7\ H_2O$. The chickens were killed by decapitation, and the blood was used for the determination of the basic values. The livers were weighed as a whole, then comparative samples were taken. Iron was determined in individual samples (a smaller part) and also in pooled livers from six chickens. Dry matter, sulphate ashes and, in the latter with orthophenanthroline, the iron content were determined.

The values obtained in the different age groups were subjected to statistical analysis.

The iron content of the liver consists of two components: the iron of the liver tissue and that of the blood remnants after bleeding.

The present values can be compared with others obtained from experiments carried out with the same methods.

Results and discussion

The statistical evaluation of the results is shown in Table 1 and Fig. 1.

Table 1

Changes in the iron content of the liver of chickens in the first month after hatching

Age in days	Weight of liver, g	Dry weight of liver, per cent		n	mg per 100 g liver	Iron in the liver, mg per 100 g dry weight			Total iron per liver, μg
		$\bar{x}$	$\hat{s}$			$\bar{x}$	$\hat{s}$	V	
1	1.078	36.4	0.57	42	4.11	11.28	1.88	9.2—14.1	44
3	0.9977	31.9	0.91	18	5.55	17.4	1.2	16.1—18.4	55
4	1.2863	32.5	1.5	25	5.65	17.9	5.0	13.1—25.0	73
7	2.0037	27.4	1.4	12	3.77	13.75	1.6	12.6—14.9	76
11	1.9305	26.0	0.52	24	4.72	18.15	3.7	16.4—24.3	91
18	3.7613	25.5	0.23	14	5.64	22.13	4.4	18.4—34.5	212
23	5.0958	26.8	0.7	5	6.55	24.46	8.8	13.8—36.9	334
25	5.7971	24.8	1.2	2	5.95	24.0	5.8	19.9—28.1	345
31	7.4555	27.55	0.95	5	5.92	21.5	4.7	17.4—29.3	441

Accordingly, the weight of livers increased with age, but their dry matter expressed in per cent was highest at the time of birth, obviously because of the little quantity of water at disposal. In the first 4—7 days the values of the dry matter in per cent gradually decreased due to the water absorbed from the intestines. Between days 7 and 31 the percentage of the dry matter was approximately stabilized. The quantity of iron determined in the livers, be it in values expressed in mg per 100 g of dry matter or mg per liver, or in absolute Fe values per liver confirms EWING's (1951) data on the minimum supplies of iron in hatched chicks. The absolutely lowest values were found on the 1st day (11.28 mg iron per 100 g dry matter and 0.044 mg of iron per liver). The value of 11.28 mg iron per 100 g dry matter of liver for one-day-old chicks is very close to the data of ELVEHJEM et al. (1929), who demonstrated 13.4—15.1 mg iron per 100 g dry matter of yolk. Up to day 4 there occurs a rise in the quantity of iron in the liver as shown by both the ration mg iron per 100 g

of liver or dry matter, and in absolute values (Fig. 1). In the period from day 4 to day 7 almost a doubling of the liver weight took place, which was partly caused by the resorption of the yolk sac. In consequence of this the uptake of nutrients from the yolk sac by the intestine and liver stops, and the only source of iron is the feed. However, in the period from day 4 to day 7 the liver's iron supply is approximately unchanged. Consequently, the concentration of

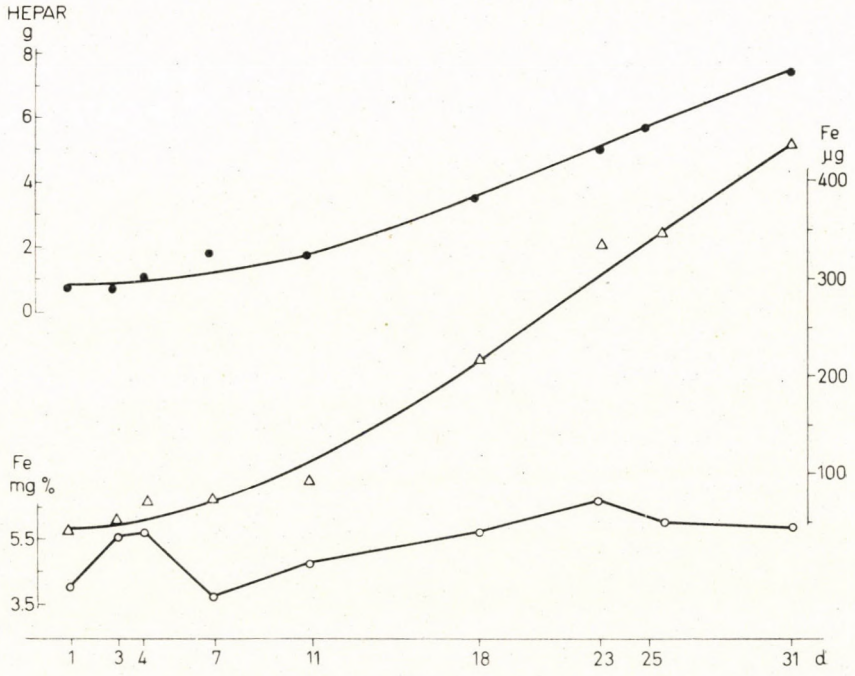

Fig. 1

iron in the liver, expressed in mg per 100 g dry matter, shows a marked fall. From day 11 on this index and the absolute weight of the liver begin to rise and both values are doubled by day 18. However, there are marked individual differences in the iron content of the liver, partly due to the unequal uptake of iron by individual birds. The absolute quantity of iron per chicken was 10 times higher on day 31 than on the day of hatching. During the same period the increase of the average weight of the liver was sevenfold.

Conclusion

The iron was determined in the livers of 147 WL chickens aged from 1 to 31 days. On the first day the average iron content of the livers was 44 μg, *i.e.*, 11.28 $\pm$ 1.88 mg per 100 g dry matter. By the 4th day of age the latter ration

rose to 17.9 mg per 100 g dry matter. In the period from day 4 to day 7 the absolute quantity of the liver iron remained unchanged while the average weight of the livers doubled. From day 18 to day 31 the absolute quantity of the iron in the liver rose nearly parallel with the weight of the liver, *i.e.*, the percentage concentration of iron remained almost unchanged.

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COPPER IN THE LIVER OF WHITE LEGHORN CHICKENS IN THE EARLY POST-INCUBATION PERIOD

By

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In the framework of a study of changes in the early ontogenesis we examined the copper content in the liver of chickens from hatching until the age of one month.

Copper is an indispensable component or activator of a number of enzymes (MAYNARD and LOSLI, 1956), it is an indispensable component of feeds (AL-UBAIDI, 1963) required for the growth, and pigmentation of the plumage. Its quantity in the egg was determined by GRENDEL (1930), in the liver by UNDERWOOD (1965). Copper absorption from the feed ration after an addition of antibiotics was examined by KIRCHGESSNER and GIESSLER (1961), the problem of different Cu concentrations in the feed ration and of their influence on the Cu content in the liver, other organs, and in body fluids was investigated by CARLTON and HENDERSON (1965), TOMOV and NAJDENOV (1963), RUSZYC and GLAPS (1963), ALVARES (1964), and by GIESSLER (1959). The interrelations existing between the quantity of Cu in the feed ration and hatchability or laying were examined by KRIEG (1965), LIEBSCHER (1937), SALEJEV (1961), and NORRIS (1966). Finally, MILTMORE et al. (1964), DICK (1952), KURNICK et al. (1958), HENDERSON (1963) and AL-UBAIDI (1963) studied the effect of a Cu deficiency in the feed on the state of health of poultry.

Material and method

Copper was determined in the liver samples from the chickens referred to in our preceding work (SOVA et al., 1969). The chickens were fed a complete feed mixture for chicken containing a 1% admixture with a 1% mineral supplement including 6% $CuSO_4 \cdot 5\ H_2O$. The number of examined animals is shown in Table 1. The Cu content was determined in the sulphate ashes after a preceding determination of the dry matter. The modified method with dicural was applied.

Results and discussion

The results obtained have been compiled in Table 1 and Fig. 1.

The average value of Cu in whole livers of hatched chickens was 20.2 μg. This means that of the total quantity of approximately 60 μg of Cu present in the egg (GRENDEL, 1930) at hatching one third was in the liver. In the course of development a decline both of absolute and relative Cu values was observed in the liver. This is evidently caused by the higher demands of the freshly hatched chick on the one hand, and, by the imperfect capacity of the intestinal epithelium to absorb Cu from the $CuSO_4$ in the feeds, on the other hand.

Table 1

Values of copper in chickens' liver in the first month of age

Age in days	Weight of liver, g	Dry weight of liver, per cent		n	Cu mg per 100 g liver	Copper in the liver, mg per 100 g dry weight			Total copper per liver, µg
		$\bar{x}$	s			$\bar{x}$	s	V	
1	1.078	36.4	0.57	24	1.86	5.12	2.5	2.03—8.25	20.2
3	0.998	31.9	0.91	18	1.01	3.17	0.51	2.58—3.50	11.0
4	1.286	32.5	1.5	18	1.23	3.79	2.1	1.56—5.73	15.8
7	2.004	27.4	1.4	12	1.58	3.78	3.3	2.55—9.00	31.7
11	1.931	26.0	0.52	24	1.00	3.85	2.5	1.50—6.35	19.3
18	3.761	25.5	0.23	14	0.71	2.79	0.91	1.90—3.78	27.8
23	5.096	26.8	0.7	5	1.21	4.53	1.1	3.40—5.92	72.2
25	5.797	24.8	1.2	2	1.55	6.23	1.3	5.35—7.20	89.6
31	7.456	27.55	0.95	5	0.96	3.50	0.88	2.56—4.57	71.7

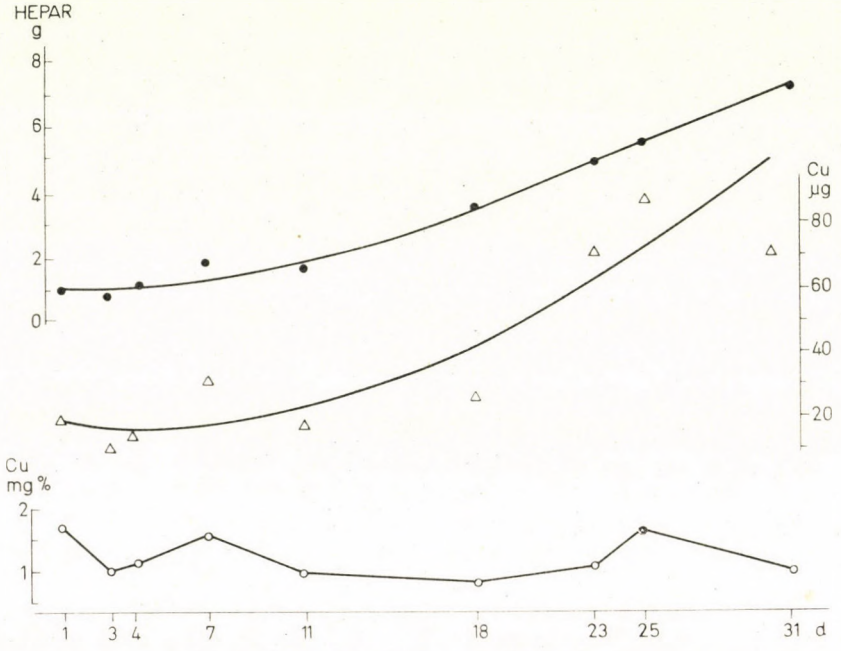

Fig. 1. Copper in the liver in the first month of age

In Fig. 1 the curve illustrating the absolute quantity of Cu in the livers shows two ascending peaks, one on the 7th day and the other on the 25th day of age, which are in good correlation with the relative values expressed in mg

per 100 g liver. Fig. 1 also shows that peaks on days 7 and 25 are lacking in the weight curve of the liver. In day-old chicks the quantity of Cu expressed in mg per 100 g of dry matter fluctuated within considerable ranges (see Table 1), *e.g.*, on day 1 between 2.01 and 8.25 mg per 100 g, on day 7 from 2.55 to 9 mg per 100 g, and on day 11 from 1.56 to 6.35 mg per 100 g, the highest value amounting to 410, 310 and 400%, respectively, We attribute these considerable differences to the imperfect dispersion of $CuSO_4$ in the mineral supplement of the feed mixture. Thus, in accordance with the observation of CARLTON and HENDERSON (1965) and TOMOV and NAJDENOV (1963), Cu accumulated in the liver in individual chickens to a variable degree. At a higher age (after day 18) the individual differences were no longer so marked, the highest values being about twice the lowest ones.

As we have already published (SOVA et al., 1969), the average iron content in the livers of the same chickens showed a 10-fold, the weight of the liver a 7-fold increase in the period from hatching to the end of the first month. The present data show that during the same period of time the copper content displayed only a 3.5-fold increase.

Conclusion

The quantity of Cu in the livers of 122 WL chickens divided into several age groups from hatching to 31 days of age was investigated. Immediately after hatching the whole livers contained on the average 20.2 μg of Cu, *i.e.*, 5.2 $\pm$ 2.5 mg per 100 g liver dry matter. This value fell to 11 μg by the 3rd day and rose to 15.8 μg by the 4th day of age. Between days 7 and 31 the concentration of Cu in the livers ranged within the limits 0.71 and 1.55 mg per 100 g dry matter. The lowest concentration of Cu in the liver was observed in 18-day-old chickens, whose average liver weight (3.761 g), compared with day-old chicks (1.078 g), reached 346%, whereas the absolute quantity of Cu rose only to 134% of the initial value (from 20.2 μg on day 1 to 27.8 μg on day 18). While the weight of liver increased 7 times, their copper content increased only 3.5 times to 4 times in the 1st month.

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STUDY OF GASTRIC FUNCTION IN SWINE WITH GASTROENTERITIS

By

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In 1921, WHITING, DOYLE and SPRAY described under the name swine dysentery a disease associated with mucous bloody stool discharge and necrosis of the large intestinal mucosa. Later on MANNINGER (1944), HEGYELI and HIRT (1942) and MANNINGER and CSONTOS (1943) clarified in great detail the epidemiology, and clinical and pathological pictures of the condition called by them "infectious swine gastroenteritis". Recently MATTHIAS and BECKER (1960) and SOFRENOVIĆ (1963) have reported the occurrence of the condition.

In the meantime, many attempts have been made to precisely clear the aetiology of the disease, but opinions are still divergent on this point. All authors agree, nevertheless, that the conditions of feeding and management play a decisive role in the severity of the disease.

Regardless of the uncertain aetiology, the great losses involved in the outbreaks of the disease have necessitated a closer study of its pathogenesis as well as an improvement of the control measures. MÉSZÁROS and PESTI (1966) described its epidemiology and pathology; PESTI (1966) examined the changes taking place in the intestinal bacterial flora, whereas LOMNICZI et al. (1967) and JUHÁSZ et al. (1967) studied the alterations of the intestinal tract and pancreas during the pathological course.

In the majority of cases, the post mortem examination of swine died of gastroenteritis revealed a stomach full of dried out food of acid odour. The gastric mucosa was dark red, swollen, sometimes covered by a pseudomembrane.

The present experiments were carried out to clarify the changes occurring in the gastric secretion of hydrochloric acid and pepsin from the onset until death, as well as the influence on gastric and intestinal motility of the acidity of gastric juice.

Our examinations covered two forms of swine gastroenteritis: the arrival diarrhoea, which sets in with frequent discharge of watery stool on the 3rd to 5th day after arrival in the fattening plant, and the "infectious" gastroenteritis described by MANNINGER (1944), which has been recently denominated by many authors as "swine dysentery".

Materials and methods

Experimental animals. Sampling. Samples of gastric juice from diseased swine, 20 with gastroenteritis and 74 with arrival diarrhoea, as well as from

carcases of 54 swine died of "infectious" gastroenteritis, were examined for acidity and pepsin activity. To assess normal values, we examined gastric juice samples from 92 clinically healthy swine and carcases of 32 slaughtered healthy swine. From the living animals samples were taken partly after fasting for 24 hours, or half to 2 hours after feeding, by means of a gastric tube. Post-fasting samples were pure and mucus-free, whereas the post-feeding samples contained residual food. In the latter case, the sample was centrifuged prior to assay. White bacon pigs, 20—60 kg in weight, were used throughout.

The relationship between the pH of gastric juice and the motility of the gastrointestinal tract was examined on 10 healthy white bacon pigs, 15—40 kg in weight. To neutralize the gastric acid, aluminium hydroxide gel, freshly prepared from 47.4 g alum [$KAl(SO_4)_2 \cdot 12\ H_2O$] and washed, was given twice daily for 5 days, through a gastric tube. The experiment was repeated administering instead of alum one mol of MgO and, in a third experiment, one mol of $NaHCO_3$. The animals received parenterally several doses daily of Ringer's solution. The changes of gastric secretion and the possible occurrence of diarrhoea were watched throughout the experiment.

Determination of acidity and pepsin in the gastric juice. Free acid and total acid of the gastric juice were determined by titration in a pH-range between 3.5 and 8.2, using methyl orange and phenolphthalein indicators. In accordance with BOCK's suggestion (1962), both the gastric hydrochloric acid and total acid were expressed in terms of milliequivalents of hydrochloric acid per litre. Hypo-acidity was expressed in the same way, using minus symbols. Hypo-acidity is understood as the deficiency in the amount of acid required to reach titration endpoint at pH 3.5 with a methyl-red indicator.

The acetic acid and lactic acid contents of the gastric juice were determined by LEPPER's method as described by SCHMIT and OTT (1953). Pepsin activity was measured by ANSON and MIRSKY's haemoglobin digestion method (1933).

The pepsin values were expressed in terms of milligramme per litre, as derived from comparison with crystalline pepsin preparations on the basis of BITSCH's (1966) and our own relevant examinations.

Results

The acidities of the gastric juices from fed and fasting healthy swine and diseased swine are compared in Table 1. No notable difference was found in the total acid values between clinically ill and healthy animals. The healthy fasting animals had no free acid, exhibiting rather a hypo-acidity. The amount of free acid measured in the gastric juices of healthy and fed swine corresponded

Table 1

Examination of gastric juices from healthy swine and those with gastroenteritis

Condition	Total acid, mEq/l			Free acid, mEq/l			Student's *t*-test
	n	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*	
Healthy fasting	25	2190	±1613	25	−1533	±1314	$P < 0.1$ %
Healthy fed	67	4453	±2336	67	+1423	±1131	
Diseased with gastroenteritis	20	3467	±1496	20	−1168	±1387	$P < 0.1$ %

n, number of examined animals; $\bar{x}$, arithmetic mean; *s*, standard deviation.

to the physiological value. In contrast, the diseased animals had hypo-acidity even with half-full or full stomach. The differences between the free acid values of fasting and fed swine, on the one hand, and between those of healthy and diseased swine, on the other, proved to be statistically significant.

Table 2 shows the acidity values measured in the gastric juice of healthy slaughtered swine, and those died of arrival diarrhoea and "infectious"

Table 2

Examination of gastric juices from healthy slaughtered swine and carcases of swine died of gastroenteritis

Condition	Total acid, mEq/ml			Free acid, mEq/ml			Student's *t*-test
	n	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*	
Healthy slaughtered	32	2628	±2482	32	+1204	±1168	$P < 0.1$ %
Arrival diarrhoea	54	2372	+2098	53	−2956	+1898	
"Infectious" gastroenteritis	38	6533	±3285	38	−3467	±2154	$P < 0.1$ %

n, number of examined animals; $\bar{x}$, arithmetic mean; *s*, standard deviation.

gastroenteritis, respectively. Total acid did not differ notably in this relation either, but it was conspicuously high in the gastric juices of swine died of gastroenteritis, as contrasted to the disappearance of free acid in the initial stage of the disease. Free acid was absent throughout the pathological course. Differences in the total acid and free acid levels of healthy swine and those with gastroenteritis were statistically significant.

To clarify the origin of the high total acid levels of swine died of gastroenteritis, organic acids in the gastric juices were analysed (Table 3). It was

Table 3

Acidity of gastric juices from healthy swine and those with gastroenteritis

Gastric acidity, mEq per litre	Slaughtered healthy swine		Arrival diarrhoea		"Infectious" gastro-enteritis	
	$n = 27$		$n = 11$		$n = 91$	
	x	s	x	s	x	s
Total acid	3869	±2445	3832	±1898	5840	±3321
Free acid	+1241	±1277	−1679	±2226	—	—
Acetic acid	1533	±1095	2007	±1350	3504	±1350
Lactic acid	1058	± 584	1022	± 912	1825	±1350

n, number of examined animals; $\bar{x}$, arithmetic mean; s, standard deviation.

found that while in healthy animals the total acidity was represented by hydrochloric acid and small amounts of acetic and lactic acids, in animals with arrival diarrhoea and still more in those with gastroenteritis, the high total acidity was due entirely to acetic acid and lactic acid. As a matter of fact, the acidity of the gastric juice was due not only to hydrochloric acid and organic acids, but very likely also to salts and buffer systems. The accumulation of acetic acid and lactic acid in the gastric juices of swine died of gastroenteritis lowered the pH of gastric acid to 1.0.

The decrease of gastric pepsin activity takes place parallel with the decline of hydrochloric acid production (Table 4). Pepsin activity was high

Table 4

Pepsin concentration in gastric juices from healthy swine and those with gastroenteritis

Condition	Pepsin, mg/l			Student's t-test
	n	$\bar{x}$	s	
Healthy slaughtered	32	530	±280	$P < 0.1$ %
Arrival diarrhoea	20	140	±270	
"Infectious" gastroenteritis	16	60	±100	$P < 0.1$ %

n, number of examined animals; $\bar{x}$, arithmetic mean; s, standard deviation

in healthy swine, reduced in those with arrival diarrhoea and practically absent in swine with gastroenteritis. Pepsin activity was significantly lower in the gastric juice of healthy swine than in that of swine with gastroenteritis.

On attempting to bind gastric acid by administering aluminium hydroxide gel through a sound, all the 10 experimental animals developed diarrhoea on the 3rd day. By this time gastric acidity had gradually declined until it disappeared because the gel had adsorbed the acids. Some days after the discontinuation of treatment on the 5th day the diarrhoea stopped, and the gastric hydrochloric acid and enzyme secretion gradually normalized. The same was found on treatment with magnesium oxide, which needs a dilute acid milieu to be dissolved. Experiments with the well-adsorbable sodium hydrocarbonate had to be interrupted on the 3rd day, owing to alkalosis.

Discussion

The present experiments have revealed that in the initial stage of gastroenteritis, simultaneously with the development of diarrhoea, free acid disappeared from the stomach and the gastric pH rose above 5.0. But in the advanced stage, gastric acidity reached an unusually low pH value in the range of 1.0. The question is, what relationship the rise of gastric pH above 5.0 and the disappearance of free acid may have with the rapid emptying of the stomach and the diarrhoea developing in the initial stage of the disease, and to what extent the high total gastric acid level and the organic acids may contribute to the development of gastric atonia and the consequent worsening of the condition.

It is known that in man, anacid stomach content is emptied more rapidly than the hyperacid one; hyperacidity being often characterized by severe retention. JÁVOR et al. (1965) have shown that in man the neutralization of acid solutions administered into the stomach brought about in 15 minutes a more than fivefold acceleration of the elimination of fluid. Though the experimental data obtained with human subjects do not necessarily apply to swine, it may well be supposed that the diarrhoea at the onset of the disease is a consequence of the rapid elimination of the nearly neutral stomach contents. In fact, lasting neutralization of the gastric acid led to diarrhoea in all of our experimental pigs. Experimenting on human subjects, several authors (SHAY and GERSON-COHEX, 1943; VAN LIERE and SLEETH, 1940; PATHAK, 1959; HUNT and PATHAK, 1960) have shown that the higher the acid content of a test meal, the lower its elimination. Obviously, the acid substances carried into the stomach pass sooner or later into the duodenum, where they effect a lowering of the pH and, consequently, reduce the rate of the passage through the pylorus. HUNT and KNOX (1962) have arrived at the conclusion that the passage is inhibited at a duodenal pH 6.0 or lower.

In swine, the rapid emptying of the stomach at the onset of gastroenteritis results in the passage of a large amount of unprepared food into the

duodenum. The gastric atonia developing with the progress of the disease is very likely a protective mechanism. Atonia is followed by fermentation of the retained food resulting in release of acetic acid and lactic acid, with a consequent downward shift of pH. Passage into the duodenum of the highly acid gastric juice further increases gastric atonia.

SUMMARY

Samples of gastric juice from diseased swine, 20 with gastroenteritis and 74 with arrival diarrhoea, as well as from carcases of 54 swine died of "infectious" gastroenteritis, were examined for total acid, free acid, and pepsin activity. In part of the swine with gastroenteritis also lactic acid and acetic acid were determined in the gastric juice. Experiments were carried out on 10 swine to clarify what effect the neutralization of gastric juice with acid-binding substances may have on the function of the gastrointestinal tract.

It was found that, parallel with the progress of diarrhoea, the free acid disappeared and the pH of the gastric juice rose above 5. With the progress of the disease the hydrochloric acid disappeared, pepsin activity stopped and, by fermentation, considerable amounts of acetic acid and lactic acid were produced until gastric acid pH had fallen to about 1.0. The authors believe that diarrhoea, setting on in the initial stage of the disease, is brought about by the rapid elimination of stomach contents, and interpret the ensuing gastric atonia as a protective mechanism to prevent the passage of unprepared stomach contents into the duodenum. Fermentation of the stomach contents with release of acetic-acid and lactic-acid fermentation, being a consequence of atonia, contributes to its maintenance and aggravation.

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GROUPING OF MUSCLES BASED ON CONNECTIVE TISSUE CONTENT IN CORRELATION WITH THE MEAT QUALITY

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The quantity and quality of the connective tissue in the muscles influence the meat quality significantly. Therefore, both morphological and chemical methods are employed in qualifying the connective tissue content. The morphological methods are principally based on staining techniques used in the histology, *i.e.*, in homogenized samples the connective tissue elements are demonstrated by a specific staining method, and their percentages are calculated. As for chemical methods, the hydroxyproline content of the connective tissue is determined.

Chemical analyses have suggested that the meat quality is influenced not only by the quantity but also by the quality of the connective tissue (Lőrincz and Szeredy, 1959).

The investigation methods used currently have been developed by numerous authors (Kotter et al., 1958; Kotter et al., 1963; Linke and Fleischmann, 1946). Determination of the residual collagenous tissue, resistant to trypsin, is accepted as the testing method for meat and meat products (Schönberg and Lochman, 1957).

Comparative studies on the connective tissue content of different muscles have been carried out by Ramsbottom (1945) and Steinhauf (1961). These authors found that among the muscles of one and the same animal the m. psoas major contained the least and finest collagenous fibres, being therefore the softest of all of the muscles.

By light- and electron-microscopy the inner structure of the sarcolemma was studied and related to the meat quality (Wang, 1965; Kono, 1961; McCollester, 1962; Lőrincz and Losonczy, 1966).

Another widely-used method is the instrumental measuring of the toughness or tenderness of the meat. Several apparatuses have been constructed for this purpose. These apparatuses imitate masticatory movements including tearing, chewing, lacerating and pressing actions. The best apparatus is Warner-Bratzler's shearer, developed in the U. S. A. in 1930, as modified by Hurwitz and Tischer (1954). Various chewing apparatuses (Volodkevich, 1938; Winkler, 1939) and electric grinders (Schoman et al., 1960) imitating masticatory movements are also used for the instrumental measuring of the toughness of meat. Recently, several papers on the employment of various apparatuses in ranking meats have been published (Ritchey, Hostetler, 1964; Bourne et al., 1966; Alsmeyer et al., 1966).

In this paper comparison is made between the kinds of meat based on the quantity of the endomysium and the internal perimysium and according to the structure of the connective tissue muscles of similar property are grouped. Grouping of this kind has been completed with data obtained by instrumental measuring of the toughness of meat.

Materials and methods

Sixteen muscle samples from both cattle and pigs were tested. Muscle samples were taken 72 hours after slaughtering from carcasses kept at +4 to +6 °C, then fixed in formalin, embedded in paraffin, cut and stained according to Mallory.

Table 1

Tested muscles of cattle and their commercial names

Muscles	Commercial name
M. iliopsoas	Fillet
M. gracilis	Topside
M. semimembranaceus	Silverside
M. semitendineus	
M. longissimus dorsi	Loin and Wing-end
M. intercostalis internus	Fore Ribs
M. latissimus dorsi	
M. rectus femoris	Thick Flank
M. biceps brachii	Bladebone
M. supra spinam	
M. infra spinam	Part of Top Ribs
M. triceps brachii	Shin and Shank
M. gastrocnemius	
M. fibularis tertius	
M. extensor carpi radialis	
M. extensor carpi ulnaris	

The samples were selected so that they should correspond to the different qualification types commonly used in the meat industry (Table 1).

On comparing the different histological pictures, the state of the muscle fibres was also taken into consideration. On evaluating the matter of the muscle fibres looseness or compactness and the size of the fibres were considered, too.

To determine the toughness of the meat, the modified Höppler's consistometer was used. A plate with 1 cm long edge was fitted to the device cutting 1 cm wide slices at right angle to the muscle fibres. The different units of the gradually increasing loads of weight were allowed to act on the meat for 30 second periods each. Turning moment values necessary for complete cutting were expressed in kg. The data are mean values calculated from 10 measurements.

Results

Based on the histological pictures the bovine muscle samples tested could be grouped in 4 types (Table 2).

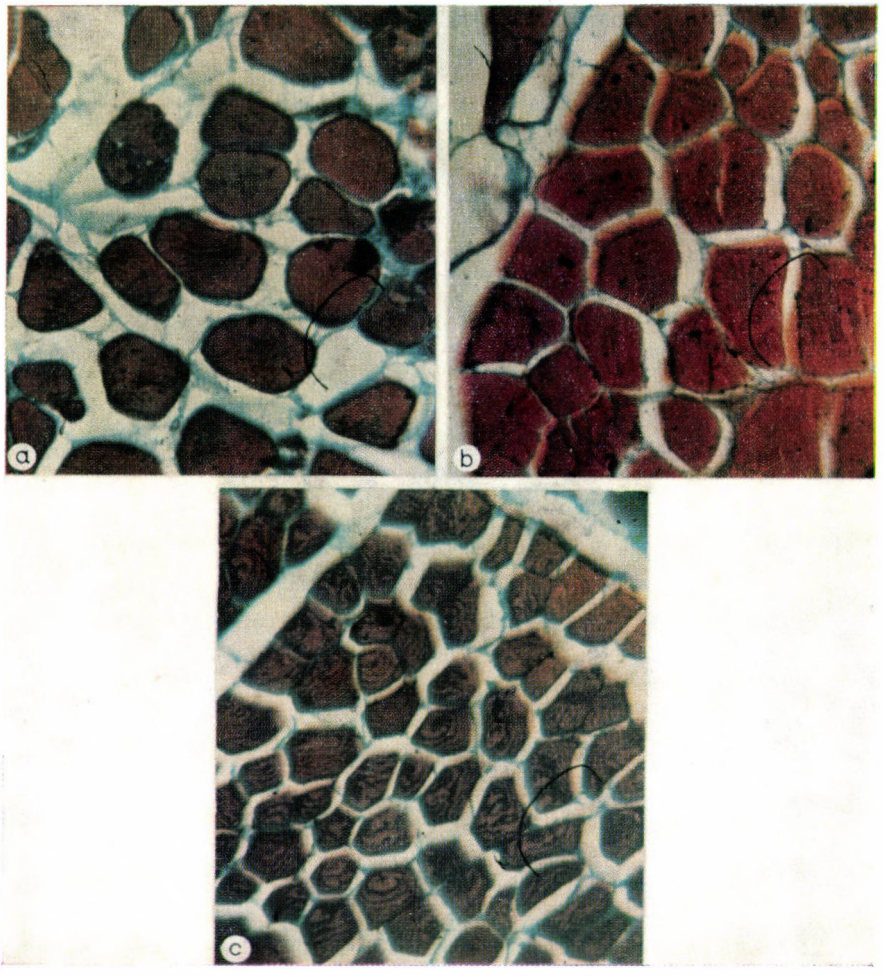

Fig. 3. Cross section of a porcine m. semimembranaceus (a), m. rectus femoris (b) and m. iliopsoas (c). Mallory staining. × 300

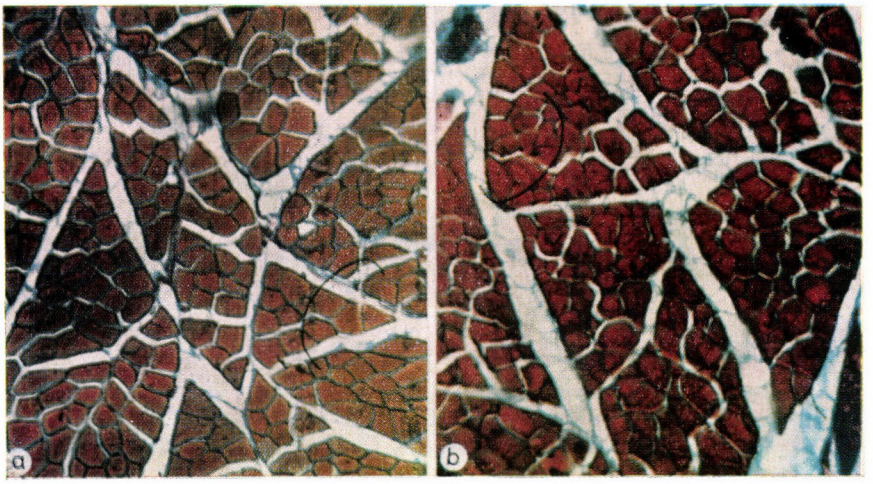

Fig. 4. Cross section of a porcine m. gastrocnemius (a) and m. extensor carpi ulnaris (b). Mallory staining. × 120

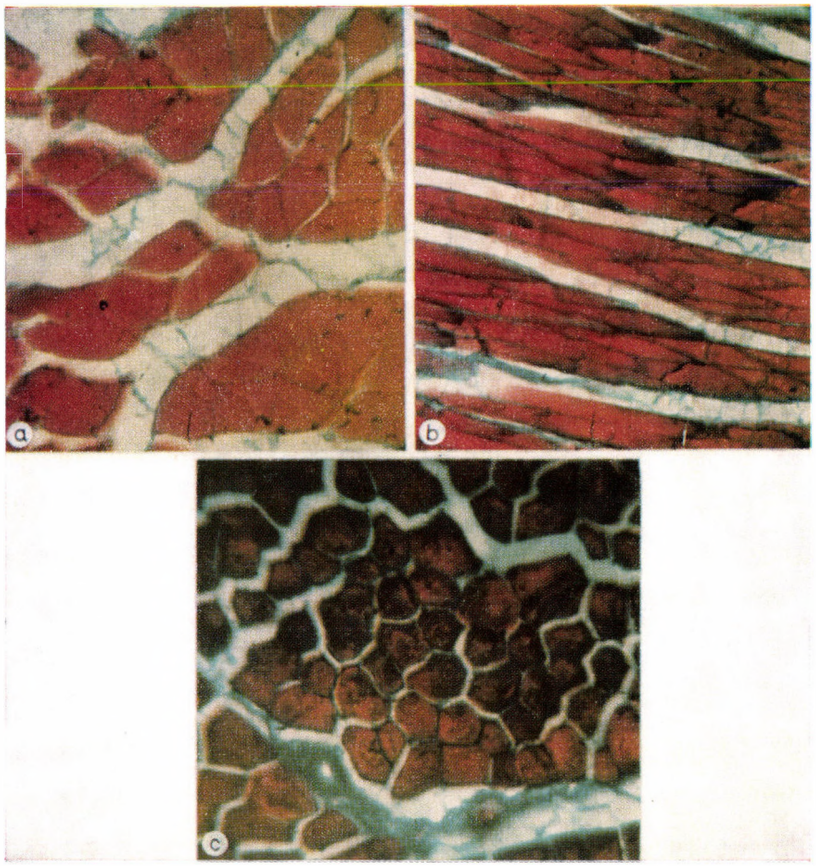

Fig. 1. Cross section of a bovine m. intercostalis internus (a), m. supra spinam (b) and m. iliopsoas (c). Mallory staining. × 300

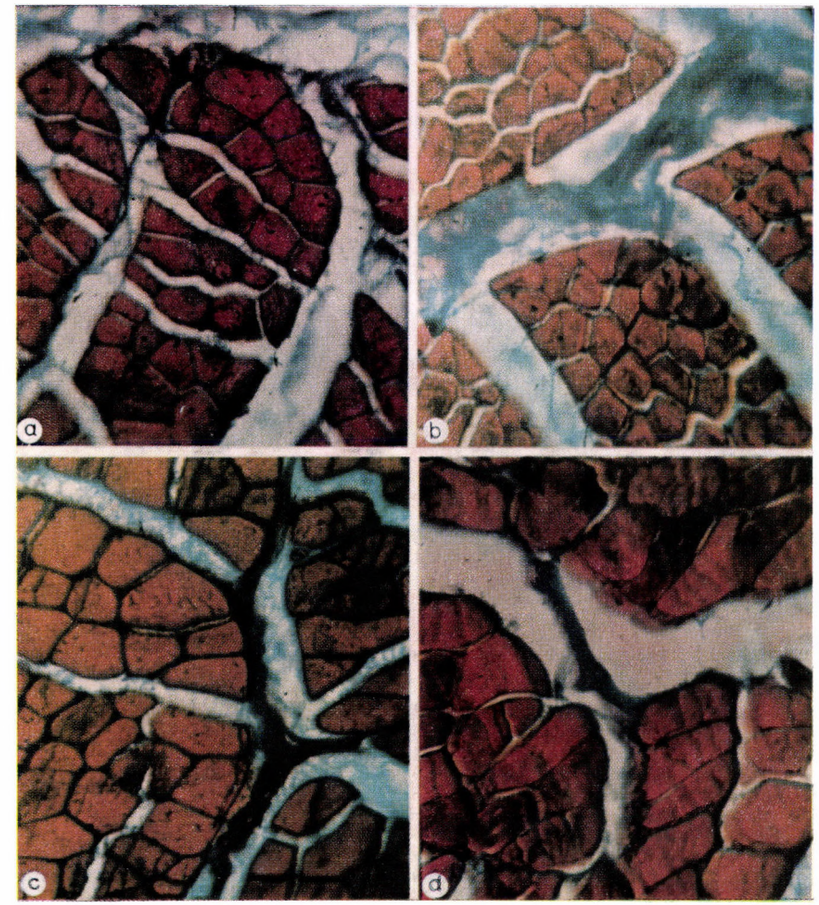

Fig. 2. Cross section of a bovine m. rectus femoris (a), m. biceps brachii (b), m. fibularis tertius (c) and m. extensor carpi radialis (d). Mallory staining. × 300

Table 2

Consistometric data for bovine muscles grouped according to their connective tissue component

Type A	Consistometric cutting values
M. semimembranaceus	5.4±0.6
M. semitendineus	5.8±0.4
M. supra spinam	
M. inercostalis internus	
Type B	
M. iliopsoas	3.6±0.3
M. longissimus dorsi	3.8±0.5
Type C	
M. rectus femoris	8.8±0.6
M. gastrocnemius	
M. biceps brachii	9.1±0.5
M. gracilis	
M. triceps brachii	
M. latissimus dorsi	
Type D	
M. fibularis tertius	9.2±0.5
M. extensor carpi radialis	9.6±0.4
M. extensor carpi ulnaris	

Muscles classified in group "A" are characterized by muscle fibres medium in size and easy to tear, and by little and fine endomysium as well as by a loose structure of the perimysium internum. The conclusion may be drawn that this histological picture indicates a good meat quality. (Fig. 1a, b).

In the muscles classified in group "B" thin muscle fibres close to one another, thus giving compact structure, can be found. The collagenous fibre network interlacing the muscle fibres is little and extremely fine. The muscle fasciculi are large, the perimysium internum is medium fine in structure and thin (Fig. 1c).

Based on the industrial qualification and the chemical analysis, due to the low level of the connective tissue nitrogen, the m. iliopsoas belonging to this group is considered the best quality.

In the muscles classified in group "C" a similar compact structure can be seen. The muscle fibres, however, are thicker than the respective formations of the former groups (Fig. 2a, b).

Table 3

Consistometric data for pig muscles grouped according to their connective tissue component

Type A	Consistometric cutting values
M. semimembranaceus	3.8±0.3
M. semitendineus	4.1±0.3
M. supra spinam	
M. infra spinam	
M. triceps brachii	
M. latissimus dorsi	
Type B	
M. iliopsoas	3.1±0.3
M. longissimus dorsi	2.8±0.4
M. rectus femoris	
M. gracilis	
M. intercostalis internus	
Type C	
M. biceps brachii	5.8±0.4
M. extensor carpi radialis	6.2±0.3
M. extensor carpi ulnaris	
M. gastrocnemius	
M. fibularis tertius	

The muscle fibres in group "D" are thicker, their fasciculi more compact and the perimysium internum thick and compact. From the connective tissue of group "C", which is thick but loose in structure, that of quality "D" differs mainly in being compact, even "rope-like" (Fig. 2c, d).

Each of 2 samples from the respective groups was measured consistometrically. Muscles of groups "A" and "B" showed significantly lower resistance against cutting than those of groups "C" and "D".

Obviously, groups "A" and "B" indicate higher quality as compared to groups "C" and "D".

When pig muscles were grouped according to similar principles, only 3 groups could be established (Table 3), for the connective tissue seen in the cross sections of pig muscles is less variable in structure than that of the bovine muscle. Consequently, from the aspect of meat qualification pig muscles are more uniform than the muscles of the cattle.

Muscles that belong to group "A" consist of loose muscle fibres surrounded by a fine connective tissue (perimysium internum) (Fig. 3a).

In addition to m. iliopsoas and m. longissimus dorsi 3 kinds of muscle can be classified in group "B". The fibre bundles of these muscles are much less compact than those of the group "B" bovine muscles and the amount of the connective tisssue is markedly less in them. The collagenous fibre network is almost lacking (Fig. 3b, c).

Porcine muscles classified into group "C" correspond to the group "C" bovine muscles. Connective tissue septa as tick as common in the bovine group "D" muscles are never found in pig muscles.

Two samples of each of the representative groups for pig muscles were measured consistometrically. Cutting resistance is more uniform than in case of the bovine muscles, whereas muscles of group "C" are significantly tougher than the other muscles.

It can be concluded that, based on the histological picture, several quality groups can be established from the different muscles. These groups differ from one another in the cutting resistance, too.

In their industrial names Fillet, Silverside and Loin and Wing-end are, as based on their histological features, superior to the others.

SUMMARY

Based on the properties of the interfibrillar connective tissue and the muscle fibres, muscles can be grouped. Of the 16 different muscle samples tested 4 and 3 groups of cattle and pig muscles, respectively, could be established. Cutting resistance values measured by the modified Höppler's consistometer also revealed quality differences among the muscles grouped in the different types.

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INVESTIGATIONS ON THE INCIDENCE OF MYCOPLASMA IN SWINE AND THEIR SIGNIFICANCE IN ENZOOTIC PNEUMONIA AND ATROPHIC RHINITIS

By

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There has been extensive review on the incidence of mycoplasma in pigs and on the aetiological relations of mycoplasma in enzootic pneumonia (EPS) as well as in atrophic rhinitis (AR) (DINTER, 1964; L'ECUYER et al. 1961; GOODWIN et al., 1965; HARTWICH and MÜLLER, 1966; HARTWICH and NIGGERLSCHULZE, 1966; HARTWICH, 1965; KLEY and MAYR, 1965; MARÉ and SWITZER, 1965; MONREAL et al., 1964; SCHIMMEL and HUBRIG, 1965; SWITZER, 1963).

So far 8 *Mycoplasma spp.* have been described. *Mycoplasma hyorhinitis* Switzer, 1955 and the so-called SEP-Agent (BAKOS and DINTER, 1963) are presently considered identical (DINTER et al., 1965). It is known that *M. granularum* (SWITZER, 1962) and *M. hyocarthrinosa* (SWITZER, 1963) are involved in the aetiology of chronic joint inflammations. The role of the "B-strains" (DINTER et al., 1965) is still in doubt. *M. hyogenitalium* produces a clinical syndrome characterized by the metritis-mastitis syndrome (MOORE et al., 1966). In recent years 2 other *Mycoplasma* species have been described: *M. hyopneumoniae* (MARÉ and SWITZER, 1965) and *M. suipneumoniae* (GOODWIN et al., 1965) which supposedly cause pneumonia like that seen in EPS.

In Germany, MONREAL et al. (1964) detected *Mycoplasma spp.* in 53 of 80 pigs affected with EPS or AR by cultivation in chicken embryos. SCHIMMEL and HUBRIG (1965) isolated mycoplasma from 64% of 236 atrophical nasal conchae and from 32% of 254 unchanged nasal conchae; KLEY and MAYR (1965) detected mycoplasma in 12 of 94 cases by nasal swab tests and in 17 of 22 cases in lung tests.

Our studies were conducted on a total of 179 young pigs. Lungs and nasal mucosa, and in some cases other organs, were examined bacteriologically and particularly for mycoplasma. All organs were examined histologically.

Materials and methods

Group 1: 106 young pigs from 10 herds which were affected with EPS and/or RA.

Group 2: 59 young pigs from 20 herds which had exhibited no clinical symptoms or signs of these diseases for approximately 4 years.

Group 3: 14 young pigs removed by Caesarean section and raised in isolation (SPF).

Hen eggs, which had been incubated at 37 °C for 5 to 7 days were used for cultivating mycoplasma. The inoculum was prepared from parts of organs

homogenized in phosphate-buffered (0.02 *M*, pH 7.6) saline. One thousand units of penicillin and 500 μg of streptomycin were then added. Material from each organ was inoculated into 5 chicken embryos at 0.2 ml *via* the yolk sac. After the inoculation, incubation of the chicken embryos at 37 °C was immediately continued. The embryos which died 1 to 2 days after the injection were discarded. Those which died subsequently and those surviving 10 days after injection were harvested under sterile conditions. The material was subsequently transferred to PPLO-plates which consisted of Bacto PPLO Agar (Difco)*, 0.1% glucose and 0.5% Bacto Yeast Extract (Difco). After sterilization 20% of horse serum was added to the medium. Incubation at 37 °C was maintained for at least 48 hours.

A relative humidity of approximately 85% and a CO_2 concentration of 20% enhanced the growth of mycoplasma colonies. As a rule, isolation of mycoplasma was achieved only by using staphylococci as feeder organisms.

The bacteriological examination was conducted using a combination of nutrient agar, sheep-blood agar and heated (80 °C, 5 min) sheep-blood agar, the latter with staphylococci to support the growth of *Haemophilus suis*.

At autopsy lungs, nasal mucosa, liver, kidneys, spleen, brain and musculature were examined. Lung samples were obtained from the cardiac, apical and diaphragmatic lobes. The organs were fixed in 10% formaldehyde. Paraffin sections were stained with haematoxylin-eosin.

Results

Incidence of infection

Group 1 (pigs with EP and RA): *Mycoplasma spp.* were detected in 74 (70%) young pigs (Table 1); 32 pigs (30%) did not yield mycoplasma.

Group 2 (without clinical symptoms): Forty-one of 59 animals (69%) were infected with *Mycoplasma spp.*, 18 pigs (31%) were negative (Table 1).

Group 3 (specific pathogen-free pigs): In one of the 14 SPF pigs examined, *Mycoplasma spp.* were detected (Table 1).

All of the isolated mycoplasma strains had essentially the following growth-properties: They could not be detected on solid medium, when inoculated directly from organs. They first required an egg passage for multiplication and adaptation. Then they acquired the ability to form colonies on our PPLO agar in the vicinity of staphylococcus colonies. The mycoplasma colonies were comparable in form, structure and size to those described by Bakos and Dinter (1963) and Switzer (1955).

* Difco Laboratories, Detroit 1, Michigan, USA.

Table 1

Results of *Mycoplasma spp.* isolation attempts

Group	Number of pigs	Lungs	Nasal mucosa	Spleen	Kidneys	Liver
I	26/74	+	+	○	○	○
	22/74	+	−	○	○	○
	7/74	−	+	○	○	○
	12/74	+	○	○	○	○
	2/74	○	+	○	○	○
	3/74	+	+	+	+	+
	1/74	+	−	−	−	−
	1/74	−	−	−	−	−
II	31/41	+	+	○	○	○
	5/41	+	+	+	+	+
	2/41	+	+	−	+	−
	1/41	+	+	+	+	−
	1/41	+	+	−	−	−
	1/41	−	−	−	+	−
III	1/14	−	+	−	−	+

+, *Mycoplasma spp.* were detected; −, *Mycoplasma* were not detected; ○, organs were not examined.

The following bacteria were isolated from lungs and nasal mucous membranes: *Group 1:* α-haemolytic streptococci in 77%, *Escherichia coli* in 75%, *Micrococcus spp.* in 67%, *Bacillus spp.* in 13%, *Haemophilus suis* in 5%, each of *Bordetella bronchiseptica* and *Proteus vulgaris* in 3%, each of *Klebsiella spp.* and *Pasteurella multocida* in 2%, and each of β-haemolytic *Streptococci*, *Streptomyces spp.*, *Corynebacterium spp.* and *Pseudomonas spp.* in 1% of the samples. *Group 2: Micrococcus spp.* in 70%, α-haemolytic *Streptococci* in 64%, *Escherichia coli* in 46%, *Bacillus spp.* in 4%, each of *Streptomyces spp.*, *Corynebacterium spp.*, *Haemophilus suis* and *Bordetella bronchiseptica* in 1% of the samples. *Group 3: Micrococcus spp.* in 70%, *Escherichia coli* in 21%, each of *Bacillus spp.* and α-haemolytic *Streptococci* in 20% and *Staphylococcus spp.* in 7% of the samples.

Pathological findings

Group 1 (pigs with (EPS and/or RA): Pulmonal changes like those occurring in EPS were observed in 65% of the cases. The lesions usually could be recognized distinctly in the form of catarrhal or catarrhal-purulent bron-

chitis or bronchopneumonia with formation of peribronchial cell accumulations and/or hyperplasia of the lymph follicles. No detectable lung lesions were found in 35% of the pigs. Rhinitis was shown in 50%, in an equal number of cases of atrophic rhinitis and rhinitis without lesions of the conchae. Done's inclusion bodies were found in 25% of cases. With few exceptions, rhinitis occurred parallel with inflammatory changes in the lungs.

Table 2

Isolation of *Mycoplasma spp.* from swine lungs

	Cases with inflammatory lung changes	Cases without particular changes	Isolation of *Mycoplasma spp.* from the lungs	
			Cases with inflammatory changes of the lung	Cases without inflammatory changes of the lung
Group 1	65%	35%	76%	35%
Group 2	48%	52%	53%	61%
Group 3	21%	79%	—	—

Table 3

Isolation of *Mycoplasma spp.* from swine nasal mucosa

	Atrophic rhinitis	Rhinitis without atrophy of the conchae	No inflammatory changes	Detection of *Mycoplasma spp.* on the nasal mucosa		
				Atrophic rhinitis	Rhinitis without atrophy	No inflammatory changes
Group 1	25%	25%	50%	50%	59%	25%
Group 2	—	33%	67%	—	—	52%
Group 3	—	—	100%	—	—	7%

Group 2 (pigs without clinical symptoms): In contrast to Group 1, the lungs in this group showed, as a rule, no gross lesions. However, 48% of the pigs had peribronchial cell accumulations, hyperplasia of the lymph follicles, widening of the alveolar septa and in some instances changes due to a light inflammatory exudate. In 52% the lungs were free of pathological processes or showed only isolated interstitial cell proliferation. The latter could not be directly associated with EPS. Rhinitis without atrophy of the conchae was found in one third of all cases. The majority of these cases was observed in young pigs with inflammatory processes in the lungs. Done's inclusion bodies were detected in only one case.

Group 3 (SPF pigs): Slight inflammatory changes were found in the lungs of 3 of the 14 SPF pigs. In one case the lesions were macroscopic. Rhinitis did not occur.

The incidence of mycoplasma in the lungs or nasal mucosa — with or without inflammatory processes — is given in Tables 2 and 3.

Discussion

Mycoplasma infection is of high incidence in swine herds in Germany. Approximately 70% of the pigs in herds with EPS had mycoplasma (Group 1), this figure being 8% higher than that described by BAKOS and DINTER (1963) for herds in Sweden. A similarly high incidence of mycoplasma infection was found in clinically healthy herds (Group 2), where mycoplasma could be isolated from about 69% of the pigs. Mycoplasma was isolated even from 1 of 14 SPF pigs.

Initially we examined only lungs and nasal mucosa; subsequently other organs were also examined and it was found that mycoplasmas can also be detected in other organs.

Comparing the histological findings with the mycoplasma isolations, one may come to the following conclusions. In the group of clinically healthy pigs inflammatory lung changes could be shown histologically. However, it must be emphasized that these processes were less pronounced both qualitatively and quantitatively than those observed in Group 1. Slight inflammation was also found in the lungs of some SPF pigs. There was no connection between the pulmonal lesions and mycoplasma detection in the lungs.

Although mycoplasmas were demonstrated more frequently in lungs with more severe inflammatory changes than in lungs with a lesser degree of inflammation, they nevertheless occurred in 61% of the lungs in Group 2, which showed no inflammatory reaction. No connection could be found between the detection of mycoplasma and the presence or absence of rhinitis.

From these findings we cannot conclude on a direct aetiological relationship between *Mycoplasma spp.* isolated in chicken embryos and the pathological changes.

As far as histological findings are concerned, correlation exists between the severity of bronchopneumonia and the extent of the changes of the nasal mucosa. It can therefore be concluded that the likelihood of inflammatory sinus changes will become greater with increasing severity of the pulmonal changes.

It is difficult to account for the mild pneumonia in some of the SPF pigs. They could have been caused by disinfectants (peracetic acid) which were inhaled into the lungs.

The possibility of a virus as aetiological factor still remains worth to be considered. Virological examinations were not conducted by us.

We agree with MARÉ and SWITZER (1965, 1966a, b, c) and GOODWIN (1965) that mycoplasma might be involved in the aetiology of EPS. This apparently does not hold true for mycoplasma strains which can be cultivated in the embryonated hen egg.

SUMMARY

Studies on young pigs in various herds in Germany have revealed that *Mycoplasma spp.* are widely distributed. Mycoplasmas were isolated in chicken embryos. The incidence of mycoplasma infections in herds with enzootic pneumonia of pigs (EPS) and/or atrophic rhinitis (AR) was similar to that in herds without these diseases, namely, 70% and 69%, respectively. *Mycoplasma spp.* were also isolated from one of 14 specific pathogen-free (SPF) pigs.

Morphological examinations revealed inflammatory processes in the lungs or nasal mucosa of animals from clinically healthy herds as well. However, these changes were significantly less pronounced in these herds than in those with sick pigs. Light pneumonia was observed even in some SPF pigs. It is concluded that the *Mycoplasma spp.* isolated by us in chicken embryos have no particular significance in the aetiology of EPS and AR.

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EXPERIMENTAL INFECTION OF PIGS WITH MYCOPLASMA STRAINS

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In recent years, extensive studies have been conducted to determine the significance of mycoplasma in the aetiology of EPS (GOODWIN et al., 1965; MARÉ and SWITZER, 1965; 1966a, b, c). However, there is still uncertainty about the pathogenicity of those *Mycoplasma spp.* which are cultivable in the chicken embryo. In an effort to obtain further information on this question we infected 60 pigs with 3 mycoplasma isolates.

Material and methods

1. Strain "E 9" (HARTWICH et al., 1967) was isolated in chicken embryos from the affected lung of a pig with typical manifestations of EPS. With this strain, 20 mycoplasma-free and 2 specific pathogen-free (SPF) pigs were infected. Thirteen mycoplasma-free and 2 SPF pigs were used as control.

2. Strain "F 121" (SCHEER et al., 1968) was similarly isolated in chicken embryos from the lung of a pig with EPS and atrophic rhinitis (AR). With "F 121" 17 mycoplasma-free and 2 SPF pigs were infected. Ten mycoplasma-free and 2 SPF pigs served as control.

3. The strain "SEP-Agent" (SCHEER et al., 1967) was isolated by Dr. BAKOS in Stockholm in chicken embryos from the lung of a pig with EPS. With the "SEP-Agent" 19 mycoplasma-free pigs were infected. Ten pigs served as control.

The mycoplasma-free pigs were either raised by us or bought from a nearby herd which had been found free of *Mycoplasma spp.* in repeated examinations. The animals of this herd showed no clinical or pathological manifestation of EPS or AR. All animals were weaned shortly after birth. At the age of 1 to 8 days they were infected. The inoculum consisted either of a mixture of egg yolk and amnio-allantoic fluid (AAF) containing Mycoplasma or of Mycoplasma in Bacto PPLO broth (Difco)* with 0.1% glucose, 0.5% yeast extract (Difco)* and 20% horse serum. The mycoplasma count in both inocula was determined (SCHEER et al., 1967) and bacteriological sterility tests were conducted on sheep-blood agar.

* Difco Laboratories, Detroit 1, Michigan, USA.

The colonies of mycoplasma were comparable in form, structure and size with those described by BAKOS and DINTER (1963) and SWITZER (1955).

The intranasal route of exposure was chosen since an aerosol spread of EPS is presumed. Three ml per pig were applied. In order to further determine whether or not there is an increase in pathogenicity after direct pig-to-pig passage of the strain, mycoplasma-free pigs were placed together with siblings which had been infected by intranasal exposure. We also infected some pigs intraperitoneally (i. p.), since by inoculation of pigs under 6 weeks of age SWITZER (1955) and ROBERTS et al. (1966a, b) could regularly produce fibrinous pericarditis, pleuritis, peritonitis and occasionally polyarthritis. In control experiments pigs were injected i. p. with the respective volumes of sterile yolk or PPLO broth. The controls were siblings which were kept separately but under otherwise similar conditions. Approximately 40 days after exposure, the pigs were killed and dissected. Nasal mucosa, lung (cardiac, cranial and main lobes), liver, kidney, spleen, brain, joints, synovial fluids, musculature, and testicles were examined bacteriologically under particular consideration of Mycoplasma and examined histologically. For bacteriological examination sheep-blood agar and heated (80 °C, 5 minutes) sheep-blood agar were used, the latter with staphylococci to support the growth of *Haemophilus suis*.

Histological examinations were conducted on lung, nasal mucosa, liver, kidney, spleen, brain, and joints. The organs were fixed in a 10% formaldehyde solution. Paraffin sections were stained with haematoxylin-eosin.

Results

1. Strain "E 9"

By intranasal exposure of 16 pigs to this strain, no clinically apparent EPS could be produced (Table 1). In 2 pigs fibrous pleuritis and pericarditis without pathological changes in the lung were noted. The histological examination revealed that in one of these 2 pigs a small fibrous purulent area was present in the left main lobe of the lung directly below the inflamed pleura.

In the group of pigs exposed by contact there were no histological changes in the lung. In the group of pigs exposed by i. p. infection, serositis (pleuritis, pericarditis, peritonitis) was noted in all 4 pigs (Table 2). Three of these animals had marked manifestations of polyarthritis. Only one of these 4 animals had pneumonia in the main lobes (catarrhal-purulent bronchopneumonia). In the lungs, liver, kidneys and spleen of this animal *Escherichia coli* and β-haemolytic streptococci were present in great numbers.

Table 1

Intranasal infection of young pigs with *Mycoplasma spp.*

Strain	Number of infected pigs	Age at infection (days)	Inoculum, quantity, organisms per ml	Duration of experiment (days)	Pathologic findings	
					Macroscopic	Microscopic
"E 9"	14	1—8	2 ml of PPLO broth or egg yolk/AAF 3.5×10^4 to 4.8×10^8	17—48	negative	negative
	1	1	2 ml of PPLO broth 3.5×10^4	45	Fibrous pleuritis	Fibrous pleuritis
	1	5	2 ml of egg yolk/AAF 4.8×10^8	35	Fibrous pleuritis	Local fibrous purulent pneumonia
"F 121"	11	3—8	2—3 ml of PPLO broth 5.9×10^7 to 5.2×10^8	21—35	negative	negative
	1	4	2 ml of PPLO broth 9.7×10^7	39	negative	Mild local peribronchitis
	1	3	2 ml of PPLO broth 5.2×10^8	45	negative	A few bronchi with peribronchitis
"SEP-"Agent"	9	2—5	2—3 ml of PPLO broth 3.7×10^7 to 6.1×10^8	38—45	negative	negative
	2	2	2 ml of PPLO broth 1.3×10^8	44	Local pleuritis, Pneumonia, atelectasis	Bronchitis Pneumonia

2. Strain "F 121"

The 13 pigs infected intranasally with this strain (Table 1) developed neither clinical manifestations nor pathological changes characteristic of EPS. Whether or not the mild peribronchitis which was demonstrated histologically in 2 pigs resulted from the intranasal infection, cannot be said with certainty. Of the 6 pigs infected i. p. with "F 121", only 2 remained healthy

Table 2

Intraperitoneal inoculation of young pigs with *Mycoplasma spp.*

Strain	Number of infected pigs	Age at infection (days)	Inoculum, quantity, organisms per ml	Duration of experiment (days)	Pathological findings	
					Macroscopic	Microscopic
"E 9"	2	5	1 ml of egg yolk/AAF 4.8×10^8	29 and 32	Serositis Polyarthritis	Serositis Arthritis
	1	2	1 ml of PPLO broth 3.4×10^4	8*	Serositis Local atelectasis in main lobe	Serositis Catarrhal-purulent pneumonia
	1	2	1 ml of PPLO broth 3.4×10^4	40	Serositis Polyarthritis	Serositis; Arthritis
"F 121"	2	3	1 ml of PPLO broth 5.2×10^8	53	negative	negative
	2	4—8	2 ml of PPLO broth 8.3×10^7	43 and 51	Serositis	Serositis
	1	4	1 ml of PPLO broth 8.3×10^7	43	Serositis Pneumonia in main lobe	Serositis Catarrhal purulent pneumonia
	1	8	1 ml of PPLO broth 9.7×10^7	51	Serositis, Pneumonia in apical lobe	Serositis Peribronchitis, Catarrhal pneumonia
"SEP-Agent"	3	3—5	1—1.5 ml of PPLO broth 2.1×10^8 6.1×10^8 to	38 and 40	negative	negative
	1	2	1 ml of PPLO broth 1.3×10^8	19	Serositis Polyarthritis	Serositis Arthritis

* Killed because of septicemia

and free of significant pathological change (Table 2). The other 4 pigs had subacute to chronic inflammatory changes in the serous membranes (peritoneum, pleura). In 2 pigs, small circumscribed inflammatory lesions were observed in the lungs. In these lesions *Escherichia coli* and β-haemolytic streptococci were present in great numbers. Arthritis was not observed.

3. *Strain "SEP-Agent"*

After intranasal infection, none of the pigs developed clinical signs of EPS (Table 1). Upon post mortem examination of the 11 pigs, 2 had pleuritis with adhesions, pericarditis, and circumscribed lesions in the cardiac and main lobes of the lung. Histological examination of these areas revealed varying degrees of purulent bronchopneumonia. Peribronchitis and enlargement of peribronchial lymph nodes were apparent. From the lungs and from the other organs β-haemolytic streptococci and *Escherichia coli* were cultured.

In the 4 pigs exposed to infection by contact, neither clinical nor pathological manifestations of EPS were observed. The 4 pigs infected i. p. (Table 2) also showed no pathological changes in the lungs, but in one of these pigs peritonitis and polyarthritis were noticed.

Mycoplasma was re-isolated from all of the animals inoculated with, or exposed by contact to, either of the 3 strains. The order of Mycoplasma isolation efficiency was brain, nasal mucosa, musculature, spleen, liver, kidney, lung, joint and testicle. These isolations were made even though there were no significant pathological changes in the respective organs. The only connection between Mycoplasma and disease was established in the pigs with polyarthritis.

None of the control pigs developed any significant clinical or pathological manifestation of EPS and yielded neither Mycoplasma nor pathogenic bacteria.

Discussion

MARÉ and SWITZER (1965; 1966a, b, c) reported that *Mycoplasma spp.* could take part in the pathogenesis of EPS. Their Mycoplasma isolates, however, contrary to ours, were not cultured and could not be adapted to chicken embryos.

Experimental infections with our isolates clearly indicated no aetiological significance of Mycoplasma in EPS. Whether or not the slight pathological changes in the lungs which developed after intranasal exposure of the pigs were actually caused by the Mycoplasma remains unanswered. Considering the bacteriological findings, these slight changes might have resulted from some

sort of syngergism between Mycoplasma and bacteria. It is, however, not possible to deny that the 3 tested Mycoplasma strains were not pathogenic. Particularly, after i. p. infection, there was a marked degree of inflammation in the serous membranes.

For the sake of completeness, it should be mentioned that the pigs exposed to infection by contact remained healthy, even though the Mycoplasma could be re-isolated from them.

SUMMARY

Pigs were experimentally infected with 3 Mycoplasma strains which had been isolated in chicken embryos from pigs with enzootic pneumonia of swine (EPS). The strains caused neither primary pneumonia nor rhinitis. However, they frequently caused serositis and/or arthritis after intraperitoneal injection. This indicates a similarity between the strains and *Mycoplasma hyorhinitis*.

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MORPHOLOGY AND DEVELOPMENT OF THWAITIA KOTLANI SP. N. (PHILOMETRIDAE, NEMATODA)

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The occurrence and development in home freshwater fishes of some roundworm species belonging to the family *Philometridae* have been described in previous papers (MOLNÁR, 1966a, b; 1967). In accordance with the earlier nomenclature, the above species have been uniformly classified as belonging to the genus *Philometra* Costa, 1845, which is still preferred by the majority of authors to the generic name *Thwaitia* proposed in RASHEED's revision (1963).

Since in the present work RASHEED's classification is followed, it should be mentioned that the previously described species *Philometra ovata* (Zeder, 1803), *Ph. rischta* Skrjabin, 1917, and *Ph. abdominalis* Nybelin, 1928, should be further on classified into the genus *Thwaitia* Rasheed, 1963, whereas *Ph. sanguinea* (Rud., 1819) into the genus *Philometroides* Yamaguti, 1935, and accordingly will be heretofore referred to as *Thwaitia ovata* (Zeder, 1803), *T. rischta* (Skrjabin, 1917), *T. abdominalis* (Nybelin, 1928) and *Philometroides sanguinea* (Rud., 1819), respectively.

In the course of faunistic studies, under the serosa of the air bladder of *Aspius aspius*, immature male and female roundworms, 2—2.5 mm long, have often been found. These parasites bore a striking resemblance to the developmental stages of *Thwaitia rischta*, but the males available for measurement differed from it in dimensions and neither developing, nor adult females were encountered.

Considering that as a predatory fish, *Aspius aspius* feeds mainly on *Alburnus alburnus* which is often harbouring *T. rischta*, it was supposed that the parasites in question were either *T. rischta* worms, whose development had come to a halt in the nonspecific host, or represented a new, hitherto undescribed species.

To clarify this problem, 41 *Aspius aspius* fishes were autopsied. Out of them 5 harboured either mature or immature females in their abdominal cavities. The morphological examination of the parasites as well as the studies of the course of their developmental cycle have evidenced that the *Thwaitia* species parasitic in *Aspius aspius* is not identical with *Th. rischta*, but actually represents a new species, the description of which is given below.

Habitat

The majority of the *Aspius aspius* fishes examined originated from the Lake Balaton and the river Tisza, whereas the minority from the river Duna

(Danube). The lengths of the fishes were between 280 mm and 570 mm, which corresponds to 2—6 summers. No correlation was found between the size of the body and the intensity of the infection.

Method

The parasites recovered either from the abdominal cavity or from the subserosa of the air bladder were placed in saline, where they remained viable and retained their colour for 2—3 days, depending on the temperature. Part of the examinations were carried out on living worms, part on lactophenol preparations fixed either in ethanol or in formalin.

Description

The only fully developed female found in our material was 61 mm in length, 1 mm in diameter, and of red colour. The body cavity was filled out by the uterus, full of larvae. The head seemed rounded off, with 4 in-

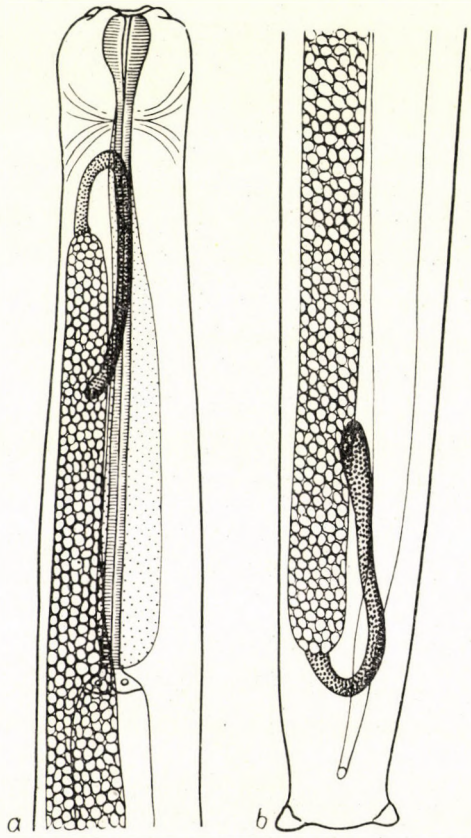

Fig. 1. a, Anterior end of a *T. kotlani* female, 20 mm long; *b*, posterior end of a *T. kotlani* female, 20 mm long

conspicuous papillae and three lips. The body was cylindrical in shape, very slightly tapering towards the anterior and posterior ends. The caudal end was rounded off, showing in dorsoventral position two small papillae-like processes on both sides. The length of the fixed larvae was 0.44—0.46 mm. Their anterior end appeared rounded off, whereas their posterior end tapering.

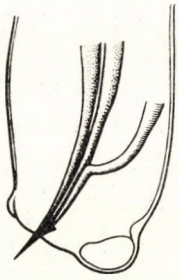

Fig. 2. Posterior end of a *T. kotlani* male

Table 1

Comparative data of *T. kotlani* and *T. rischta*

Measurements	*T. kotlani*	*T. rischta*
♀♀ length	61 mm	30—32 mm
diameter	1 mm	0.8— 1 mm
♂♂ length	2.1—2.6 (2.3) mm	1.7—2.0 (1.8) mm
diameter	0.041—0.062 (0.045) mm	0.022—0.039 (0.036) mm
spiculum I, length	0.090—0.108 (0.100) mm	0.045—0.057 (0.051) mm
spiculum II, length	0.082—0.098 (0.092) mm	0.045—0.053 (0.050) mm
diameter of spicula at base	0.005 mm	0.003 mm
diameter of spicula, median	0.004 mm	—
length of gubernaculum	0.053—0.066 (0.060) mm	0.031—0.041 mm

The immature 20—30 mm long females showed papillae-like processes on the caudal end. The anterior papillae were more distinct and conspicuous than those seen in older females (Figs 1a and b).

The structure of the internal organs (MOLNÁR, 1964a) was in every respect similar to that of *T. ovata* (Zeder, 1803).

The transparent males were 2.1—2.6 mm (av. 2.3 mm) in length and 0.041—0.062 mm (av. 0.045 mm) in diameter. The head was rounded off, without papillae. The body was slightly tapering toward both ends. The caudal end was blunt, with two lateral papillae-like protrusions, connected dorsally by a narrow inconspicuous membrane. The two narrow, relatively short

spicula were nearly equal and governed by a gubernaculum. The gubernaculum was represented by a bent chitinous plate, with a spear-like end. At about its middle, the gubernaculum bent dorsally, then dorso-cranially (Fig. 2). The body dimensions of males and females, as well as the measurements of the male chitinous organs are compared with the corresponding dimensions of *Thwaitia rischta* in Table 1.

Host—parasite relationship

As shown in Table 2, despite the relatively high intensity of infestation, migration of the females from the air bladder to the abdominal cavity took place in only few *Aspius aspius* fishes and less than half of the parasites had

Fig. 3. Cross section of a *T. kotlani* female, encapsulated by connective tissue, but freely moving in the abdominal cavity. Note the uterus, oesophagus and ovary in the body cavity (H. E. stain)

reached full maturation. The mature females moved freely in the host's abdominal cavity; their body surface was smooth and their colour was vivid red. The development of the other females seemed to be suppressed, presumably by the resistance of the host. The body of the retarded specimens was encased partly or entirely, by connective tissue (Figs 3 and 4). Their colour was either white or pale pink, their movements were limited; some were even unable to change place as their connective tissue capsule was adherent to the surface of intestines or abdominal organs. Part of the encapsulated 10—30 mm long females deteriorated and the only indication of their

Table 2

Infection of *Aspius aspius* fishes with various developmental stages of *Thwaitia kotlani*

Habitat	Infection			♂♂			Mature or developing ♀♀			Retarded ♀♀ bladder		in air	Retarded ♀♀ in abdominal cavity			Deteriorated ♀♀ encapsulated in abdominal cavity		
	Number of fishes examined	Number of infected fishes	Per cent infected	Infected hosts		Intensity of infection	Infected hosts		Intensity of infection	Infected hosts		Intensity of infection	Infected hosts		Intensity of infection	Infected hosts		Intensity of infection
				No.	%		No.	%		No.	%		No.	%		No.	%	
Balaton	23	18		16			4			10			3			4		
			78.3		69.5			17.4			43.4			13			17.4	
						1—20			1—3			1—7			1—3			1—8
Tisza	12	8		7			1			5						1		
			66.6		58.3			8.3			41.6		—	—	—		8.3	
						1—8			1			1—5						1
Duna (Danube)	6	3		2						2								
			50		33.3		—	—	—		33.3		—	—	—	—	—	—
						2—6						1—2						
Total	41	29		25			5			17			3			5		
			70.7		61			12.2			41.5			7.3			12.2	
						1—20			1—3			1—7			1.3			1—8

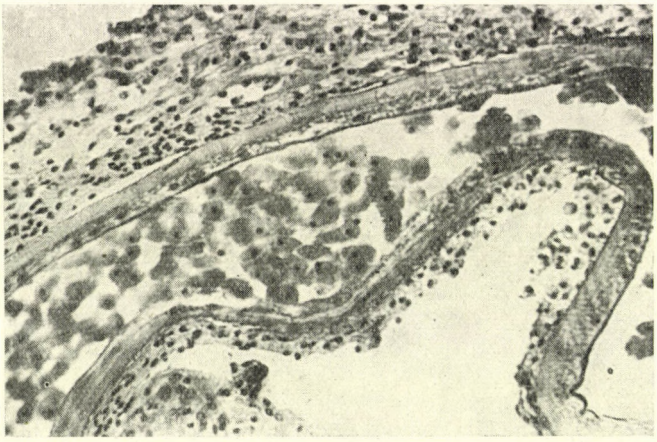

Fig. 4. Longitudinal section through part of the body of a freely moving, encapsulated *T. kotlani* female. The body cavity is full of immature eggs. (H. E. stain)

Fig. 5. Cross section of an encapsulated, nonviable *T. kotlani* female (Farkas—Mallory's stain)

Thwaitia nature was the dried, shrunken cuticle (Fig. 5). Another part of the encapsulated worms reached maturity but were unable to release their larvae. Deteriorated, encapsulated females full of larvae were still found in the host's abdominal cavity in June.

In the majority of the cases not even the above outlined incomplete development took place; like the males, the retarded females were retained in the air bladder all the year round; they remained viable but did not grow larger than the males, having been incapable of further development.

Developmental cycle

Since only a few of the examined parasites were normally developed, the life cycle could be followed up only in rough outlines. Anyway, it seems fairly certain that like the other *Thwaitia* parasites known in Hungary, also *T. kotlani* has a regular one-year developmental cycle. Mature females full of larvae have been found exclusively in May. The length of such females is approx. 60 mm. The larvae swarmed into the water — presumably after release of the females — continue their development in cyclopses. The *Aspius aspius* fishes become infected during the summer, supposedly by eating the infected cyclopses discharged from the digestive tract of smaller plankton-eating fishes. The time of the female's migration from air bladder to abdominal cavity is as yet unknown, but presumably takes place during the early autumn or even earlier, as young 15—20 mm females are found in the host's abdominal cavity already in the early spring. The uteri of such females are filled out by many juvenile eggs. By early April the females reach 25—30 mm in length and the eggs become mature; by the end of April juvenile larvae are apparent in the eggs. The mature larvae hatch in May. This cycle of *T. kotlani* differs notably from that of *T. rischta*. After copulation by about the end of summer, the males and females of *T. rischta* uniformly leave the air bladder; the females settle subcutaneously in the anterior part of the host's body, primarily under the skin covering the gills. The *T. kotlani* males, however, remain in the air bladder throughout the year and the fertilized females capable of further development settle in the abdominal cavity.

SUMMARY

A new roundworm parasite of the fish *Aspius aspius* is described under the name *Thwaitia kotlani* sp. n. The males settle under the serosa of the air bladder, whereas the females are capable of further development in the abdominal cavity.

T. kotlani has a season-governed one-year developmental cycle. Mature females full of larvae are found exclusively in May.

In part of the hosts the development of the parasite is suppressed, thus retarded or deteriorated specimens are often encountered.

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OCCURRENCE OF ENCEPHALITIS CAUSED BY INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN CALVES IN HUNGARY

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Infectious bovine rhinotracheitis (IBR) virus may cause different clinical symptoms. The virus was first isolated from animals suffering from a disease of the upper respiratory tract (MADIN et al., 1956) (infectious bovine rhinotracheitis: IBR), later on the causative agent of IBR was found identical with that of the infectious pustular vulvovaginitis (KENDRICK et al., 1958). More recently it has been revealed that the virus can cause other clinical pictures, too, *viz.*, conjunctivitis of cattle (ABINANTI and PLUMER, 1961), abortion of cows (CRANE et al., 1964), and encephalomyelitis of calves 1 to 5 months of age (JOHNSTON et al., 1962; FRENCH, 1962).

Of the syndromes induced by the IBR virus the occurrence of the so-called infectious bovine rhinotracheitis (DOMÁN and FODOR, 1961), infectious pustular vulvovaginitis (MANNINGER et al., 1962) and conjunctivitis have been demonstrated in Hungary. Encephalitis, however, had not been observed in Hungary so far. Its occurrence has been reported in Australia and in the United States of America.

In this paper the occurrence of encephalomyelitis caused by IBR virus in a calf herd, the course of the disease and control measures are reported.

Materials and methods

For isolation of the virus monolayer cultures of calf kidney and testicle cells were used. It was attempted to isolate the virus from the nasal and ocular discharge, brain, nasal mucous membrane, tonsils, naso-lacrimal duct, lungs, spleen and liver of affected animals. Discharges were taken by swabs. The discharges and homogenized organs were diluted 1 to 10 with Hanks' solution. Following centrifugation each suspension was treated with antibiotics, then 4 cell cultures were inoculated, with 0.1 ml each. The inoculated cultures were observed daily for cytopathic (CP) changes. If CP changes had appeared, the culture fluid was subinoculated into fresh cell cultures on the same day. Five blind passages were done with each sample before discarded as negative.

The isolates, before subjected to the virus neutralization test, were tested for resistance to chloroform, heat and trypsin, and stability in the presence of *M* $MgCl_3$. The quality of the CP changes was also taken into consideration. All the properties thus revealed were characteristic of the

Herpesvirus group. Thus, the neutralization test was carried out with antisera to members of this group of viruses.

The 2nd passage of one of the strains isolated from the brain (Na/1967) was used for experimental infection. Two calves 4 weeks of age were instillated intranasally and inoculated intramuscularly with 2 ml culture fluid each. Temperature was taken daily, and the clinical symptoms were recorded regularly. Serum samples for neutralization test were taken on the day before, and on the 8th, 14th and 44th days following experimental infection.

Paired serum samples were collected from some of the calves, cows and heifers housed in the infected premises.

After the definite diagnosis had been established IBR vaccine of the Diamond Laboratories Inc. (Des Moines, Iowa, USA) was employed, which was put at our disposal for experimental purposes by the Department of Animal Health, Ministry of Agriculture and Food, Budapest. Calves were inoculated with 2 ml each.

History and course of the disease

In a newly-established calf house of a cooperative farm 290 calves in 8 groups were housed under excellent conditions. The calves were grouped according to their age, *i.e.* 10 to 30 days, 4 to 7, 8 to 10 and 12 to 15 weeks of age in groups 1, 2, 3 and 4, respectively. Calves older than 3 months were divided in groups 5 to 8. Each group comprised 35 to 40 calves. In 1964 and 1965 virus diarrhoea and a respiratory disease caused by adenovirus, respectively, occurred among the calves, whereas in 1966 no symptoms indicative of an infectious disease of the digestive or respiratory tract were observed.

On April 22, 1967, 2 calves two weeks of age were affected. They lost appetite and showed serous nasal discharge, lacrimation, salivation and forced respiration. Their muffles were reddened. On the 3rd or 4th day thereafter symptoms indicating central nervous involvement appeared intermittently. They showed dullness, muscular tremor and sometimes excitement and blindness. Symptoms which took 5 to 10 minutes passed off for hours. Nervous symptoms, however, turned persistent by the 5th day, and at the end of the 5th day the calves were unable to stand, they lay in opisthotonic position and showed uncoordinated movements, and finally died on the 6th or 7th day.

As a total 8 calves, all from group 1, had died with similar symptoms by the 10th May. Initially all affected animals showed general symptoms, which were followed by nervous disorders. It is worth mentioning that all affected animals were calved by first-pregnant heifers.

At post mortem examination no signs characteristic of any definite disease were found. In some of the calves mild gastroenteritis or localized

catarrhal bronchopneumonia was found. The bacterial examination gave negative results in every case.

Histopathological examination, revealing lymphocytic infiltrations in both the grey and white matter of the brain, indicated some viral disease.

To clear up the aetiology, investigation on the spot was decided on the 10th May. It was found that calves of groups 1 to 4 had been removed to a new calf house which was thought free of the infection. Each calf remained in its original group. On the 8th May, one day following the resettlement, half of the calves in groups 1 and 2 were already affected. They showed general symptoms including inappetence, nasal discharge, respiratory disturbances and reddening of the muffle. The general symptoms could still be observed on the 10th May. Profuse salivation and reddening of the muffle were the most striking symptoms. In a calf 3 weeks of age definite signs of nervous involvement were seen; the calf was hardly able to stand, intermittent trembling, drawing forwards of the head and neck, then collapse and opisthotonus were seen. In 2 other calves only marked dullness indicated nervous disorder but the attendants reported on trembling, too.

The severely affected calf was slaughtered in emergency, but no lesions could be found at the post mortem examination. The organs including brain were brought for virus isolation. Nasal and conjunctival discharges were collected for virus isolation from 2 calves that showed nervous symptoms and from 3 further calves with general symptoms.

Following isolation and identification of the IBR virus the premise was visited again on the 18th May in order to immunize the herd with the IBR vaccine produced by the "Diamond Laboratories". In the meantime 6 calves died of the disease and 40% of the calves in groups 3 and 4 (18—15 weeks of age) were involved, too. Some of the died animals had been calved by older cows.

Calves in groups 5 to 8 (3 to 6 months of age), which remained in their original house, and those of groups 2, 3 and 4 were vaccinated, while group 1 was left unvaccinated. All calves in the vaccinated groups were injected regardless of their state of health. At the time of vaccination not a single calf in these groups showed nervous symptoms, and the general symptoms shown were mild. Reddening of the muffle started fading and the superficial layer of the epithelium began to desquamate.

No postvaccination reaction was observed. One calf in group 3 died 5 days after vaccination, showing characteristic nervous symptoms. In the control group (group 1) 7 calves died by the 3rd June, when the calves still alive in group 1 were vaccinated. In this group one calf showed nervous symptoms (dullness, convulsions, disturbances in swallowing) 6 days following vaccination, but recovered subsequently.

As a total, about 70 calves between 2 to 15 weeks of age showed general

symptoms, 22 of them suffering from nervous disorders. The latter died in 6 to 7 days. On the 5th day following vaccination 1 calf died; another was affected, and despite nervous involvement, recovered.

Results

From the brain, nasal mucous membrane, tonsils and nasolacrimal duct of the calf that showed nervous symptoms before being emergency-slaughtered IBR virus was isolated in calf testicle and kidney cell cultures. Isolation of the virus from the spleen, liver and lungs failed. The virus could be isolated from the nasal discharge of 3 of the affected calves. The isolates showed characteristic CP effect already in the 1st passage.

The first signs of the CP effect could be observed 48 to 72 hours following inoculation. This included syncytium formation and rounding of the infected cells. In stained preparations the changes described in an earlier paper as characteristic of the IBR virus could be seen.

The virus proved chloroform-, heat- and trypsin-labile. It was not stabilized by *M* $MgCl_3$ against the action of heat. Based on these properties and the CP changes, the isolate was identified as a member of the *Herpesvirus* group. The isolated strains were neutralized by the specific anti-IBR serum alone. Thus, identification of the isolate was confirmed serologically.

Calves experimentally infected intranasally or intramuscularly with 2 ml of the virus (2nd passage) responded with general symptoms 5 to 7 days following infection. Temperature over 40 °C and dullness were observed. Initially serous, later on mucous, nasal discharge, conjunctivitis, profuse salivation and lacrimation were seen. The calf infected intranasally showed enteritis, too. Its respiration rate increased from the initial 30 to 50. The calves recovered between the 12th and 14th days following injection. No nervous involvement occurred.

The virus could be reisolated from the nasal discharge 5, 7 and 9 days following infection. No virus could be isolated from any organ of the calves slaughtered on the 32nd or 44th day following experimental infection.

Serum samples from 12 cows in the affected premises were tested for virus-neutralizing antibodies. The calves of 7 of the tested cows (5 heifers and 2 cows) had died of the disease. The calves of the remainders had escaped the infection. None of the sera taken from the heifers contained specific antibodies, whereas specific antibodies of high titre (1 : 64, 1 : 256) could be demonstrated in the sera of the 7 older cows. In sera taken 10 days following recovery from 5 calves with general symptoms antibodies could be demonstrated in very low titres (1 : 4, 1 : 8), if any. It may be noted that the antibody levels in the sera of the vaccinated calves were higher than in those of the calves infected naturally and recovered.

During the 2-week period of observation in the unvaccinated group 7 calves died, while in the 7 groups of vaccinated calves only one case became fatal (on the 5th postvaccination day).

Discussion

It is known that among the clinical forms caused by the IBR virus in Hungary infectious pustular vulvovaginitis is more common than infectious bovine rhinotracheitis. Another form involving conjunctivitis has been found occasionally. Encephalitis caused by this virus, however, had not been observed in Hungary. This condition which had first been reported in Australia (FRENCH, 1962) and in the USA (BARENFUS et al., 1963) has not been found in any other country.

The encephalitis outbreak under study, caused by the IBR virus, occurred in a herd in which none of the forms of the IBR syndrome had been observed earlier. It might be assumed that the causative agent had been present in the herd without having caused an illness manifested in clinical symptoms. This is indicated by the neutralizing antibodies found in sera of some of the cows. Dilutions of the sera as high as 1 : 64 to 1 : 256 were able to neutralize 100 $TCID_{50}$ of the virus. This level of antibodies is considered noteworthy, for such a high antibody level cannot be observed in sera taken from convalescent animals that had survived the respiratory form of the disease; in the convalescent sera of cows with infectious pustular vulvovaginitis even no antibodies can be demonstrated.

Antibodies were not present in the sera of the growing calves and cow-heifers, indicating that the offspring in the calf houses and the growing animals had escaped infection. The virus must have been introduced just before the outbreak.

In the cases described by the Australian and American authors no symptoms indicating respiratory, or any other form of the IBR syndrom, were observed in the calves besides encephalitis. We, on the other hand, raised the suspect of an IBR virus infection because some of the calves affected by encephalitis had suffered from respiratory disease prior to the appearance of nervous symptoms. These symptoms included dullness, inappetence, forced respiration, reddening of the muffle and profuse salivation. Such symptoms were not reported by the Australian and American authors. Nevertheless, the nervous symptoms, the 100% fatality among the calves showing nervous involvement, the age of the affected calves and the protracted course of the cases agreed well with the literary data.

Owing to the isolation of the virus from the brain, nasal mucous membrane, lacrimal duct and tonsils as soon as in 3 to 4 days, an early diagnosis could be established. The virus could be isolated from the nasal discharge

even in some cases showing only respiratory symptoms. The lymphocytic infiltrations in both the grey and white matter supported the viral origin of the disease. Isolation of the virus along with the histopathological changes in the brain proved obviously that the nervous symptoms observed in this herd were ascribable to the IBR virus.

As a result of the early diagnosis animals could soon be vaccinated. Being of low virulence, this vaccine can be used safely in young calves. In pregnant cows the vaccine may cause abortion. In other countries the same vaccine had been used successfully in preventing the respiratory form of the disease. It has been claimed that in an affected herd the animals had developed a satisfactory immunity 10 days after vaccination. Consequently all but 1% of the vaccinated animals remained healthy.

As far as we know, the vaccine had not been employed in preventing encephalitis caused by the IBR virus. Taking into consideration that an adequate immunity develops in 10 days, and the virus spreads among calves slowly, the use of the vaccine was decided. Since the vaccine was supplied by the Animal Health Department of the Ministry of Agriculture and Food for experimental purposes only, the youngest calves, belonging to the age group that had been affected most severely, remained unvaccinated. The uninfected growing calves 4 to 6 months of age (groups 5 to 8) and those kept in the infected groups (groups 2 to 4) were all vaccinated, regardless of having been infected or not. None of the calves showed nervous symptoms at the time of vaccination.

The active immunity developed soon, as proved by the fact that no additional cases among the vaccinated calves were reported, except one that died as early as on the 5th day following vaccination. In the control group, however, 7 calves died of nervous symptoms in 2 weeks. The result of the vaccination obtained in group 2 can be evaluated well, because before vaccination the clinical symptoms and losses were as severe in this group as in group 1. In groups 3 and 4 the disease was first observed 2 weeks before the vaccination and some of the animals had died. Since 7 calves died in the control group 2 weeks following vaccination while only 1 calf died in the 3 vaccinated groups, the vaccination must be considered as successful.

Taking into account that in groups 5 to 8 no prevaccination case of the disease had been observed, the apparently good result in these groups cannot be attributed to the vaccination.

Since the results of the vaccination had convinced us of the good preventive effect of the vaccine, we vaccinated the 24 calves still alive in group 1. Six of the calves showed respiratory symptoms at the time of vaccination. Nervous symptoms were not observed, but one calf showed nervous involvement 6 days following vaccination. Unlike our experiences and those obtained abroad the calf recovered in 6 weeks. No additional losses occurred in the group.

Thus, it can be stated that the vaccine can successfully be used, even if encephalitis caused by the IBR virus has occurred in an affected herd. (In our case the calves were vaccinated 1 month following the onset of the first case.) One question has remained to be cleared: What is the reason that of the several infected herds the virus had induced encephalitis in none but this herd. Encephalitis could neither be induced in 2 calves infected experimentally.

These observations enable us to suppose that, in addition to the adaptation of the virus to the central nervous system, other factors must be involved in the manifestation of encephalitis.

SUMMARY

In a large-scale farm an outbreak of encephalitis caused by the IBR virus was observed among calves. Calves of 10 to 30 days of age were most affected. General symptoms (forced respiration, fever, nasal discharge, salivation, reddening of the muffle) were observed initially, followed by nervous symptoms in some of the affected calves. The latter died in 5 to 7 days. Mortality amounted to 15%. No macroscopic post mortem changes could be observed. Histologically, lymphocytic infiltrations in the white and grey matter of the brain were demonstrable.

The virus was isolated in tissue cultures from the nasal discharge of affected animals and the brains of died animals. The calves infected experimentally showed only general symptoms.

Three of the infected 4 groups were vaccinated with a live IBR vaccine (Diamond Laboratories, USA). One group served as unvaccinated control. Only 1 of the 112 vaccinated calves died of the disease 5 days following vaccination as compared to the 7 of the 31 unvaccinated controls.

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REACTION OF THE INTESTINE OF THE CARP (CYPRINUS CARPIO L.) TO ENVIRONMENTAL STRESS

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The environmental — stress — effects affecting the carp, such as transport, pH change, fluctuations in O_2 saturation of the water, *etc.*, have been referred to by several authors (RICHLICKI and ŻARNECKI, 1956; LASSLEBEN, 1964), but their consequences have hardly been analysed. So far chiefly speculative conclusions have been drawn, *e.g.*, it has been supposed that the elimination of stressors would notably reduce the losses by certain fish diseases, above all by dropsy (LASSLEBEN, 1959).

In this laboratory, studies on the aetiology and pathogenesis of dropsy have been extended to a closer examination of the factors damaging the intestinal mucosa of the carp, with regard to the observation that in the majority of the cases outbreaks of acute dropsy occur after harvesting, transport or stocking, although *Aeromonas punctata*, the postulated causative agent is demonstrable in the intestine of many carps, independent of season (SCHÄPERCLAUS, 1954; KOCYLOWSKI and MIACZYNSKI, 1963; BUZA and SZAKOLCZAI, 1968). It has, therefore, been supposed that the above environmental changes may act as stress factors and, in addition to their influence on other organs, affect the permeability of the intestinal mucosa.

Experimental

Materials and methods. Intestinal changes were examined in relation to harvest, abrupt rise of temperature and transport, on 77 2-year-old (C_2) and 12 3-year-old (C_3) carps.

I. *Effect of harvest.* The test material was obtained from a pond (water temperature 6—8 °C) of each of two farms, where the autumn harvest lasted 6—7 days. Two apparently healthy C_2 were secured from each of the two ponds on the 1st, 3rd and 6th days of the harvest. The fishes were killed immediately. Full parasitological examination of all organs and bacteriological examination of the intestines and liver were performed. Specimens from the anterior, medium and posterior part of the intestine and, for information, also from the liver and kidneys were processed for histological examination. Frozen sections fixed in formalin and paraffin-embedded sections were prepared in the Department of Pathohistology of this Institute. The sections were stained with haemalaun-eosin, Sudan III or PAS.

II. *Effect of abrupt temperature elevation.* Ten C_2 fishes, 200—250 g in weight, were kept throughout the winter in running tap water of 10 °C in a tank, without food. At the beginning of the experiment, 2 fishes were killed to serve as controls, and 2 others were placed in another tank under the same conditions. Then the water of the tank accommodating the remaining 6 fishes

was warmed to 20 °C in 5 hours (raising the temperature by 2 °C per hour). Two fishes were killed in the 6th, 7th and 8th hours following the heating of the tank water. In the 8th hour also the 2 survivors, maintained throughout at 10 °C, were killed. The organs of all fishes were processed in the same way as described under I.

III. *Effect of transport.* These experiments were carried out in March. From a winter pond (water temperature 3—5 °C) in each of 5 farms, 5 C_2 fishes, 200—250 g in weight, were collected. Two fishes from each group were killed on the spot and the remaining 3 were transported to the laboratory under the usual conditions, in 4—8 hours. They were killed in succession in the 24th, 96th and 120th hours after collection and their organs were processed as described under I. In the case of the fishes killed on the spot, often more than 3—5 minutes elapsed between catching and processing.

The 3 transported fishes killed in the 24th hour had jellinaceous clear intestinal contents, varying in amount from 0.5 to 3.0 ml. This material was placed in test tubes, and after adding 100 μg/ml of terramycin to prevent eventual bacterial growth, it was stored in the refrigerator until use.

After mass transports (6—24 hours) to 10 pond farms in the spring (March, April) and to 2 in the summer (June), on a total 30 C_2 and 12 C_3 fishes were collected from the 12 farms and processed as described in the foregoing. Clear jellinaceous contents were found in the intestines of 12 fishes from 5 pond farms stocked in the spring. In this experimental series we have missed controls from the pre-transport period.

Precipitation test. Ten healthy C_2 fishes were bled. The sera collected after clotting were pooled and their protein contents were determined by refractometry at 20 °C as 3.52 g%. Using 2 ml doses of the serum, a rabbit was immunized on 4 occasions at 1-week intervals. Seven days after the last immunization the precipitin titre of the rabbit serum was determined and since it was satisfactorily high, the rabbit was exsanguinated. After clotting and separation, 1 : 10,000 Thymerosal was added for preservation and the immune serum was stored in the refrigerator until use.

Agar plates were prepared by Ouchterlony's method in Petri dishes 9.5 cm in diameter, using 20 ml 1.5% agar solution with 0.62% NaCl. The wells, of 5 mm diameter each, were spaced at 5 mm distance. In part of the wells 0.05 ml homogenized, undiluted jellinaceous intestinal contents, in part fish serum was placed to serve as control. The 1 : 10 diluted rabbit immune serum was placed in the central well. The precipitation reaction was read every 24 hours until the final reading on the 10th day.

Results

Parasitological examinations have led to practically negative results. Only a few Trichodina of Dactylogyrus specimens were found on the gills.

In the course of bacteriological examinations, isolation from the liver was invariably negative. The intestines of the carps maintained in tanks without food during the winter and also of those collected in the spring from the winter ponds were sterile in 10—15% of the cases. In the intestines of the rest both the number of bacterial species and bacterial counts were very low. The majority of the bacterial population was identified as *Aeromonas punctata*.

Histological examination

I. In every case, the intestinal lumen was empty. Lesions were found in the intestinal mucosa, progressing parallel with the duration of harvest.

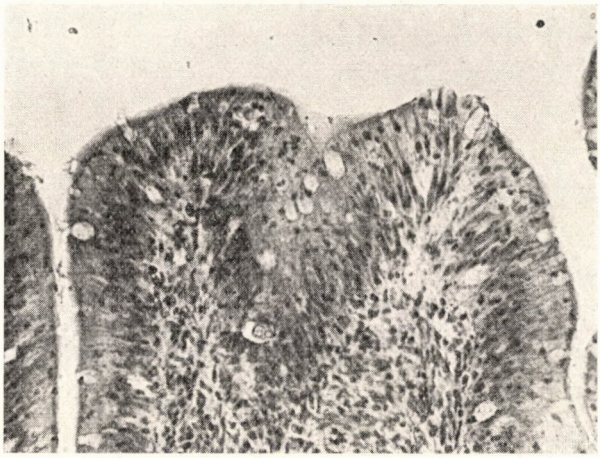

Fig. 1. Mucosal folds in healthy intestine. Frozen section. Haemalaun-eosin stain. × 200

Slight detachment of columnar epithelium cells from the basement membrane over the ridges and sides of the mucosal folds was apparent most often in the anterior and medium, but sometimes also in the posterior portion of the intestine. Goblet cells decreased in number. The capillaries passing immediately below the cell bases and basement membrane were distended and full with red blood cells. Owing to the epithelial damage, the structure of the propria became somewhat loose. In the tissue gaps an eosinophilic, structureless substance — serum — and a few leukocytes were seen. Lymphocytes occurred scattered both in epithelium and propria. On staining with Sudan III, finely granular fat infiltration became often apparent in the space between the epithelial layer and basement membrane, less often between loosely arranged epithelial cells. In the muscle layer there was a very moderate degree of hyperaemia (Figs 1, 2, 3, and 4).

Fig. 2. Goblet cells in healthy intestine. Paraffin-embedded section. PAS-stain. × 200

Fig. 3. Post-transport hyperaemia and detachment of columnar epithelium from the basal membrane over the ridges of the mucosal folds. Paraffin-embedded section. Haemalaun-eosin stain. × 200

In the liver, hyperaemia, moderate vacuolation of the cells and mild fatty infiltration, whereas in the kidneys nephrosis of some of the tubules were seen.

II. In every case, the intestinal lumen was empty. In the intestines of the control fishes no lesions whatever were found, whereas those of the experimental fishes showed the changes described above. Also the muscle layer of the intestine was hyperaemic.

The liver and kidneys were hyperaemic and many eosinophils were found between the renal tubules.

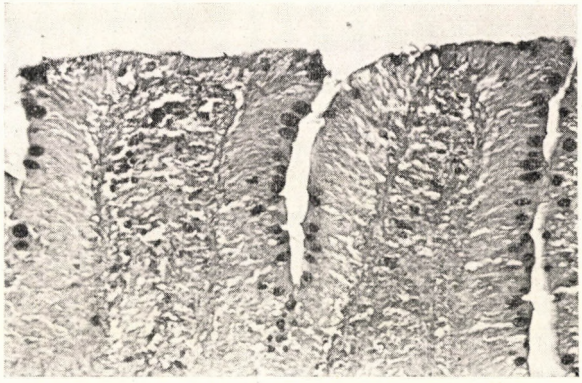

Fig. 4. Post-transport decrease of goblet cells in the epithelium. Paraffin-embedded section PAS-stain. × 200

III. The intestines of the fishes collected from the winter ponds and processed on the spot were empty. On microscopic examination the intestine, liver and kidneys proved to be normal, with sporadic minimal hyperaemia.

Lesions in the intestines of fishes killed 24 hours after collection were of the above types, but more distinct. In 3 cases an eosinophilic substance, staining yellow with Sudan III, was apparent also in the intestinal lumen.

In the carps killed in the 96th and 120th hour after collection, the lesions had become milder and hardly noticeable.

In the carps selected from the lots subjected to mass transport, the above lesions were found in every case where the intestine was either empty or contained a clear jellinaceous substance only. The severity of the lesions increased in direct relation to the prolongation of transport time or the elevation of the temperature above 8—10 °C.

If the carp's intestine contained natural or artificial food, in addition to the above lesions also necrosis of the epithelium and propria were found, particularly if fishing and transport were made at a higher temperature (15 °C). In the necrotic areas many neutrophils and lymphocytes were seen, with degenerated or fully disintegrated nuclei (Fig. 5). The liver and kidneys were hyperaemic and showed necrobiotic fatty infiltration and necrosis.

Precipitation tests. The titre of the precipitating immune serum was 1 : 10,240. It gave 11 precipitation lines with the fish serum, against which it was prepared as well as with sera from healthy carps, while only 3—7

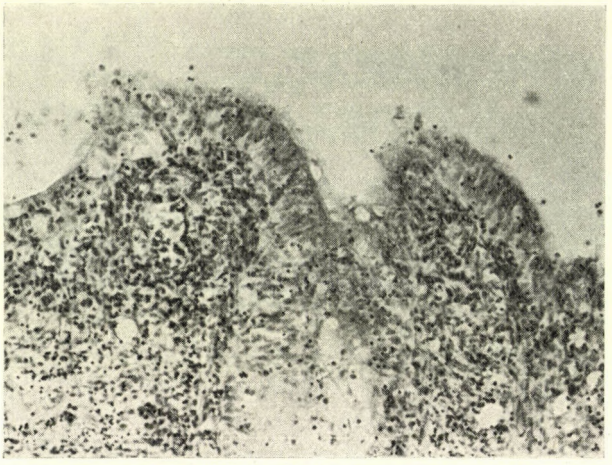

Fig. 5. Necrotic phenomena in the intestinal mucosa of a carp transported after feeding. Frozen section. Haemalaun-eosin stain. × 200

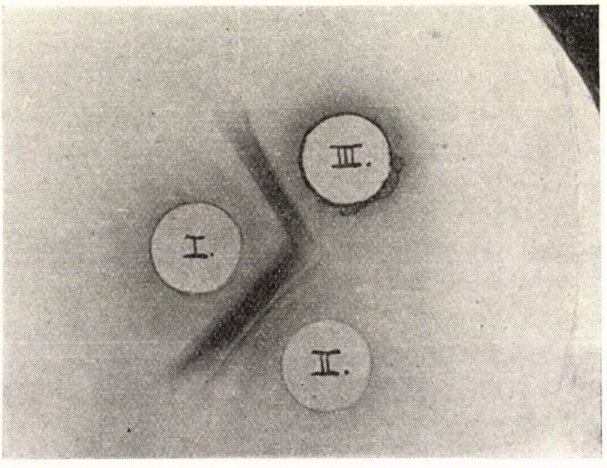

Fig. 6. Agar-gel precipitation test. I. Rabbit immune serum. II. Carp serum. III. Water-clear jellinaceous intestinal contents. Dried preparation stained with fuchsin

precipitation lines with the jellinaceous intestinal contents (Fig. 6). Parallel to the quantitative increase of intestinal contents, the number of the precipitation lines tended to decrease. The highest number of precipitation lines was obtained with the cylinder-shaped coherent clear jellinaceous intestinal contents.

Discussion

Based on the results of parasitological and bacteriological examinations, the carps studied could be justly regarded as healthy.

Microscopic examination of organ specimens from the carps has shown that the various environmental effects (harvest, abrupt rise of temperature, transport) affect the fishes in a uniform way, causing the same type of lesions in their intestines. Naturally, mucosal hyperaemia and extravasation of serum may be attributed to the activity of intestinal bacteria as well. This is, however, contradicted by the fact that the lesions in question were encountered also in intestines with no or very low bacterial contents.

The lesions indicated an alteration of the mucous membrane's permeability, at least in the direction toward the intestinal lumen. It remains to be investigated whether or not this permeability change involves also abnormal conditions of absorption.

We have failed to offer a feasible explanation for the decrease, or even for the disappearance of goblet cells within the areas of epithelial lesions. Further studies are to be carried out to clarify whether the absence of goblet cells is primary — whereby the mucosa-deprived epithelium becomes a "locus minoris resistentiae" — or secondary, representing the first sign of the onset of a pathological process.

According to the results of the precipitation tests, the jellinaceous substance occurring in the intestines can be considered serum protein. As the serum protein components do not occur in full range in the intestines and with the progression of the lesions they decrease also quantitatively, their identification tends to become more and more uncertain.

SUMMARY

The effect on the carp's intestine of environmental (stress) factors such as harvest, abrupt rise of temperature and transport was examined. These factors were found to consistently elicit detachment of mucous epithelium, mucosal hyperaemia and extravasation of serum from the mucosal capillaries into tissue gaps and intestinal lumen. The extravasated serum was identified by precipitation test. It is supposed that the lesions affect the permeability of the intestinal mucosa and can thereby pathologically alter the conditions of absorption.

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ERGEBNISSE EINIGER KLINISCH-DIAGNOSTISCHER UND THERAPEUTISCHER UNTERSUCHUNGEN BEI DER SPONTANEN GEHIRNLISTERIOSE DES SCHAFES

Von

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(Eingegangen am 23. November 1968)

Listerioseenzootien stellen nach wie vor einen bedeutsamen ökonomischen Faktor in der Schafhaltung dar. Neuere Erkenntnisse über Epizootologie und Pathogenese dieser Erkrankung sowie eine in den letzten Jahren laufend verbesserte intravitale und postmortale Diagnostik haben zwar dazu beigetragen, daß die Ausbrüche klinisch manifester Erkrankungen auf zumeist wenige Tiere einer Herde beschränkt bleiben, jedoch bestehen bei der besonderen Häufung von Erkrankungen des Zentralnervensystems beim Schaf mit unterschiedlichen Ursachen häufig differentialdiagnostische Schwierigkeiten bei der klinischen Differenzierung einiger unter nahezu gleicher Symptomatologie einhergehender cerebraler Störungen. So beobachten wir häufig im gleichen Bestand das Auftreten von Bornascher Krankheit (enzootische Virusenzephalomyelitis), Cerebrokortikalnekrose und Listeriose, außerdem vermögen Stoffwechselstörungen, wie die Ketose des Schafes und die Pansenazidose, wie auch die Clostridienintoxikationen ähnliche Krankheitsbilder zu erzeugen.

Diese bemerkenswerte Häufung von Krankheiten des Schafes, die mit annähernd gleicher Symptomatologie zumeist perakut oder akut verlaufen, machen eine rasche, weitgehend objektiv gesicherte und hochwahrscheinliche intravitale Diagnose notwendig. Sie schafft die Voraussetzung für das sofortige Einsetzen von Maßnahmen im Bestand, die beispielsweise in einer Eliminierung der als listeriosefördernd bekannten Faktoren und dem frühzeitigen therapeutischen Eingreifen bei erkankten Tieren besteht.

Wir haben in einem Zeitraum von zehn Jahren umfangreiche epizootologische, klinisch-diagnostische und therapeutische Untersuchungen über die Schaflisteriose im Bezirk Leipzig vornehmen können, über die teilweise in den nachfolgenden Ausführungen berichtet werden soll. Unser Hauptaugenmerk richteten wir dabei seit 1958 außer auf die Frequenz dieser Erkrankung in den ständig kontrollierten Schafherden besonders auf die klinische Diagnostik und die Erarbeitung einer praxisreifen Therapie.

Aus den Untersuchungen von LEHNERT (1964, 1968) wissen wir, daß in unserem Raum *Listeria monocytogenes* als ubiquitär verbreiteter Keim im Erdboden, in Deck- und Randschichten der Silage und im Tierkörper (nachweisbar im Nasensekret) vorkommen. Nur ein kleiner Teil der latent infizierten Schafe erkrankt an der klinischen Verlaufsform, für deren Zustandekommen verschiedene Faktoren verantwortlich gemacht werden. Wir haben für unser Gebiet einige dieser Umstände charakterisiert (URBANEK und LIPPMANN, 1962), und in der Folgezeit versucht, diese Erkenntnisse in Verbindung mit ersten therapeutischen Versuchen zur Minderung der ökonomischen Verluste durch Listerioseenzootien in Schafbeständen zu nutzen.

Wie sich das Geschehen im Verlauf des letzten Jahrzehnts entwickelt hat, vermittelt die Tabelle 1.

Diese Zusammenstellung zeigt, daß in den von uns regelmäßig untersuchten Schafherden (durchschnittliche Tierzahl 409 je Herd), deren Anzahl zwischen 91 und 177 Beständen schwankt, im 10jährigen Durchschnitt in 23 Herden (= 17%) klinisch-diagnostisch und histologisch gesicherte Listerioseausbrüche auftreten. Obwohl die jährliche Frequenz stark schwankt — nicht zuletzt ist dieser Umstand wohl auf die Qualität der eingesetzten Futter-

Tabelle 1

Listeriosefrequenz in Schafbeständen des Bezirkes Leipzig und Anteil der Klinikpatienten mit Listeriose im Verlauf von 10 Jahren

	1958	1959	1960	1961	1962
Anzahl der Schafherden mit Listerioseenzootien	11	16	26	21	33
Anteil der Listerioseherden an der Gesamtzahl untersuchter Herden in Prozent	14,9	17,6	25,0	16,9	21,9
Verlustzahlen bei Listerioseenzootien in diesen Schafherden in Prozent (Schwankung u. Durchschnitt)	0,2—3,0 (1,3)	0,3—5,0 (1,4)	0,5—22,0 (1,9)	0,3—6,5 (1,4)	0,2—4,6 (1,6)
Klinikpatienten (mediz. Tierklinik Leipzig)	14	32	67	22	19
Anteil der Listeriosepatienten an der Gesamtzahl der Klinikpatienten (Schafe) in Prozent	7,6	16,8	33,0	23,4	13,8

	1963	1964	1965	1966	1967
Anzahl der Schafherden mit Listerioseenzootien	24	23	12	22	39
Anteil der Listerioseherden an der Gesamtzahl untersuchter Herden in Prozent	14,8	10,4	6,5	16,9	23,3
Verlustzahlen bei Listerioseenzootien in diesen Schafherden in Prozent (Schwankung u. Durchschnitt)	0,5—6,0 (1,2)	0,2—7,9 (0,7)	0,2—2,5 (0,35)	0,2—6,2 (1,2)	0,2—3,3 (1,1)
Klinikpatienten (mediz. Tierklinik Leipzig)	12	18	16	10	6
Anteil der Listeriosepatienten an der Gesamtzahl der Klinikpatienten (Schafe) in Prozent	10,2	18,6	17,0	7,6	6,2

mittel zurückzuführen — bewegen sich die Verlustziffern zwischen 0,2 und 5% des Bestandes (im 10jährigen Mittel beträgt die Mortalität 1,2%). Diese Zahlen entsprechen auch der Häufigkeit im Patientenmaterial der Medizinischen Tierklinik Leipzig, die bei einem Anteil von 27,6% Schafen im 10-jährigen Durchschnitt am Gesamtkrankengut durchschnittlich 15,4% Schaf-

listeriose diagnostizierte. Von den in der Klinik festgestellten 216 Listeriosen beim Schaf war nur ein Lamm an septikämischer Listeriose erkrankt, die übrigen 99,6% zeigten die cerebrale Verlaufsform.

Klinische Laboratoriumsdiagnostik

Die klinischen Merkmale einer Listerienenzephalitis unterscheiden sich nur geringfügig von den bei anderen Nervenkrankheiten des Schafes zu beobachtenden Störungen von Sensorium, Sensibilität, Motilität und Reflexerregbarkeit. Pathognomonische Symptome fehlen bis auf eine nahezu regelmäßig auftretende hämorrhagische Konjunktivitis bei der Schaflisteriose, die zumeist in Verbindung mit einem ausgeprägten Opisthotonus auftritt (LIPPMANN, 1966). Eine Objektivierung der intravitalen Diagnose ist nur durch Einbeziehung labordiagnostischer Untersuchungen möglich. Nach unseren Erfahrungen eignet sich hierzu bei manifester Erkrankung in hervorragender Weise die Liquordiagnostik, über deren Ergebnisse im folgenden berichtet wird. Der Blutstatus gibt nur unspezifische Resultate, wenn auch im Einzelfall die diagnostischen Möglichkeiten bereichert werden. Von den biochemischen Blutuntersuchungen liefert nur die Serumeisen-Bestimmung beweisende Resultate. Über die bei der Listerienenzephalitis regelmäßig und frühzeitig mit hoher Sicherheit vorhandene Hyposiderose ist von uns an anderer Stelle berichtet worden (LIPPMANN, 1966; LIPPMANN, 1968).

Die Untersuchung des Liquor cerebrospinalis dagegen erfüllt die Anforderungen an eine sehr rasche und objektive Sicherung der intravitalen Diagnose, wenn Symptomatologie und Epizootologie berücksichtigt werden. Wir wenden die Postoccipitalpunktion unter Barbituratnarkose in der von HIEPE (1958, 1959) beschriebenen Technik zur Entnahme des Untersuchungsmaterials aus der Cisterna cerebellomedullaris an.

Das folgende Liquorsyndrom bei der ovinen Gehirnlisteriose begründet sich auf die Auswertung von 253 Punktionen erkrankter Tiere. Vergleichsweise sind diesen Werten die Ergebnisse von 289 Liquoruntersuchungen bei klinisch gesunden Schafen gegenübergestellt, die während des gleichen Zeitraumes mit der gleichen Entnahmetechnik und Labormethodik gewonnen wurden. Beide Kollektive erfuhren eine Auswahl in der Weise, daß in den Zahlen nur Tiere im Alter über 3 Monate enthalten sind, um die größere Variationsbreite der Parameter bei Sauglämmern auszuschalten. Zum anderen wurden nur Erstpunktionen zur Auswertung herangezogen, d. h., bei gesunden Schafen wurde jeweils nur einmal Liquor entnommen; bei Gehirnlisteriose sind nur die Werte verarbeitet worden, die bei akut erkrankten Tieren am 1. oder 2. Krankheitstag gewonnen wurden, alle Verlaufsuntersuchungen blieben unberücksichtigt. Verwendet wurden nur benzidinnegative, also weitgehend blut-

freie Liquoren. Hinweise auf die Untersuchungsverfahren vermittelt die nachfolgende Tabelle 2.

Tabelle 2

Liquorsyndrom bei der Listerienenzephalitis des Schafes

	Angab. z. Methodik	Klinisch gesund	Listeriose
Farbe Aussehen		farblos wasserklar	farblos o. leicht getrübt; selten weißtrüb mit Flocken
Zellzahl $\left(\frac{n}{3}\right)$	Fuchs—Rosenthal-Zählkammer	0—12 ($\bar{x} \pm S = 6{,}35 \pm 4{,}68$)*	300— < 12 000 ($\bar{x} \pm S = 2864 \pm 1344$)*
Zellbild	Forster	lymphozytär (< 85% Lymphozyten)	neutrophile Z. (40—90% Neutr.)
Gesamteiweiß (mg%)	Kafka	18—40 ($\bar{x} \pm S = 27{,}43 \pm 12{,}93$)*	48—140 ($\bar{x} \pm S = 93{,}02 \pm 41{,}31$)*
Albumine (mg%)	Kafka	12—36	19,2—84,0
Globuline (mg%)	Kafka	2,4—19,2 ($\bar{x} \pm S = 10{,}76 \pm 6{,}52$)*	22,6—96,0 ($\bar{x} \pm S = 51{,}10 \pm 23{,}20$)*
Eiweißquotient	(Glob.: Alb.)	$\approx$ 0,35	$\approx$1,0
Quantitative Globulinreaktionen	Pandy; Nonne—Apelt; Weichbrodt	negativ (—) bis schwach positiv (+)	verschiedengradig positiv (+ bis +++)
Mastix-Lumbotest	Emanuel u. Rosenfeld	negativ (bis 1—1—0—0 . . . .)	Linkskurven (⌀ 4—3—2—1—0—0. . ..)
Goldsolreaktion	Lange—Borowskaja	negativ (bis 1—1—1—1—1—0 . . .)	Links bis Mittelkurven (⌀ 1—2—2—3—4—4——3—0 . .)
Glukose (mg%)	Fermognost (enzymat.)	20—70 ($\bar{x} \pm S = 43{,}2 \pm 12{,}1$)*	40—180 ($\bar{x} \pm S = 107{,}6 \pm 19{,}8$)*

* Statistisch gesicherte Signifikanz ($p < 0{,}01$)

Die angegebenen Zahlen besitzen den Wert von Approximationen; bis zu 5% der objektiven Daten liegen bei unserem Material außerhalb dieser Variationsbreite. Zur Charakterisierung der Liquorsyndrome und damit zur klinischen Differenzierung der Krankheiten sind sie ausreichend, zumal ein Teil des Syndroms ohnehin subjektiv beurteilt wird. Für objektiv zu bestimmende Werte, wie Zellzahl, Gesamteiweiß- und Globulinkonzentration und Glukosegehalt haben wir unser Material einer statistischen Bearbeitung

unterzogen und dabei für die genannten Parameter eine hohe Signifikanz nach STUDENT (RICHTERICH, 1965) gefunden. Der Zellgehalt in der Zählkammer und die Eiweißwerte nach KAFKA wurden doppelt bestimmt und das Mittel zur Auswertung benutzt, um den methodischen Fehler zu verkleinern.

Aus der Tabelle 2 geht hervor, daß sich die Summe der Parameter des Liquorsyndroms bei der spontanen Gehirnlisteriose signifikant von den physiologischen Werten unterscheidet. Unsere für gesunde Schafe festgestellten Werte entsprechen nahezu den im Schrifttum mitgeteilten Befunden, geringfügige Unterschiede bezüglich Zellzahl und Eiweißgehalt sind auf einen unterschiedlichen Entnahmeort (BAGEDDA, 1948) oder rassemäßige Verschiedenheiten (CHANDLER, 1957; EL AMROUSI u. Mitarb., 1966) zurückzuführen. Für die Gehirnlisteriose sind uns im zugänglichen Schrifttum keine anderweitigen Untersuchungen bekannt, ein Vergleich ist daher nicht möglich.

Das Liquorsyndrom bei der ovinen Listerienenzephalitis ist auf Grund der Auswertung von 253 Einzeluntersuchungen erkrankter Schafe charakterisiert durch eine hochgradige Pleozytose vorwiegend neutrophiler Natur, einem signifikanten Anstieg des Gesamtproteingehaltes unter besonderer Bevorzugung der Globulinfraktion, als deren Folge sich der Eiweißquotient von im Mittel 0,35 bei gesunden Tieren auf Werte um 1,0 erhöht. Die Kolloidreaktionen ergeben daher Links- und Mittelkurven. Die qualitativen Globulinfällungsreaktionen nach PANDY, NONNE—APELT und WEICHBRODT fallen verschiedengradig positiv aus und sind lediglich im Sinne eines Suchtestes zu verwerten. Bemerkenswert ist die signifikante Erhöhung der »wahren« Glukose im Liquor, die mit einer Verschiebung des Liquor-Blutzucker-Quotienten auf 1,1 (von normal 0,9) einhergeht. Listerien wurden im Ausstrich seltener als erwartet (etwa 10% der Fälle) nachgewiesen.

Signifikante Unterschiede bestehen hinsichtlich der Liquorbestandteile besonders bei der in unserem Gebiet häufigen Bornaschen Krankheit (HIEPE, 1958, 1960) sowie bei den nichtinfektiösen und sekundären Erkrankungen des Zentralnervensystems beim Schaf (LIPPMANN, unveröffentlicht). Darin drückt sich der besondere Wert der Liquoruntersuchung in der intravitalen Listeriosediagnostik aus.

Therapeutische Untersuchungen

Im Schrifttum (ÖZGENY, 1952; SEELIGER, 1958, 1961; LINZENMEIER und SEELIGER, 1953; KARASEK, 1964) ist ausgewiesen, daß Listerien sowohl sulfonamid- als auch antibiotikaempfindlich sind, wobei sich innerhalb der einzelnen Wirkstoffgruppen teilweise erhebliche Unterschiede bei der in-vitro-Testung ergeben. Das trifft insbesondere für Sulfonamide zu, die bereits 1939 von PORTER und HALE (zit. nach SEELIGER, 1958) zur Therapie der experimentellen Listeriose eingesetzt wurden und für deren unterschiedliche Wirkung wahrscheinlich Resistenzprobleme verantwortlich sind (ÖZGEN, 1952). Von den Antibiotika läßt sich bezüglich der in-vitro-Wirkung und demzufolge der klinischen Erfolgsaussichten eine absteigende Empfindlichkeit der Listerien gegenüber den Tetrazyklinen, dem Penizillin, Streptomyzin und Chloramphenikol feststellen (SEELIGER, 1958; KARASEK, 1964). In jüngster Zeit sind beachtliche Erfolge in der Listeriosetherapie des Menschen mit dem halbsynthetischen Penizillin, Ampi-

zillin, besonders bei Erkrankungen im Säuglingsalter, erzielt worden (SEELIGER, 1967; WEINGÄRTNER und ORTEL, 1967; WEINGÄRTNER und Mitarb., 1967).

Behandlungsergebnisse bei der spontanen Listerioseenzephalitis des Schafes finden sich nur spärlich im zugänglichen Schrifttum, sie sind meist nur an einer geringen Tierzahl gewonnen worden und überdies in ihrer Aussage widersprechend. Während SANDVIK und Mitarb. (1958) bei sehr jungen Lämmern ausgezeichnete Erfolge mit einer Streptomyzin—Penizillin-Kombination erzielten und IWANOW (1962) die Letalität durch Penizillin, Streptomyzin, Biomyzin und Sulfonamide bei rechtzeitigem Behandlungsbeginn von 100 auf 58,3% senken konnte, versagte bei den Versuchen von GRAY und MOORE (1953) die Aureomyzintherapie beim Schaf, während sie bei Rindern erfolgreich war. HIEPE (1960) hat aus unserer Klinik über die Heilung von 2 Lämmern aus einer Gruppe von 8 mit je 10 mg/kg KGW. Tetrazyklin behandelten Tieren berichtet. ULLRICH (1966) weist darauf hin, daß eine Therapie nur bei Lämmern erfolgversprechend sei, nach seinen Beobachtungen bleibt bei erwachsenen Schafen jegliche Therapie wirkungslos. Die bisherigen Ergebnisse der Antibiotikatherapie bei Schafen lassen sich dahingehend zusammenfassen, daß die Behandlungserfolge bei jungen Lämmern gegenüber erwachsenen Schafen wesentlich besser sind und anderseits die Medikamente nur in ausreichend hoher Dosierung erfolgversprechend eingesetzt werden können, jeweils vorausgesetzt, daß die Applikation möglichst frühzeitig erfolgt.

Wir haben in Vorversuchen in 6 Gruppen spontan erkrankter Schafe Sulfonamide, Penizilline, Streptomyzin sowie die Kombinationen Streptomyzin (Penizillin und Sulfonamid) Penizillin geprüft. Dabei hatten Sulfonamide und Streptomyzin sowie die Kombinationen keinen Einfluß auf den Krankheitsverlauf, während die mit Terramyzin und Penizillin behandelten Gruppen eine auffällig verlängerte Krankheitsdauer bis zum letalen Ausgang aufwiesen. Da diese beiden Antibiotika gleichermaßen Aussicht auf Erfolg versprachen, wurden sie nach Ermittlung der wirksamen Dosis in der folgenden Zeit systematisch und kontrolliert zur Therapie der ovinen Gehirnlisteriose eingesetzt. Insgesamt 95 Tiere im Alter von 4 Monaten bis 5 Jahre wurden behandelt, 102 Patienten blieben ohne jegliche Therapie und dienten als Kontrolle. Alle unbehandelten Tiere der letztgenannten Gruppe verendeten nach einer durchschnittlichen Krankheitsdauer von 1,7 Tagen. Damit wird zunächst bestätigt, daß die Letalität der ovinen Gehirnlisteriose 100% beträgt. Wir müssen somit unsere früher geäußerte Ansicht (URBANEK und LIPPMANN, 1961) über die Möglichkeit einer Selbstheilung nach Beobachtungen an einem größeren Krankengut und bei Berücksichtigung einer exakten, klinisch hochwahrscheinlichen Diagnose revidieren.

Das Ergebnis der Therapieversuche, deren Patienten keinerlei Auslese unterlagen, faßt Tabelle 3 zusammen.

Vor Beginn der Therapie wurde die klinische Diagnose durch die Symptomatik, anamnestische und epidemiologische Erhebungen sowie besonders das Liquorsyndrom und andere labordiagnostische Befunde (Blutstatus, Serumeisenspiegel) gestellt. Alle während oder nach der Antibiotikatherapie ad exitum gekommenen Schafe konnten außerdem histologisch und z. T. bakteriologisch kontrolliert werden.

Die klinische Heilung konnte in der Regel über einen Zeitraum von 3 Monaten beobachtet werden. Die Befunderhebung zeigt, daß unabhängig vom Medikament bereits am 2. Behandlungstag die anfangs fieberhafte Kör-

Tabelle 3

Ergebnisse der Listeriosetherapie mit Antibiotika

Antibiotikum	Dosierungsschema	Tierzahl	Heilungs-quote	Überlebensdauer verendeter Schafe
Penizillin	*a)* anfangs 4000 IE/kg Penizillin-Na i.v. *b)* folgend über 5 Tage je 40 000 IE/kg tägl. Penizillin i.m.	52	30,8%	7,5 Tage (2—71)
Oxytetrazyklin	über 5 Tage je 10—15 mg/i.m.	33	60,7%	6,2 Tage (2—19)
Penizillin + Streptomyzin	über 5 Tage je 20 000 IE/kg Penizillin i.m. und 20 000 IE/kg Streptomyzin i.m.	10	20,0%	5,5 Tage (1—12)

pertemperatur zur Norm zurückkehrte, die zentralnervösen Ausfallserscheinungen jedoch unterschiedlich lange, meist 7—24 Tage nach Abschluß der Therapie, noch bestehen blieben. Die Liquorwerte kehrten während der Rekonvaleszenz in der Regel nach 42—72 Tagen zur Norm zurück, ein Ansteigen von Zellzahl und Eiweißgehalt unmittelbar nach Therapieabschluß ist prognostisch ungünstig zu werten und hat bei unserem Material in jedem Falle zum Exitus letalis geführt.

Während dieser Zeit kam es bei 8 der 38 später als klinisch geheilt entlassenen Patienten zu fieberhaften Rezidiven, die auf das gleiche Medikament gut ansprachen. Diesen zweiphasischen Heilungsverlauf beobachtete auch IWANOW (1962) bei antiobiotisch behandelter Schaflisteriose, nur verendeten in seinen Versuchen bei Rezidiven 5 von 28 ursprünglich ausgeheilten Schafen. Ein solcher typischer Verlauf ist in Abbildung 1 am Beispiel des Schafes 226/66 dargestellt. Während sich aus den Parametern des Liquors (Zellzahl, Neutrophilenanteil, Gesamteiweißgehalt) kein Hinweis für eine erneute Reaktion des Zentralnervensystems ergibt, fällt der Serumeisenspiegel einen Tag vor dem Rezidiv ab, um während und nach Abschluß der 5tägigen Oxytetrazyklinbehandlung kontinuierlich anzusteigen.

Auffällig war eine erhebliche Verlängerung der Krankheitsdauer bei während und nach der Behandlung verendeten Tieren, ohne daß es vorher zu Rezidiven gekommen war.

Einschränkend muß zu den Ergebnissen der Antibiotika-Therapie festgestellt werden, daß später etwa ein Drittel der erfolgreich behandelten Schafe der Verwertung zugeführt werden mußten, weil sie infolge nervöser Ausfallserscheinungen nicht herdentauglich waren. Diese haben ihre Ursache in bindegewebiger Organisation ehemals umfangreicher Parenchymschädigungen

bei akuter Gehirnlisteriose (POTEL und SCHLEICHER, 1965, 1967) und deuten auf zu späten Einsatz der Antibiotikabehandlung hin. Von besonderer Bedeutung für den Verlauf der Rekonvaleszenz ist die symptomatische Behandlung der Sekundär- und Folgezustände der Listeriose. Anfangs haben wir aus Gründen der Versuchsanstellung auf eine solche Zusatzmedikation verzichtet, später konnten wir durch Beeinflussung der durch die fehlende spon-

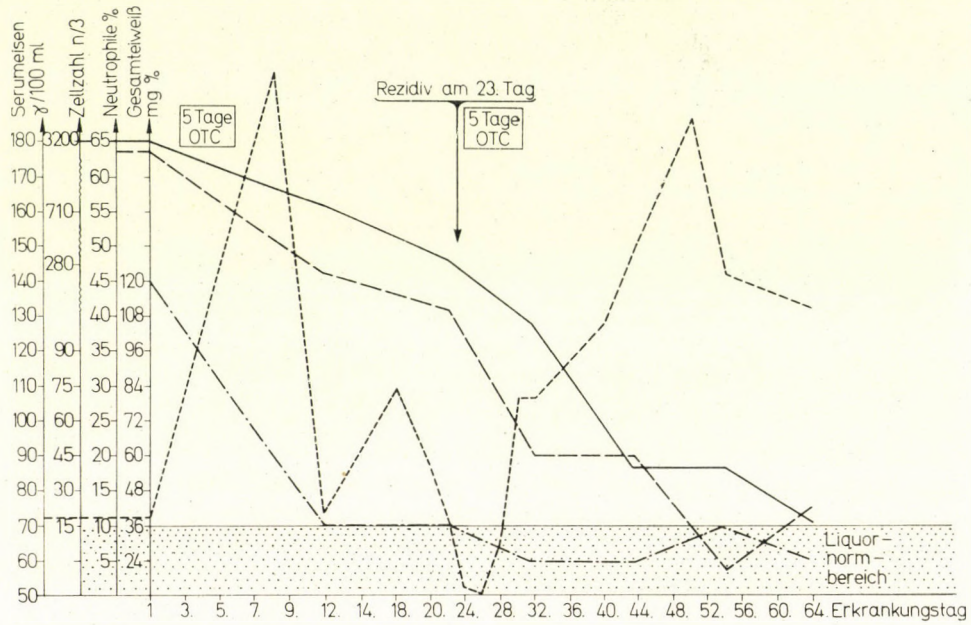

Abb. 1. Verhalten der Liquor- und Serumeisenwerte bei antibiotisch behandelter zerebraler Schaflisteriose (biphasischer Heilungsverlauf, Pat. Nr. 226/66)

tane Nahrungs- und Wasseraufnahme eingetretene Indigestion mit Pansensaftübertragung und der Exsikkose mit der intravenösen Gabe von Kochsalzlösung das Ergebnis der Therapie, ähnlich wie es JENSEN und MACKEY (1965) für das Rind empfehlen, verbessern. Die früher von uns empfohlene Infusion von Glukoselösungen (LIPPMANN, 1967) müssen wir nach Kenntnis des erhöhten Blut- und Liquorzuckerspiegels bei der Listeriose des Schafes heute als kontraindiziert ansehen.

Diese Therapieversuche an natürlich erkrankten Tieren bestätigen, daß die ovine Listerienenzephalitis mit einer 5tägigen Antibiotikatherapie beeinflußt werden kann. Eine solche Behandlung erscheint angesichts der vollständigen Letalität dieser Erkrankung bei wertvollen Zuchttieren gerechtfertigt. Bei gleichzeitiger Eliminierung listeriosefördernder Faktoren und ihrer Einflüsse gelingt es damit, die Verluste bei einer Listerioseenzootie im Schafbestand möglichst gering zu halten. Als geeignet hat sich der Einsatz von Oxytetrazyklinen und Penizillinpräparaten in einer täglichen Dosierung von

10—15 mg bzw. 40 000 IE/kg Körpergewicht erwiesen. Dabei ist der Oxytetrazyklintherapie wegen ihrer besseren therapeutischen Wirkung und der günstigeren ökonomischen Verhältnisse für Praxisbedingungen der Vorzug zu geben. Von entscheidendem Einfluß auf den Krankheitsverlauf und den Ausgang sind frühzeitiger Therapiebeginn, ausreichend hohe Dosierung und symptomatische Behandlung der Begleit- und Folgezustände.

ZUSAMMENFASSUNG

Nach einleitender Besprechung der Listeriosefrequenz im Raum Leipzig innerhalb von 10 Jahren werden Ergebnisse klinisch-labordiagnostischer und therapeutischer Untersuchungen zur Listerienenzephalitis des Schafes mitgeteilt. Aus differentialdiagnostischer Sicht läßt die Liquoruntersuchung eine rasche intravitale Abgrenzung dieser Erkrankung gegenüber anderen zentralnervösen Störungen des Schafes mit hoher Wahrscheinlichkeit zu. Nach Auswertung von 253 Liquoren spontan erkrankter Schafe wurde festgestellt, daß im Liquor cerebrospinalis eine hochgradige Pleozytose (300/3 bis 12 000/3 Zellen) vorwiegend neutrophilen Charakters auftritt. Die Gesamtproteine sind gegenüber den Werten gesunder Schafe signifikant erhöht (48—140 mg%), unter besonderer Beteiligung der Globuline (23—96 mg%). Diese Globulinvermehrung bedingt die Trübung des Liquors und führt zu einer Verschiebung des Globulin-Albumin-Quotienten auf den Wert 1 (normalerweise 0,35). Aus dem gleichen Grunde fallen die qualitativen Eiweißfällungsproben (nach PANDY, NONNE–APELT und WEICHBRODT) im Listerioseliquor verschiedengradig positiv aus und die Kolloidreaktionen ergeben Links- und Mittelkurven. Bemerkenswert ist der erhöhte Glukosegehalt im Liquor, der den des Blutes noch übersteigt. Nur in wenigen Fällen konnten im Liquorausstrich die Erreger nachgewiesen werden.

Zur Behandlung der ovinen Listerienenzephalitis haben sich Oxytetrazykline (10—15 mg/kg über 5 Tage) und Penizilline (40 000 IE/kg über 5 Tage) als geeignet und für Praxisverhältnisse empfehlenswert erwiesen.

60,7% der Tiere aus der Tetrazyklingruppe und 30,8% der mit Penizillin behandelten Schafe wurden klinisch geheilt, während alle 102 Kontrolltiere nach durchschnittlich 1,7 Tagen verendeten. Der Erfolg der Antibiotikatherapie hängt vom frühzeitigen Beginn, der ausreichenden Dosierung und der symptomatischen Beeinflussung von Indigestionen und Exsikkose ab.

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PARENTERAL INFECTION EXPERIMENTS WITH EIMERIA STIEDAI

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It is known that most coccidia enter the host organism by the oral route. In the digestive tract the oocysts give rise to sporozoites and the endogenous stages released from the latter settle and grow generally in the epithelial cells of the intestinal mucosa, but less often they invade also the epithelium of other organs (liver, kidney, *etc.*).

The possibility of parenteral infection by oocysts has been considered for several decades. Studying the developmental cycle of *Eimeria stiedai*, SMETANA investigated already in 1932 the route of coccidia from the digestive tract to the liver. He even attempted to administer *Eimeria stiedai* oocysts directly into one of the mesenteric veins. Unfortunately, the results were equivocal and SMETANA himself was unable to decide whether the subsequent liver coccidiosis was actually due to the parenteral infection or merely to an intercurrent infection of the rabbits used in the experiment.

In the 'sixties, interest was centered once again on parenteral infection with coccidia. SHARMA and REID (1962) infected chickens with doses of 1 million sporulated oocysts each by subcutaneous, intravenous, intramuscular and intraperitoneal routes. Using *E. tenella* oocysts they succeeded in eliciting a mild caecal coccidiosis. A similar condition could be induced also by the administration of 150 000 *E. tenella* sporozoites by the above routes. DAVIES and JOYNER (1962) reported similar parenteral infection experiments. The mature oocysts excysted within the organism of the chickens. The released sporozoites started to develop in the intestinal portions characteristic of their species *(Eimeria acervulina, E. maxima, E. tenella* and *E. necatrix)* and completed their endogenous cycle in due course. Nevertheless, the artificial infection was either milder than expected or sometimes failed altogether. Second stage merozoites of *Eimeria tenella* administered into the peritoneal cavity continued to develop and completed their endogenous cycle in the caecal mucosa. Experimental infection with subcutaneously or intramuscularly administered merozoites failed. In 1964, SHARMA reproduced the experiments formerly undertaken in cooperation with REID and arrived at similar conclusions. He found that the sporulated oocysts had excysted in the circulating blood within 1/2 to 2 hours without preceding disintegration and the released sporozoites gained access to the tissues characteristic of their species. Also the intramuscularly or subcutaneously administered oocysts had excysted, but deteriorated shortly afterwards; *e.g.*, the oocysts injected intramuscularly had lost their infectivity by the 7th day. The experimental coccidiosis was invariably mild. The parenteral infection of chickens evoked only a very weak immune response. FITZGERALD (1965) inoculated sporulated *Eimeria bovis* oocysts into cattle, intraperitoneally, subcutaneously, intramuscularly and intravenously. He performed similar infection experiments with nonsporulated oocysts, to investigate whether the oocysts not having undergone the exogenous developmental cycle were able to evoke an immune response. Nevertheless, exclusively the intraperitoneally administered sporulated *Eimeria bovis* oocysts were able to elicit an intestinal coccidiosis in calves and to render them moderately immune to challenge. LONG and ROSE (1965) reported intravenous infection of chickens with *Eimeria tenella* sporozoites, resulting in an immune response equivalent to that evoked by experimental oral infection. PATNAIK (1966) infected chickens by various parenteral routes and succeeded in producing a mild caecal coccidiosis. Using non-disintegrated oocysts, he holds the view that in excystation leucocytes may play an active part, while the blood plasma and the red blood cells do not.

Experimental

The present experiments were undertaken to clarify whether or not the parenterally administered *E. stiedai* oocysts can settle and give rise to an infection in the organism of the rabbit. In part of the experiments oocysts excysted in a grinder, in part of them untreated mature oocysts were used for inoculation into the auricular vein, peritoneal cavity or femoral muscle. To prevent bacterial contamination, a loopful of a penicillin—streptomycin mixture was usually added to the inoculum.

Table 1

Parenteral infection of rabbits with intact and excysted *Eimeria stiedai* oocysts*

Days after infection	Infection with excysted oocysts			Infection with intact oocysts			Control
	i.v.	i.m.	i.p.	i.v.	i.m.	i.p.	
0	Inf. dose: several millions of *E. stiedai* oocysts						
16	+++	++	++++	died	+	+	neg.
Liver weight (% of body weight)	8.8	8.3	11.6	—	5.8	5.9	4

* The degree of liver lesions is marked with 1 to 4 crosses.

To prevent premature natural infection with *Eimeria stiedai*, the experimental animals were put daily into fresh cages sterilized with heat, throughout the entire period between weaning at 18 days of age and the start of the experiment at 3—8 weeks of age. By this method, intercurrent liver coccidiosis could be excluded in the overwhelming majority of the cases. The sporadic intercurrent infections did not interfere with the valuation, as histological examination enabled a precise timing as to whether the infection was related or unrelated to the experiment.

Experiment 1. Nine rabbits, 5 weeks old, were divided into 3 equal groups. The 3 rabbits in group 1 received several millions of excysted *Eimeria stiedai* oocysts, one into the auricular vein, another into the femoral muscle and the third into the peritoneal cavity. In group 2, the same doses of untreated mature oocysts were applied by the same routes. Group 3 served as uninfected control. In group 2 the intravenously infected rabbit died of lung embolism during the inoculation and was, therefore, not available for comparison. The data pertaining to the other routes of infection are shown in Table 1.

The above data and the microscopic examinations have shown that artificial infection was most efficient with excysted oocysts by the intraperitoneal route. The liver weight of the rabbit infected by this method reached 11.6% of the body weight, as compared to the 8.8 and 8.3% in the case of

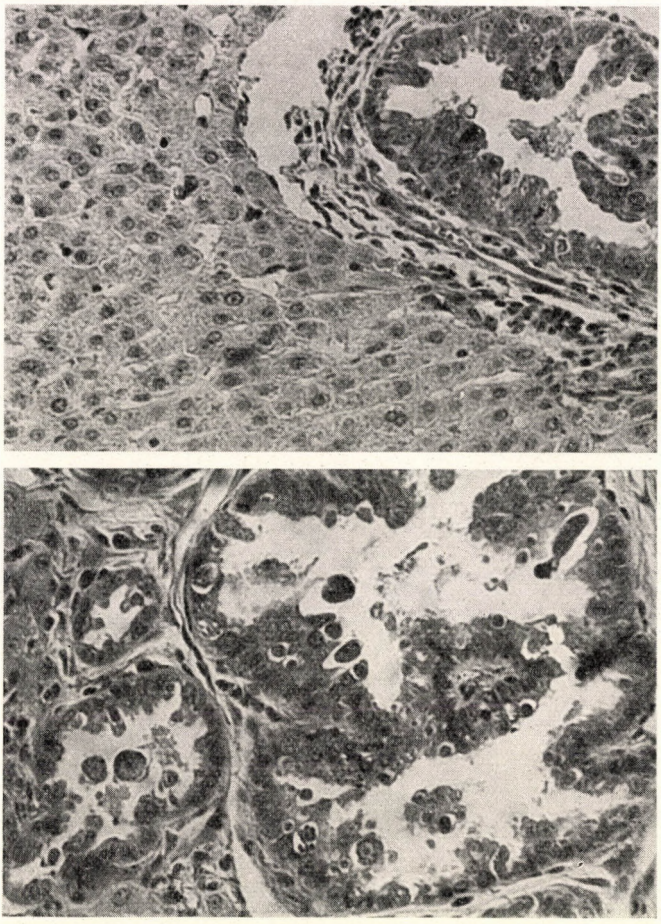

Fig. 1. Microscopic picture of rabbit liver on the 16th day after intraperitoneal infection with intact (top) and excysted (bottom) *Eimeria stiedai* oocysts. H. E. × approx. 400

the two other rabbits. Infection with intact mature oocysts caused a notably lesser damage of the liver (Fig. 1) as shown not only by the microscopic picture, but also by the lower liver weights and the lesser number of gross coccidial lesions. As compared to the perfectly normal liver of the control rabbits, the livers of the infected rabbits were somewhat larger and in relation to each other there was a slight difference in favour of the intraperitoneally infected animal.

It should be mentioned here that oral infection with 100,000—200,000 sporulated *Eimeria stiedai* oocysts produces considerably more severe gross and microscopic lesions (Fig. 2). In some cases the weight of the liver may even reach 25% of the body weight. This implies that in the parenteral infection experiment the majority of the infective oocysts had disseminated and been hold up somewhere in the organism, thus only the minority of them had reached their specific site of settlement, *i.e.*, the biliary ducts.

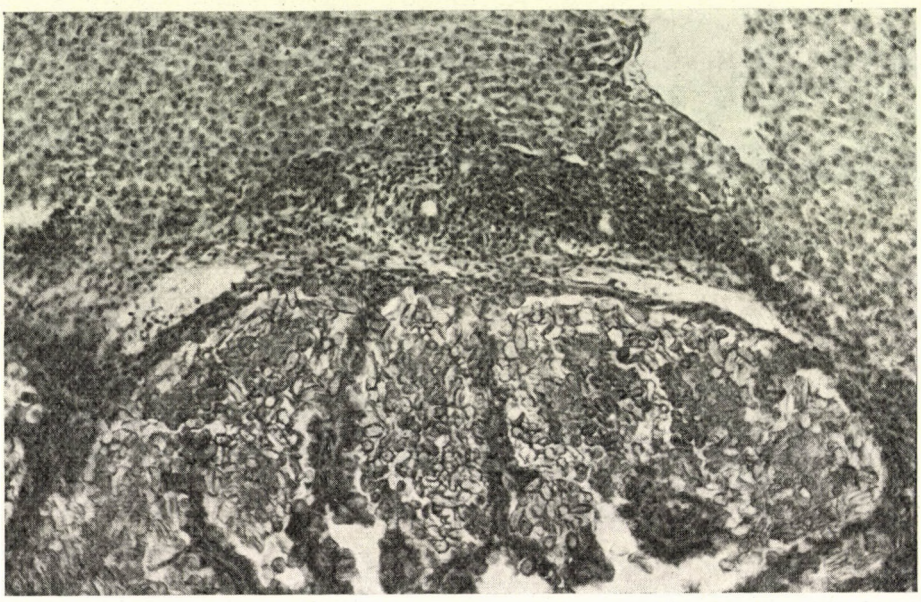

Fig. 2. Microscopic picture of rabbit liver on the 16th day after oral infection with approx. 100,000 *Eimeria stiedai* oocysts. H. E. × approx. 150

Another phenomenon, revealed by microscopic examination of the liver of the intramuscularly infected rabbit killed on the 16th day, was that instead of the gametogonic stages corresponding to this period of the *endogenous* cycle chiefly earlier schizogonic stages were present. This implies a delay for 2 or 3 days of the endogenous development, due very likely to the parasites' complicated passage from muscle to liver as compared to the peritoneum-to-liver or vein-to-liver ways. A substantial part of the intramuscularly administered oocysts was still demonstrable microscopically in the muscle tissue on the 16th day, surrounded by inflammatory cell elements, giant cells and debris (Fig. 3).

Experiment II. Dissemination in the host organism of oocysts injected into the auricular vein was examined on 4 rabbits, 4—5 weeks old. They were inoculated each with a dose of several millions of sporulated oocysts. One of

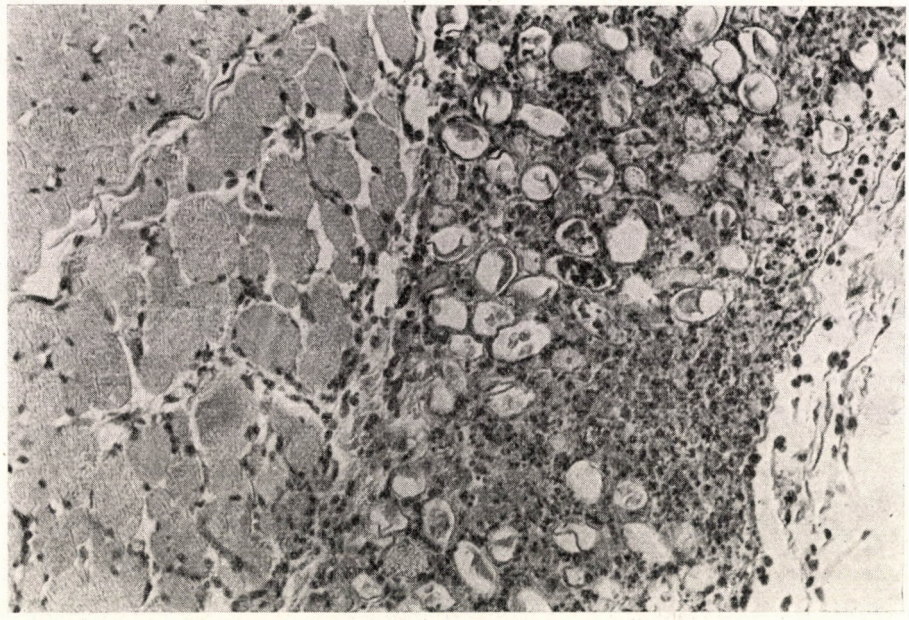

Fig. 3. Eimeria stiedai oocysts on the 3rd day after administration into the femoral muscle. The indented cyst walls are sharply defined against the inflammatory cell elements. Note the intact muscle tissue left below. H. E. × approx. 150

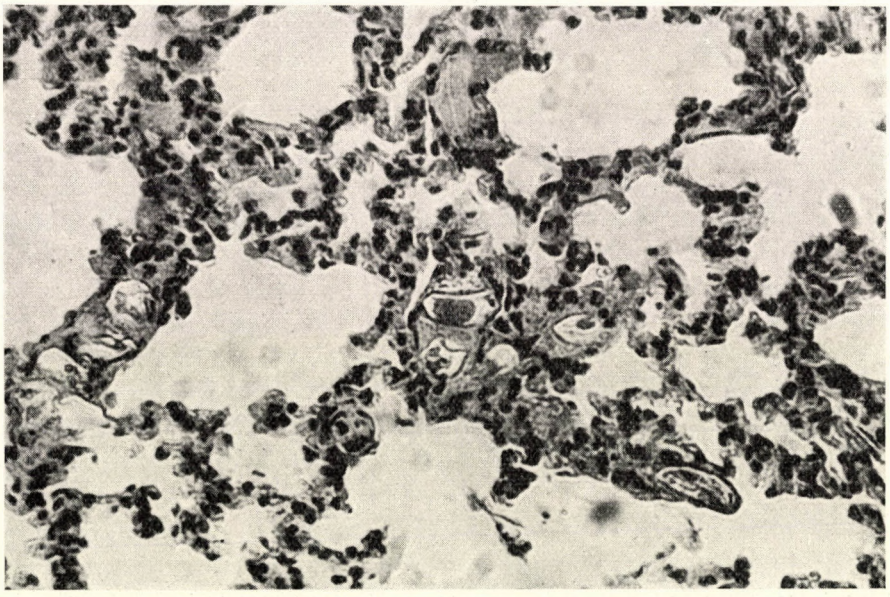

Fig. 4. Microscopic picture of the lung of the rabbit died of injection trauma (lung embolism) during the administration of oocysts. In the capillary vessels retained oocysts, with surrounding atelectatic and emphysematous lesions, are seen. H. E. × approx. 300

them died of lung embolism during the injection (Fig. 4). The remaining 3 rabbits were killed 4 hours after the injection and the passage of the oocysts in the organism was followed up in stained and unstained preparations. It was found that the first severe obstacle to the passage of oocysts was presented by the lung capillaries, where the retained oocysts gave rise to atelectatic and vicarious emphysematous lesions with inflammatory cellular reaction in the affected areas. Nevertheless, part of the oocysts and free sporozoites were capable of passing through some lung capillaries and were carried further

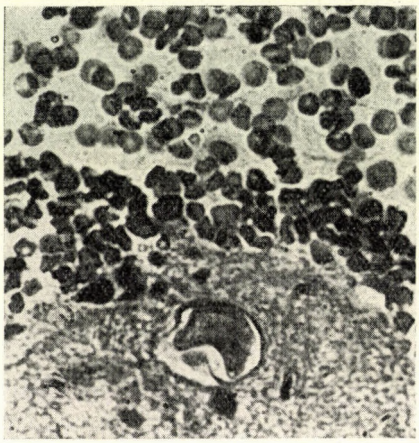

Fig. 5. Eimeria stiedai oocysts in the heart blood. Giemsa stain. × approx. 500

by the blood stream. The oocysts traced in the lungs by microscopic examination had mostly intact or indented walls. Infrequently, oocysts were seen also in other organs, as *e.g.*, an oocyst with indented wall was apparent in a renal glomerule with no surrounding inflammatory reaction present. A few oocysts were demonstrated among red blood cells in unstained blood smears from the left cardiac ventricle (Fig. 5). Larger numbers of oocysts with intact or indented walls were seen in the liver. Most of them had undergone excystation, but the release of sporozoites was, owing to their poorly defined structure, implied rather than seen from the many empty shells of sporocysts apparent in the liver. The coccidia which did not reach the liver had presumably deteriorated and their remains were eliminated in the usual way.

Experiment III. Three rabbits, 2 weeks old, were infected intraperitoneally with several hundreds of thousands of excysted *Eimeria stiedai* oocysts. Three uninoculated rabbits of the same age served as controls.

One of the infected rabbits died on the 12th day of the experiment. Its liver was slightly enlarged but no gross lesions indicative of coccidial infesta-

tion were seen. Microscopic examination, however, revealed an early stage of proliferation in the biliary capillaries and the developmental stages seen in the lining epithelium corresponded to the regular picture seen on the 12th day after oral infection.

The two surviving rabbits were killed on the 17th day of the experiment. Intraperitoneal infection with oocysts gave rise to liver coccidiosis in both cases. As in this particular experiment the infective dose contained several hundreds of thousands instead of several millions of oocysts, the liver lesions were correspondingly milder. Similarly, proliferation of the biliary capillaries was of a considerably lower degree than after oral infection of the same strength. Further to this, the success of the experimental infection was confirmed by the presence of many *Eimeria stiedai* oocysts in the bile as compared to their complete absence in the perfectly normal livers of the 3 control rabbits.

Experiment IV. This experiment was undertaken to compare the efficiency of oral and intravenous infection in inducing liver coccidiosis.

The experiment was started on 6 rabbits, 3 weeks old, out of which two died during the administration of the infective material. Thus, only the surviving 4 animals, 2 infected intravenously, 1 orally and 1 serving as control, were available for comparison.

The results were read on the 18th day after infection (Fig. 6). Relating the liver weights to body weights, the succession was 7% for the orally infected rabbit, 5.6 and 5.9% for the two intravenously infected rabbits and 4.9% for the control rabbit. The most distinct lesions were found in the liver of the orally infected animal. The Figure may be deceptive as to the enlargement of the livers, since the body weights of the animals were variable (600—750 g). Anyway, in previous oral infection experiments both the liver enlargement and the lesions were more distinct on the 18th day than in this particular instance. The lesser difference between the sizes of the livers of orally and parenterally infected rabbits in the present experiment may be explainable by the fact that the infective material had been stored in the refrigerator for about one year. Nevertheless, the available results have satisfactorily confirmed the possibility of parenteral coccidial infection.

Experiment V. This experiment was undertaken to reproduce Experiment IV with a more reliable infective material. The degree of liver lesions developed after experimental infection with equal oral and intraperitoneal doses of *Eimeria stiedai* was compared.

Out of 6 rabbits, 3 weeks old, 3 were given intravenously 100,000 freshly sporulated, excysted *Eimeria stiedai* oocysts, two rabbits received identical doses of untreated mature oocysts intravenously, the last rabbit received the same orally.

The livers of the animals, killed on the 17th day after infection, are shcwn in Fig. 7. The orally infected, emaciated control rabbit had the greatest liver weight — 17.3% of the body weight — and distinct lesions corresponding to the 17th day of the infection. Enlargement of the liver was less pronounced — 6.5, 6.9 and 7.1%, respectively — and coccidial foci were lesser in number in the case of the three rabbits infected intravenously with excysted oocysts.

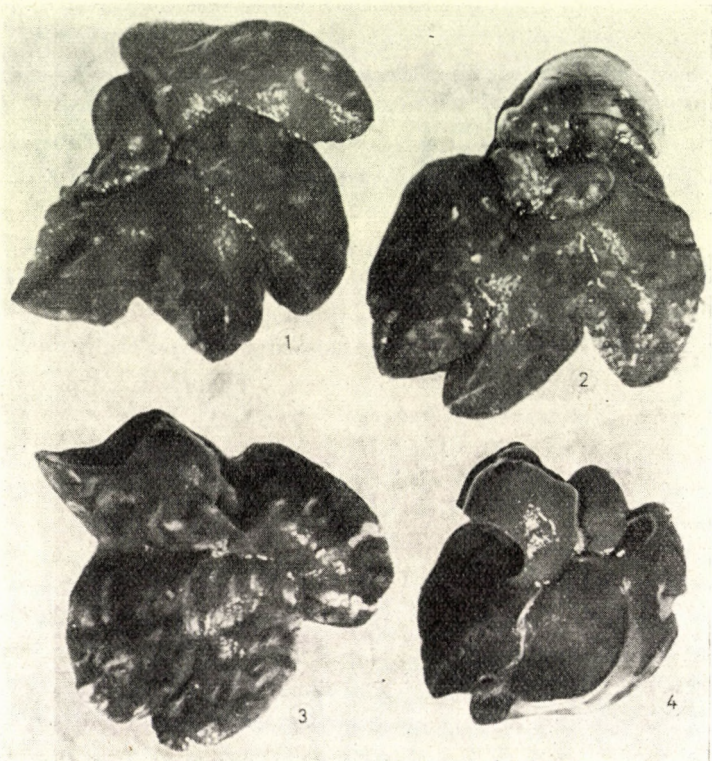

Fig. 6. Livers of intravenously infected (1—2), orally infected (3) and uninfected (4) rabbits. Approx. two thirds of the natural sizes

The livers of the two animals intravenously infected with untreated sporulated oocysts showed the least enlargement — 6.5 and 6.8% — and hardly any lesions.

In every case, *Eimeria stiedai* oocysts were demonstrable in unstained preparations of gallbladders. Histological examination revealed a uniform progress of endogenous development in all livers, indicating that the endogenous development was normal in that chiefly mature gametogonic stages and oocysts were seen in both the lumina and epithelium of the markedly

distended biliary ducts. Only the degree of the lesions differed. In the liver of the orally infected rabbit hardly any normal liver tissue was seen between the enormously proliferated biliary ducts. In the livers of rabbits infected

Fig. 7. Livers of rabbits infected orally (1), and intravenously with excysted (2—4) and intact oocysts (5—6). Approx. half of the natural size

with excysted oocysts proliferation was less pronounced and coccidial foci were scarcer. Finally, in the livers of the animals infected with intact mature oocysts of *Eimeria stiedai*, the foci were sporadic, though pronounced and sometimes of considerable size.

Experiment VI. Of 4 rabbits, 4 weeks old, 2 were infected orally each with 35,000 sporulated oocysts. On the 14th day after infection they were killed and their liver homogenates were used to infect through the auricular vein the two other rabbits kept until then in isolation. Throughout the entire procedure care was taken to keep the liver homogenate at 37 °C, to prevent the merozoites' damage by cooling. On microscopic examination of the homogenate, a considerable number of endogenous stages typical of the 14th day of infection — schizonts containing 6 or 7 merozoites — were seen.

One of the two rabbits infected with merozoites through the auricular vein had died on the day after infection. As post mortem findings were indicative of trauma and icterus, this animal was excluded from the evaluation.

The remaining single rabbit, infected with merozoites gained on the 14th day of the infection, was examined at a time, when the completion of the endogenous cycle would have been due in the case of an oral infection. This term being normally 17 days, the animal infected with merozoites was killed after 3 days, postulating that if endogenous development had continued uninterruptedly after the transfer, final stages (oocysts) should have been found in the liver.

But the histological findings failed to confirm this hypothesis. The proliferation of the biliary ducts was moderate, although the invading merozoites (trophozoites) were clearly apparent within, and between, the epithelial cells of the biliary ducts and the surrounding inflammatory cell reaction showed an incipient liver damage. This indicated that though the 14-day-old endogenous stages of *Eimeria stiedai* transferred from a donor were able to settle in the liver of the recipient, there was a delay in the developmental cycle. This appears to be explainable by the fact that the merozoites had to pass from the blood capillaries to the biliary ducts which was a task not normally encountered in their life cycle. The performance of this unexpected task had obviously deteriorated many merozoites. Another explanation offered for the delay of endogenous development may be that in the intact biliary ducts where the proliferation had not yet started the merozoites did not immediately find the circumstances favouring their development.

*Experiment VII.** Three rabbits, 8 weeks old, were used. Two of them were given excysted *E. stiedai* oocysts into the mesenteric vein, while the third served as untreated control. The control animal died of an intercurrent disease after a few days. The gross and microscopic findings of its liver were negative in respect of coccidiosis, serving as a reliable proof that also the two other animals were free of coccidia prior to infection.

* This and Experiment VIII were performed in cooperation with Dr. U. DÜRR, assistant professor of the Justus Liebig-University, Giessen, German Federal Republic.

The two rabbits infected through the mesenteric vein were killed on the 18th day after infection. Both had moderately enlarged livers with coccidial foci on the surfaces and in the incisions. Oocysts were demonstrated also in the bile. The histological picture was typical of an 18-day infection: proliferation of biliary ducts, surrounding inflammatory reaction and coccidial foci were seen. Relatively many empty shells of oocysts were found perivascularly and the adjacent haemorrhages indicated that the oocysts had obstructed the capillaries and gained access to the liver tissue after the breaking of the capillary wall.

Thus, it has been evidenced that liver coccidiosis can be elicited by infection with excysted *Eimeria stiedai* oocysts through the mesenteric vein. The experimental coccidiosis was mild as compared to that induced by oral infection with a similar dose of oocysts. An explanation for this difference has been offered in context of Experiment VI.

Experiment VIII. Experiment VII was repeated on 2 rabbits with the difference that the oocysts excysted by grinding were treated additionally with pancreatin. It was assumed that this way a greater number of sporozoites would release and bring about a more massive infection. But the gross and microscopic examination of the livers of the animals killed on the 17th day failed to confirm this supposition as no difference was found in comparison to the lesions caused by infection with oocysts not treated with pancreatin.

Discussion

According to literary data, chickens developed coccidiosis after parenteral administration of oocysts. SMETANA (1933) performed similar experiments on rabbits, though with equivocal results. The aim of his experiments was to follow up the intestine-to-liver passage of *Eimeria stiedai*, his parenteral infection experiments on chickens had the practical aim to examine the possibility of active immunization against coccidiosis.

The present experiments have shown that in rabbits, experimental liver coccidiosis can be uniformly induced by intravenous, intraperitoneal or intramuscular administration of *Eimeria stiedai* oocysts. In every case, the condition was much milder than that elicited by the oral route, owing very likely to the fact that the parenterally administered coccidia have to overcome several obstacles during their passage to the liver and many of them die in the meantime. Also, it has been evidenced that intraperitoneally administered sporulated oocysts cause a liver coccidiosis. The mode of the passage of oocysts through Glisson's capsule, however, remains to be clarified.

Interestingly enough, no particular difference has been found between the results of parenteral infection with intact and artificially excysted oocysts.

This circumstance appears to support the view of SHARMA (1964) and others that intact sporulated oocysts excyst in the blood stream.

Parenteral infection was successful not only with oocysts, but also with intravenously administered merozoites obtained from the livers of rabbits on the 14th day of infection. The particularity of this way of infection is that the endogenous development does not continue immediately in the recipient rabbit, but only after certain delay. Apparently, it takes some time until the originally intact biliary ducts reach a state which favours the development of coccidia and until the resistance of the epithelial cells is overcome by the parasite. Last but not least, the merozoites originating from the donor's biliary ducts need time to find their way from the recipient's capillary vessels to the biliary ducts.

SUMMARY

Infection experiments by intravenous, intramesenteric and intramuscular administration of intact and excysted *Eimeria stiedai* oocysts have shown that *Eimeria stiedai* is parenterally transferable to susceptible rabbits. The experimental liver coccidiosis was in every case milder than that elicited orally by similar doses. The condition was mildest on intramuscular infection, owing apparently to the difficult passage of the oocysts from the femoral muscle to biliary ducts. Intraperitoneal infection was particularly successful, sometimes even more so than the intravenous infection. No notable difference was found between the results of parenteral infection with intact and excysted oocysts, indicating that excystation may actually take place in the blood stream (SHARMA, 1964). A susceptible rabbit developed liver coccidiosis on administration into a mesenteric vein of liver homogenate from a donor rabbit infected 14 days earlier. The homogenate contained numerous schizonts and merozoites. In the recipient, the developmental cycle was somewhat delayed, owing very likely to the merozoites' slow passage from capillary vessels to biliary ducts, a task not encountered in their cycle under normal conditions.

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OCCURRENCE OF INFLUENZA A/EQUI-2 INFECTION OF HORSES IN HUNGARY

By

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In autumn 1968, a highly contagious febrile respiratory disease appeared among horses in Hungary. In this paper clinical symptoms, epidemiological observations and aetiological studies concerning the disease are reported.

Equine respiratory disease caused by influenza virus was first observed in Sweden in 1955 (Heller, Espmark and Viriden, 1956, Espmark and Salenstedt, 1964), but the causative agent was isolated by Sovinova et al. (1958) during an epizootic among horses in Czechoslovakia in 1956. The isolated strain was designated influenza A/equi/1/Prague. The result of a world wide serological survey organized by the WHO (Kaplan and Payne, 1959) evidenced that the infection is widely distributed in the world, including Hungary (Romváry et al., 1966).

In 1963 a highly infectious respiratory disease was observed among horses wintered in Miami, USA. The clinical symptoms started with temperature elevation which was followed by coughing and catarrhal rhinitis. All horses became affected regardless of age, breed and sex. From this outbreak a new subtype of influenza A virus was isolated which was designated influenza A/equi/2/Miami (Waddell et al., 1963). In the same year the virus was introduced into Canada (Marois et al., 1963). In 1965 the disease appeared in Europe in Great Britain (Rose, 1965), France (Brion et al., 1966) and Switzerland (Gerber et al., 1966). In the late summer of the last year the virus reached the southern border of Hungary.

History

The disease was first observed at nearly the same time in three southern counties from where it was spread to all over the country in four to five months, as illustrated in Fig. 1. In the autumn the rate of spreading was faster than in early winter, according to the moving rate of horses. In the rapid spreading of the disease transportation of agricultural products played the primary role. Horses from different farms came in contact at the product-collecting centres and after returning they infected not only their stable mates, but also horses kept in other plants of the respective farm. From one county to the other, or even farther the virus was carried by racing horses and by those of itinerate vendors. Into Budapest and its surroundings the virus was introduced early in the autumn, possibly by horses used for transportation of vegetables and fruits from villages even far from the city.

The incubation period lasted usually for 2 to 3 days, in extreme cases 1 to 5 days. Among horses kept in a common air-space the disease frequently appeared explosion-like, because the virus was excreted by infected horses already

before clinical symptoms appeared. Usually the stock housed in common stable became infected within 48 to 72 hours. Nevertheless, horses kept in isolated plants of the farms escaped the disease, if direct contact with the affected horses could be avoided. Depending on the number and distribution of the horses in the farm, the acute disease ran its course in 2 to 4 weeks.

The disease started with a rise of temperature up to 41.5 °C, dullness and transient or complete inappetence. The conjunctiva and nasal mucous membrane became hyperaemic, and lacrimation and serous nasal discharge appeared.

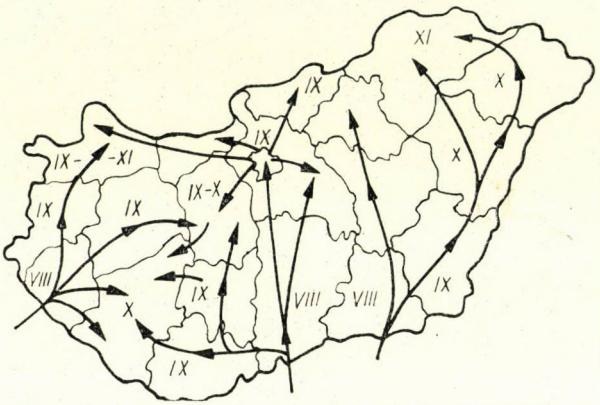

Fig. 1. Spreading of the disease in Hungary from the middle of August 1968

Two days following the onset of the disease spontaneous dry cough set in. Usually one day later the character of the cough changed to moisty, the nasal discharge became of mucous and later of muco-purulent character. In horses rested from the onset of symptoms the fever lasted usually for 4 to 5 days and coughing persisted for about 2 weeks. In some horses kept in work during the febrile period, fatal complications as pneumonia, pleurisy and acute emphysema joined the acute symptoms in the 2nd week.

The same symptoms and course of the disease could be observed in donkeys, except that involvement of the upper respiratory tract was more pronounced and severe complications could be observed more frequently than in horses.

Materials and methods

Virus isolation. Nasal, pharyngeal and conjunctival specimens were taken by swabs from horses at the initial stage of the disease. Lungs of two aborted foetuses, one of them with unknown history were also investigated. Swabs were put in tubes containing Hanks' solution to which 1000 IU penicillin and 100 gamma streptomycin per ml was added and kept in the ice box until prepared

for inoculation. After rinsing in Hanks' solution samples were centrifuged at 3500 rpm for 20 minutes and each of the supernatants were inoculated both into the amnion and allantoic cavity of 5 to 6 embryos preincubated for 11 days, according to the method described by PACCAUD et al. (1966). The inoculated hen's eggs were further incubated at 33 °C for 72 hours, then cooled at +4 °C for 12 hours. Death of embryos in the first 24 hours was considered due to bacterial contamination, therefore such eggs were discarded.

Amnion and allantoic fluids were collected separately and tested for the presence of a hemagglutinating (HA) agent. In the HA test guinea pig and chicken red blood cells were used, and Takátsy's plates and microtitrator were employed. Subinoculations were made in the same way with 0.2 ml egg fluid diluted to 10^{-1} to 10^{-2}, according to the HA titre, into the allantoic cavities of a new series of embryonated eggs. Egg fluids negative in the HA test were pooled and subinoculated as before. At least 5 blind passages were carried out with each of the samples before discarded as negative.

Serological test. Paired serum samples were collected at an interval of 14 to 21 days from 8 studs. Only convalescent sera were taken in another 37 stocks kept in different regions of the country. Sera of attendants being in contact with diseased horses and showing respiratory symptoms and those of cattle suspected of infection were also tested. Serum samples were kept at −20 °C until tested. To eliminate unspecific inhibitors all sera were treated with M/60 KJO_4 and inactivated at 56 °C for 30 minutes before used in the haemagglutination inhibition (HI) test. In doubtful cases treatment of sera with KJO_4 was made at 37 °C instead of room temperature.

HI test was performed at +4 °C according to Lannette's method and using Takátsy's microtitrator. The test was carried out using 4 HA units of virus and chicken red blood cells. Sera were tested both against one of our isolates (5690/7) and equi A/1/Prague strain. The highest serum dilution yielding complete inhibition was regarded as titre value.

Results

Isolation of the virus was attempted from 31 samples taken in 7 studs seated in different regions of the country. As a total 18 strains from 6 studs were isolated. One of the strains was isolated from the nasal discharge of a donkey. The virus strains agglutinated both guinea pig and chicken red blood cells. Chloroform added in 20% concentration to the infective allantoic fluid inactivated the virus almost completely.

In lack of corresponding type sera, one of our isolates (5690/7) was sent to the WHO World Influenza Centre, London where it was identified *Myxovirus influenzae* A/equi-2 subtype. [On studying the same epizootic ROMVÁRY et al. (1968) reported in a preliminary paper the isolation of the same subtype.]

The isolates did not produce any cytopathic effect, and no haemadsorption was revealed in monolayer cultures of calf kidney and testicle, rabbit kidney and chicken kidney tissue inoculated with the virus. From the lung of the aborted foetus with unknown history rhinopneumonitis virus was isolated. Virological investigation of a second aborted foetus gave a negative result.

The serological survey revealed that all horses in the country had been susceptible to the A-2 subtype before this epizootic. This was evidenced by the fact that no specific antibodies could be demonstrated in the serum sam-

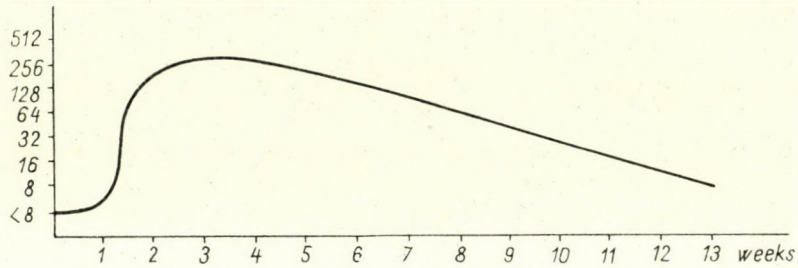

Fig. 2. Changes in average titre values of the HI antibodies as plotted against time

ples taken in the acute phase of the disease. Antibodies against equi A-2 virus were not found in any sera collected in 1967 either. ROMVÁRY et al. (1966) reported also that all horses in Hungary had been fully susceptible to equi A-2 subtype. The recent epizootic was caused by this subtype alone, because titre values of the paired sera from all studs had shown at least fourfold increase as compared to the initial titre of <8, and in serum samples collected during the reconvalescence in the additional 37 stocks distributed in different regions of the country, antibodies against equi A-2 subtype could be demonstrated.

Antibodies demonstrable in the HI test appeared in the second week of the disease and after reaching an average level of 1 : 256 in the 2nd to 3rd week, the titre gradually dropped to a value of 1 : 16 three months later. At this time in some of the sera antibodies could not be demonstrated any more, even in a dilution of 1 : 8 (Fig. 2).

Antibodies against equi A-1 subtype were demonstrated in 80% of the studs tested, but only in sera of horses older than 6 years of age.

In the sera of men and cattle which suffered from respiratory disease at the time or shortly after the affection of horses, no antibodies could be demonstrated either against equi A-1 or equi A-2 virus.

In Figs 3 and 4 results of HI tests with some of the paired serum samples are illustrated. While in the former one results obtained in a stud infected earlier with equi A-1 are shown, in the latter one those obtained in a stud which escaped equi A-1 infection are presented.

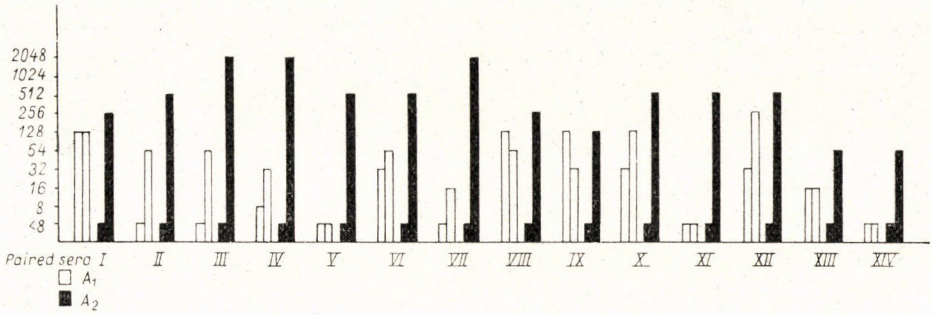

Fig. 3. HI titres to equi A-1 and A-2 subtype of paired sera from a stock previously infected with influenza A/equi-1 virus

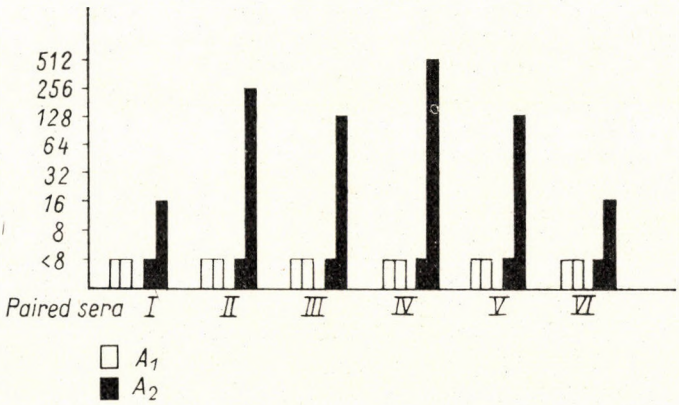

Fig. 4. HI titres to equi A-1 and A-2 subtype of paired sera from a stock free of influenza A/equi-1 infection

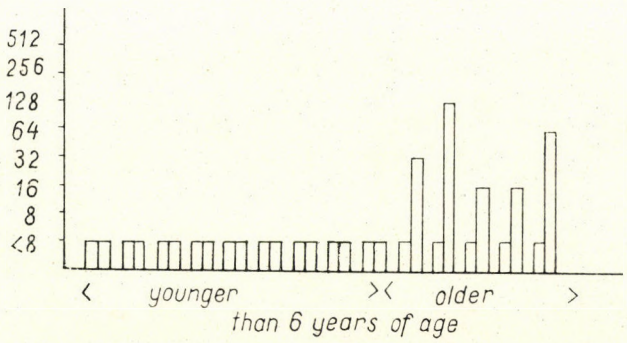

Fig. 5. Changes in HI titres to influenza A/equi-1 of sera taken from horses of various age during the epizootic caused by influenza A/equi-2 subtype

Discussion

In autumn 1968 a rapidly spreading equine respiratory disease occurred in this country. From the diseased animals *Myxovirus influenzae* A/equi-2 has been isolated. The disease differed in many respects. from equine respiratory diseases known so far in Hungary. Among these its trend to rapid spreading was the most remarkable. The first cases appeared along the southern border of Hungary in the middle of August 1968, and in 5 months the infection spread over the whole country, leaving only some stocks uninfected.

In our experiences the disease is principally spread by direct contact. Explosion-like appearance in horses kept in a common air-space points to droplet (aerogenous) infection. In carrying the virus to long distances racing and cart horses as well as those of itinerant vendors played an important role. Accordingly, new foci of infection far from each other arose from which the virus was introduced into adjacent farms by the horses used for local transportations.

The epizootiologically important question how long the infected animals would excrete the virus could exactly be cleared only by transmission experiments. No such experiments could be carried out by us, but the virus could be isolated from the nasal discharge taken as late as on the 5th day following the onset of the disease, when the fever already ceased. Probably, the virus is excreted by coughing, at least until the antibodies would appear in the blood serum. Generally, this takes 7 to 10 days. Consequently, the affected animals must be taken under restriction at least for as long as 2 weeks.

Our observations are in agreement with those of PACCAUD et al. (1966) in respect of the observation that only solipedous animals are affected. No HI antibodies could be demonstrated in serum samples of men and cattle collected parallelly with those of affected horses.

Specific antibodies against equi A-2 virus were demonstrated in the HI test in all convalescent sera. However, in some sera also antibodies to equi A-1 subtype were revealed. This heterologous antibody rise may be explained with the recalling action of the common type-specific component of the equi A-2 viral antigen in animals that had been infected with the heterologous equi A-1 strain earlier. Our suggestion on the specificity of this antigenic action was supported also by the fact that no changes in titre values to rhinopneumonitis antigen could be demonstrated in these sera. This opinion is confirmed also by the results of the serological tests performed with serum samples taken in the stock of one of the farms. Antibodies against equi A-2 subtype were found in all sera, but those against equi A-1 subtype were present in no but sera of horses over 6 years of age (Fig. 5). It was revealed that a respiratory disease resembling clinically that observed recently had occurred in the horses of this stock in 1962 (SZELÉNYI, 1968). By retrospecting the history and based on our present investigations, the causative agent of that affection must un-

doubtedly have been equi A-1 virus. The fact that antibodies to equi A-1 subtype were found only in horses older than 6 years of age of this stock, indicates that no influenza virus infection had occurred in the stock for the last 6 years. Simultaneous rise in titre of the HI antibodies to the heterologous subtype reported by MINUSE et al. (1965), PACCAUD et al. (1966) and ROSE (1966), has been explained on a similar basis.

Despite its high contagiousness the disease took a mild course. Though morbidity attained practically 100%, mortality was very low, not exceeding 0.5%. Deaths occurred mainly due to complications as pneumonia and pleurisy. Some of the old horses, however, which suffered from chronic respiratory or vascular diseases succumbed to the acute phase of the disease despite their having been rested from the very beginning of the symptoms. Generally, pregnant mares and donkeys were affected more severely.

Acknowledgement

We are greatly indebted to Dr. H. G. PEREIRA, London, for typifying our isolate and to Dr. V. POPOVICI, Bucharest, for supplying the *Myxovirus influenzae* A/equi-1/Prague strain.

SUMMARY

A highly contagious equine respiratory disease which appeared in Hungary in autumn 1968 is reported. Horses of most stocks in the country were affected regardless of age, breed and sex. The clinical symptoms, the course of the disease and epizootiological observations are described.

From 31 specimens (nasal, pharyngeal and conjunctival swabs) taken in 7 stocks kept at different regions of the country 18 virus strains were isolated in embryonated hen's eggs. The strains were identified *Myxovirus influenzae* A/equi-2.

As a result of serological tests performed with serum samples collected in the former 7 farms and in another 37 ones, it was evidenced that in the epizootic influenza A/equi-2 virus alone was involved, though in the sera of some of the horses older than 6 years of age appearance or rise of antibody titre to influenza A/equi-1 subtype could also be demonstrated. The phenomenon is explained by the stimulating action of the common type-specific antigen of equi/A viruses, because it could be demonstrated only in horses beyond a certain age, whose previous infection with influenza A/equi-1 virus was demonstrable.

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РЕЗЮМЕ

ЖЕЛЕЗО В ПЕЧЕНИ ЦЫПЛЯТ ПОРОДЫ БЕЛЫЙ ЛЕГХОРН В РАННИЙ ПОСЛЕИНКУБАЦИОННЫЙ ПЕРИОД

З. СОВА, З. МАРЕК, Й. ХОУШКА и К. КОУДЕЛА

У цыплят, распределенных в разные возрастные группы — от вылупления до 31. дня — изучено содержание железа в печени. В момент вылупления цыплят наблюдали только 44 μг железа в печени, что равняется только 11,28 $\pm$ 1,88 мг%-ам сухого вещества. До возраста 4 дней содержание железа возрастает до 17,9 мг%-ов сухого вещества. В период между 4-ым и 7-ым днем возраста при неизменном абсолютном содержании железа (к 4. дню — 73 μг, к 7. дню 76 μг) наступает удвоение веса печени. В возрасте между 18 и 31 днем имеется повышение абсолютного количества железа в печени в зависимости от роста этого органа. Таким образом количество железа в пересчете на мг% сухого вещества или на вес печени остается практически неизменным.

МЕДЬ В ПЕЧЕНИ ЦЫПЛЯТ ПОРОДЫ БЕЛЫЙ ЛЕГХОРН В РАННИЙ ПОСЛЕИНКУБАЦИОННЫЙ ПЕРИОД

З. МАРЕК, З. СОВА, К. КОУДЕЛА и Й. ХОУШКА

У 122 цыплят породы белый легхорн, распределенных в разные возрастные группы — от вылупления до 31. дня — изучено содержание меди в печени. При вылуплении печень цыплят содержала всего 20,2 μг меди, что равняется только 5,2 $\pm$ 2,5 мг%-ам сухого вещества этого органа. В первые четыре дня имеется резкое сокращение поступления меди в печень: к 3. дню наблюдали в ней 11 μг и к 4. дню 15,8 μг этого элемента. Между 7. и 31. днем содержание меди в печени колебалось в пределах 0,96 — 1,55 мг%, при чем низший показатель получен у цыплят возраста 18 дней, средний вес (3,767 г) печеней которых по сравнению с таковой печени (1,078 г) односуточных цыплят увеличился на 347%, тогда как абсолютное содержание меди увеличилось только на 134% (с 20,2 μг-ов k 1. дню возраста — до 27,8 μг-ов в возрасте 18 дней). В первый месяц жизни вес печени увеличивается в семь раз, тогда как содержание меди — только в 3,5 — 4 раза.

ИЗУЧЕНИЕ ДЕЯТЕЛЬНОСТИ ЖЕЛУДКА У БОЛЬНЫХ ГАСТРО-ЭНЕТРИТОМ СВИНЕЙ

Я. ВАРГА и Л. ПЕШТИ

Авторами изучены общая кислотность, свободная соляная кислота и пепсиновая активность 20 живых, но болеющих гастро-энтеритом и убитых из-за т. наз. поноса поступивших на откормочную базу 74 и из-за гастроэнтерита 54 животных. У части животных с гастро-энтеритом определены и содержание уксусной кислоты и содержание молочной кислоты в желудочном соке. На 10 свиньях проведен эксперимент для выявления того, к каким изменениям функции желудочного-кишечного тракта ведет нейтрализация желудочного сока кислоту-связывающими веществами.

Согласно наблюдениям авторов параллельно с наступлением поноса и желудочного сока исчезает свободная соляная кислота, pH поднимается над 5. По мере усиления гастро-энтерита наряду с исчезновением соляной кислоты понижается и потом совсем затихает пепсиновая активность, содержимое желудка начинает бродить, в нем накопляется много уксусной и молочной кислот, что в свою очередь понижает pH до I. Согласно авторам понос в начале болезни является следствием быстрого опорожнения нейтрального содержимого желудка. Атония желудка это рефлекторная защитная реак-

ция для недопущения, попадания в задние участки кишечного тракта неподготовленных кормов. Уксусно- и молочно-кислое брожение содержимого желудка, потеря им влаги — это следствие атонии, но оно в свою очередь благоприятствует ее продлению и усилению.

ГИСТОХИМИЧЕСКОЕ ВЫЯВЛЕНИЕ ГЛИКОГЕНА ПРИ СОЗРЕВАНИИ МЯСА

Г. БИРО

Гистохимическое выявление гликогена осуществлялось в кроличьей и свиной мышце при помощи реакции PAS. При равномерном содержании гликогена на поперечном среде получется равномерное темно-красное окрашивание. Изменения содержания гликогена среди мышечных волокон реакцией выявляются. При помощи гистохимического метода внутри мышечной связки можно следить за содержанием гликогена в мышечных волокнах и на основании этого можно судить о биохимическом состоянии последних.

ОБНАРУЖИВАЕМОСТЬ МИКОПЛАЗМ В СВИНЬЯХ И ИХ ЗНАЧЕНИЕ ПРИ ЭНЗООТИСЕСКОЙ ПНЕВМОНИИ И АТРОФИЧЕСКОМ РИНИТЕ

М. ШЕР, Й. ХАРТВИХ и Й. СОНДЕРСЛЕБЕН

Изучением молодых свиней из разных стад в Германии доказано, что микоплазмы широко распространены. Микоплазмы изолировались из куриного зародыша. Микоплазмы одинаково часто наблюдались как в стадах с энзоотической пневмонией свиней (70%), так и в стадах с атрофическим ринитом (69%). Микоплазмы изолированы и из 14 свободных от микробов животных.

Морфологическими исследованиями выявлены воспалительные явления в легких и слизистой носовой полости животных и из клинически здоровых стад. Но эти изменения были в них менее выражены, чем в больных животных. Легкая пневмония обнаружена и в некоторых свободных от микробов свиньях. В заключение сказано, что виды микоплазм, изолированные из куриного зародыша не играют роли в этиологии энзоотической пневмонии свиней или атрофического ринита.

ЭКСПЕРИМЕНТАЛЬНОЕ ЗАРАЖЕНИЕ СВИНЕЙ ШТАММАМИ МИКОПЛАЗМ

М. ШЕР, Й. ХАРТВИХ и Й. САНДЕРСЛЕБЕН

Свиньи экспериментально заражались тремя штаммами микоплазм, изолированными при помощи куриного зародыша из больных энзоотической пневмонией свиней. Штаммы не вызывали ни пневмонию, ни ринита. Зато они при введении в брюшную полость часто вызывали серозит или артрит. Это указывает на сходство упомянутых штаммов с **Mycoplasma hyorhinis.**

МОРФОЛОГИЯ И РАЗВИТИЕ

Thwaitia kotlani sp. n.

К. МОЛНАР

Под наименованием **Thwaitia kotlani sp. n.** описывается новый вид нематоды из **Aspius aspius.** Самцы паразитируют под серозной оболочкой воздухоносного пузыря, при чем самки способны дальше развиваться в брюшной полости.

T. kotlani характеризуется годичным, сезонами обусловленным циклом развития. Зрелые самки, полные личинками, обнаруживаются только в мае.

В одной части хозяев развитие паразита заторможено, поэтому при вскрытии часто обнаруживаются отставшие в развитии или распавшие экземпляры гельминта.

ЭНКЕФАЛИТЫ СРЕДИ ТЕЛЯТ, ВЫЗВАННЫЕ ВИРУСОМ ИНФЕКЦИОННОГО БЫЧЬЕГО РИНОТРАХЕИТА

А. БАРТА, Г. ХАЙДУ, П. АЛДАШИ и ДЬ. ПАЦОЛАИ

В поголовье телят крупного хозяйства вирус инфекционного бычьего ринотрахеита вызвал энкефалит. Болели главным образом телята 10—30-дневного возраста. Болезнь началась общими симптомами (одышка, температура, усиление слюноотделения и истечения слизи из носа, покраснение зеркальца), после чего у некоторых животных появились и симптомы поражения нервной системы. Последние животные без исключения погибли после 5—6 дней. Всего погибло 15% животных. Патолого-анатомическая картина при вскрытии отклонений от нормы не показывала. В сером и белом веществах мозга наблюдалась инфильтрация лимфоцитами.

Из носовой слизи больных и мозга павших животных удалось в тканевой культуре изолировать вирус. На заражение изолированным вирусом экспериментальные животные реагировали только общими симптомами без явлений поражения нервной системы.

Телят 3 зараженных экспериментальных групп привили живой вакциной вируса инфекционного бычьего ринотрахеита (**Diamond Laboratories, USA**), телята 4-ой группы оставлены непривитыми. Среди привитых 112 телят погиб один к 5. дню после прививки, тогда как среди непривитого 31 теленка погибло 7 животных.

РЕАКЦИЯ КИШЕЧНИКА КАРПА НА ВОЗДЕЙСТВИЕ (СТРЕССЫ) СРЕДЫ

Й. САКОЛЦАИ

Изучались последствия воздействия факторов среды (отлов, изменение температуры, транспорт) на кишечнике карпа. Обнаружено, что в качестве последствия воздействия стрессов в кишечнике, как правило, наступает отслоение эпителия, гиперимия и из капилляров плазма переходит в тканевые щели и просвет кишечника. Реакцией преципитации характер эксудата доказан. Н этом основании автор допускает, что описанные изменения изменяют проходимость через слизистую, что в свою очередь может вести к патологическому всасыванию.

РЕЗУЛЬТАТЫ КЛИНИКО-ДИАГНОСТИЧЕСКИХ И ТЕРАПЕВТИЧЕСКИХ ИССЛЕДОВАНИЙ ПРИ СПОНТАННОМ ЛИСТЕРИОЗЕ МОЗГА ОВЕЦ

Р. ЛИППМАНН

После введения, в котором обсуждается частота обнаруживаемости листериоза в области Ляйпциг за последние 10 лет, докладывается о результатах клинико-диагностических и терапевтических исследований листериоза мозга у овец. Изучением ликвора можно при жизни отдифференцировать с высокой долей надежности листериоз мозга от других поражений центральной нервной системы. При изучении 253 ликворов спонтанно заболевших овец обнаружено, что при болезни имеется высокой степени плейоцитоз (от 300/3 до 12 000/3 клеток) главным образом нейтрофильного характера. Количество всего белка в спинномозговой жидкости больных сигнификантно повышается (48—140 мг%) по сравнению со здоровыми. Большинство белка составляют глобулины (23—96%). Это повышение количества глобулинов содействует помутнению жидкости и ведет к сдвигу соотношения глобулин-албумин к значению I (нормальное значение — 0,35). Из-за этой причины пробы выпадения белка (по Панди, Нон—Апелт и Вайхброд) в разной степени положительны и коллоидные реакции образуют левые и средние кривые. Интересно уделить внимания высокому содержанию глюкозы в ликворе, превосходящему содержание глюкозы в крови. Только в редких случаях обнаружен возбудитель в мазке жидкости.

В терапии овечьего листериозного энкефалита зарекомендовали себя окситетрациклин (по 10—15 мг/кг в течение 5 дней) и пенициллин (по 40 000 МЕ/кг в течение 5 дней).

Среди обработанных животных тетрациклином и пенициллином клинически выздоровело 60,7% и 30,8% животных соответственно, тогда как все 102 контрольные животные в среднем через 1,7 дня погибли. Успех терапии антибиотиком зависит от раннего вмешательства, от применения необходимых доз и симптоматозного лечения перебоев деятельности пищеварительного тракта с профилактикой эксикоза.

ЭКСПЕРИМЕНТЫ ПО ПАРЭНТЕРАЛЬНОМУ ЗАРАЖЕНИЮ ВИДОМ КОКЦИДИИ

Eimeria stiedai

Л. ПЕЛЛЕРДИ

Эксперименты по внутривенному, внутрибрюшному и внутримышечному заражению нетронутыми и эксцистированными ооцистами **Eimeria stiedai** показали, что восприимчивых кроликов можно парэнтерально заразить этим видом кокцидий. Вызванный таким путем экспериментальный печеночный кокцидиоз был всегда более умеренным кокцидиоза, вызванного путем заражения такими же дозами этого же вида через рот. Наиболее умеренным был он при введении спорулированных ооцист в мышцу, откуда (бедренная мышца) видимо трудно попасть ооцистами в желчные ходы. Наиболее легким способом заражения явилось введение ооцист в брюшную полость. Не обнаружено заметной разницы между результатами парэнтерального заражения нетронутыми и эксцистированными ооцистами, что указывает на возможность эксцистирования в кровяном русле (Шарма, 1964). У одного кролика удалось вызвать кокцидиоз печени путем введения в брыжеечную вену растертой массы печеночной ткани кролика, инвазированного 14 дней раньше кокцидиозом печени. Растертая печеночная ткань содержала много шизонтов и мерозоитов. В инвазированном таким образом кролике цикл развития паразита, вероятно из-за медленного прохождения мерозонтов из капилляров в желчные ходы, немножко замедляется.

ЗАРАЖЕНИЕ ЛОШАДЕЙ ВЕНГРИИ ВИРУСОМ ИНФЛЮЭНЦЫ

A/equi—2

Л. ЧОНТОШ, Т. ФЕХЕРВАРИ, Й. БОКОРИ, Ш. ГЁНЕ, А. БАРТА
и Т. СЕНТ-ИВАНИ

Докладывается о сильно заразной респираторной болезни лошадей, появившейся в Венгрии осенью 1968 г. Болели все лошади, независимо от возраста, породы и пола. В статье описываются клиника, течение болезни и наблюдения по эпизоотологии.

От 31 животного из 7 поголовьев при помощи куриного зародыша изолировано (из носовой, гортанной и конъюнктивальной слизи) 18 вирусных штаммов. Штаммы идентифицированны как **Myxovirus influenzae A/equi-2**.

При помощи серологических проб с сыворотками из упомянутых 7 поголовьев и дальнейших 37 таковых доказано, что в эпизоотии участвовал только вирус инфлюэнцы **A/equi-2**, хотя в сыворотках некоторых лошадей возраста больше 6 лет обнаруживался и вирус инфлюэнцы подтипа **A/equi-1**. Явление объясняется стимулирующим эффектом типово-специфического антигена вируса **A/equi-2**, ибо он наблюдался только у лошадей выше определенного возраста, заражение которых вирусом инфлюэнцы **A/equi-1** до этого удалось доказать.

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EXPERIMENTAL STUDIES ON THE ANTIGENIC ACTION OF BIOPLAST PREPARATIONS

By

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In the various fields of surgery different materials are used for the substitution of tissues, internal fixation of fractured bones and stopping of bleedings. Besides the auto- and hetero-grafts plastics, metals and materials of animal and plant origin are used.

In some cases the implanted materials must remain in the body permanently. In other cases, however, the implant must be removed after an interval, their further presence being unnecessary or even harmful. To cover these manifold requirements, searching for materials that would absorb following their functions had been fulfilled is striven for a long time. In fixing fractured bones internally (SHERSHER, 1966) some alloys may cover this requirement.

GERENDÁS (1961) has reported that models — bioplasts — can be made from human or animal proteins by chemical treatment and pressure. The bioplast prepared from fibrin, being a natural protein, may elicit immunological response, therefore studies on their action on experimental animals were considered justified.

The antigenic action of fibrinogen, fibrin and fibrin derivatives has been studied by several authors (BAGDY and SZILÁGYI, 1953a, b; KESZTYŰS et al., 1950a, b; SZILÁGYI et al., 1955; GLYNN, 1946; SZILÁGYI and BAGDY, 1955; SZILÁGYI et al., 1955).

SZILÁGYI and BAGDY (1955), and BAGDY (1963) reported that unheated fibrin elicited an antibody response including the development of precipitin to homologous and heterologous mammalian fibrins and mammalian fibrinogens, whereas heat treatment made fibrin non-antigenic.

SZILÁGYI, BAGDY and KOCSÁR (1955) implanted heated or unheated fibrin films or foam prepared from blood of cattle, pig, horse or sheep in the peritoneal cavity of guinea-pigs and rabbits. The unheated fibrin films induced antibody production, the heated fibrin films and the fibrin foams, either heated or unheated, did not. Sensitizing action was demonstrated only when bovine fibrin plates had inadequately been pre-treated. In the sera of patients treated with fibrin derivatives as haemostatics homologous antibody could not be detected. Neither antibodies to bovine serum proteins could be detected (WINTER et al., 1952).

Experimental

In the first series of experiments unheated bioplast preparations were implanted into 10 rabbits: 5 rabbits were treated with a human, another 5 with a bovine, bioplast preparation. Fibrin plates were prepared by using fibrin powder obtained from Cohn's fraction I, and were pressed as recommend-

ed by GERENDÁS (1963). The bioplast plates were kept in 0.1% alcoholic Famosept solution until implanted. The plates were 20×5×1 mm in size.

In each rabbit two bioplasts of the same origin were implanted, in the subcutaneous connective tissue of the dorsal or lumbar region. An incision 1.5 to 2 cm long was made in the skin and a recess large enough for the bioplast preparation was formed in the subcutaneous connective tissue, and following implantation the skin was closed by sutures.

The day following implantation a swelling the size of a nut-half or a dove's egg developed around the incision. As compared to the surrounding area the swollen area was warmer, painful, and pasty on palpation. Starting from the 3rd or 4th day following operation the symptoms of the inflammation gradually diminished. During the initial period the bioplast plates could be detected through the skin by palpation. Later on the bioplast plates became gradually smaller and were completely absorbed in about 60 to 80 days.

Antibody response was examined by the two-dimension agar-gel diffusion method of OUCHTERLONY (1953). Medium of 2% agar was prepared with borate buffer of pH 8.6 and 4 ml medium was solidified on each slide. Wells 15 mm^3 in size were cut in the agar plates. Five wells at 3 mm distance from one other were made for the antigen dilutions around the central well filled with antiserum. After adding the samples the plates were kept in a moist chamber at room temperature for 40 hours, and when the precipitation lines had developed unprecipitated protein was removed by washing with saline. Finally the plates were dried and the precipitation lines were stained with 0.1% acid fuchsin dissolved in 10% acetic acid solution. Differentiation was made by rinsing the plates several times with a 5% solution of acetic acid.

Serum samples taken from the rabbits at intervals after implantation were tested for precipitins. A solution suitable as antigen in the test being hard to prepare from the solid bioplast plates, a purified bovine fibrinogen preparation (Behring) was used as antigen. A 2% solution was prepared, and from this serial dilutions were made in a borate buffer of pH 8.6. In the tests serum samples were placed in the central wells and the antigen dilutions from 1 : 2 to 1 : 32 in the peripheral ones.

Pre-implantation sera failed to react with the antigen and so did the samples taken from the rabbits 30 days after the implantation of the human bioplast preparation. At the same time 3 of the rabbits in which the bovine bioplast preparation had been implanted had precipitin detectable with the 1 : 8 diluted antigen. The precipitation lines could not be removed by washing and could easily be stained with fuchsin. Sera of the remaining two rabbits gave negative results.

On testing the sera collected 60 days following implantation only 1 of the 3 previously positive rabbits gave a slight precipitation with the antigen dilution of 1 : 4. The remainders in the "bovine" group were found negative.

Of the 5 rabbits in the "human" group 3 reacted, precipitating 1 : 8, 1 : 4 and 1 : 2 dilutions of the antigen, respectively.

On the 90th day following the operation, when the bioplast plates had completely been absorbed, the implantation was repeated in 6 rabbits. Laparotomy was made and an unheated bioplast plate of either human or bovine origin, $20 \times 5 \times 1$ mm in size was implanted in the omentum of each of 3 rabbits. Thirty days following the second implantation, *i.e.*, on the 120th day of the experiment, the sera of 2 rabbits treated with the bovine bioplast preparation gave positive reaction with 1 : 32 and with 1 : 16 dilution of the antigen respectively. The rabbits treated with human bioplast preparation remained negative.

On the 120th day, after blood sampling, 1 rabbit in each group was injected with a solution containing 30 mg bovine fibrinogen, intravenously, in order to demonstrate a possible allergic state. Body temperature, pulse rate, respiration rate and the quality of respiration were recorded before the injection and followed up over two days. Despite the high levels of precipitin the experimental animals responded with no clinical symptoms to the heterologous fibrinogen.

To control the absorption of the bioplast preparations, 1 rabbit was killed in each group. No local changes due either to human or bovine bioplast plate could be observed. The plates were found covered by the omentum, and they retained their original size. No significant inflammatory reaction could be observed.

Thirty days later, *i.e.*, on the 150th day of the experiment, the sera of the rabbits treated with the human bioplast preparation showed a weak reaction with the 1 : 4 dilution of the antigen. The same result was obtained with the sera of 2 rabbits treated with the bovine bioplast preparation, though a strong positive reaction with higher dilutions of the antigen had been observed earlier.

On the last blood sampling 30 mg purified fibrinogen (Behring) was injected intravenously into each of the 4 rabbits still alive. The experimental animals were taken under clinical control as described above. No clinical symptoms could be seen following injection of the heterologous fibrinogen.

Finally, the animals were killed. No pathological changes were seen in the parenchymatous organs. The bioplast plates were found in the peritoneal cavity, covered by the omentum. The plates were reduced in size, their originally smooth surfaces turned rough.

To study the antigenic properties of the bioplast, a further series of experiments has been conducted. Heated bovine bioplast nails 20 mm long and 2 mm in diameter were implanted in the femoral medullar cavity of 240 to 420 g male guinea-pigs. Kirschner's wires of the same size were used as controls. As a total, 21 animals were treated: 14 were implanted bioplast nails and

7 Kirschner's wire. The animals were distributed in 7 groups, 3 in each. Thus, each group comprised 2 guinea-pigs treated with bioplast nail and 1 control. One group was killed on each of the 3rd, 6th, 9th, 16th, 30th, 45th and 60th days following the operation. Just before killing blood was taken to test antibody level with the agar-gel precipitation method. Precipitin reacting with the fibrinogen was not demonstrated in any of the serum samples. Femurs of the guinea-pigs were investigated both pathologically and histopathologically, and the absorption rate of the bioplasts were recorded. (The results will be published elsewhere.)

Along with the guinea-pig experiments unheated bioplast nail was implanted into the medullar cavity of a dog's femur. The bioplast preparation was well-tolerated by the dog. The dog was killed 2 months following the operation. Its serum gave slight precipitation with the 1 : 8 dilution of the antigen. The broken diaphysis recovered with an abundant callus proliferation.

Discussion

Single subcutaneous implantation of the unheated bioplast plates elicited specific precipitin production in more than half the treated animals. Sera from the rabbits treated with the bovine bioplast preparation reacted with the bovine antigen sooner (on the 30th day) than those obtained from the rabbits treated with the human bioplast (on the 60th day). By this latter time, however, the rabbits treated with bovine bioplast being positive earlier showed only a slight, if any, precipitation reaction. The second implantations in the same animals confirmed that the bioplasts not treated with heat may elicit antibody response, but the antibody level soon declines.

In rabbits treated with bioplast plates anaphylactic shock could not be evoked with injection of heterologous fibrinogen; at post mortem examinations no changes in the organs could be seen, and clinically no symptoms indicating allergic reactions could be observed. These facts suggest that bioplasts which absorb later on cause no clinically detectable allergic condition even if implanted as unheated. Owing to the small number of experiments, further investigations are needed to clear the very action of these heated and unheated bioplast preparations as the allergic and anaphylactic reactions are concerned. Our experiments are in progress. Nevertheless, transient appearance of the precipitins specific to the fibrinogen proves that the unheated bioplast preparations contain some antigen components of the fibrin molecule in a state which may induce the production of antibodies in a heterologous organism.

Heated bovine bioplast implanted in the medullar cavity of guinea-pigs did not induce development of specific precipitins in 30 to 60 days.

In the serum of a dog in the medullar cavity of which unheated bovine bioplast had been implanted a low level of antibody was demonstrated 60 days following the operation.

It may be concluded that the unheated bioplast, being a heterologous protein, may elicit antibody response. Thus, an application of heated bioplast models seems to be more advisable. It may be expected that the risk in the use of the bioplasts will be limited. As an analogy, proteo-hormones, *e.g.*, Zn protamine insulin, may be mentioned, which although induce antibody production, may be used without considerable risk.

SUMMARY

Unheated bioplast films and heat-treated or unheated bioplast nails prepared from human and bovine fibrin were implanted in the subcutaneous connective tissue or peritoneal cavity of rabbits, and medullar cavity of guinea-pigs and a dog. Agar-gel diffusion tests have shown that antibodies appeared in the sera of the rabbits and the dog treated with unheated bovine or human bioplasts precipitate not only to the homologous but also to the heterologous mammalian fibrinogen (though in lower titre). Heated bioplast prepared from bovine fibrin did not elicit antibody production in guinea-pigs. Anaphylactic reaction could not be evoked.

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COMPARATIVE STUDY OF TISSUE REACTIONS TO VARIOUS SUTURE MATERIALS (CATGUT, SILK AND POLYESTER)

By

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Although surgical suture techniques have been applied ever since the ancient times, the ideal suture material remains to be found. The various natural materials, such as human hair, rat tail, linen and silk thread, have all shortcomings.

The catgut suture, introduced by LISTER in the second part of the past century, has been a great advance but has, nevertheless, some disadvantages. Certain authors (BORSOS, 1962; FURKA, 1964; HUDEMANN, 1959) have even supposed that its repeated application may elicit allergic reactions. Catgut is strong and readily absorbed, but the row catgut is rich in spores and the chemical sterilization applicable without impairing the tensile strength significantly, is sometimes not efficient enough to ensure sterility. On the other hand, drastic sterilization may damage the protein material to such an extent that the tensile strength may become inadequate. Recently attempts have been made to sterilize catgut radiologically or by ethylene oxide gas. Last but not least, high-quality reliable catgut is fairly expensive.

Certain filamentous suture materials, such as pure silk or linen can be appropriately sterilized in the autoclave, but they are absorbed either very slowly or not at all and, owing to the frequent presence of incrustating substances, are not fully histophilic.

The introduction of synthetic fibres into surgery seems to have brought us nearer to the discovery of the ideal suture material. Some of the synthetic fabrics used in the textile industry (e.g., syntophil, plastigut, nylon, perlon, teflon, marlex, mersilene, tevdek, etc.) have been tried and partly applied in the surgery.

The production of polyester fibres has been particularly expanded during the recent years, owing to their favourable properties.

1. Terephthalic acid-ethylene glycol polycondensates, e.g., trevira, tergal, diolene, terylene, terlenka.
2. Terephthalic acid-1,4-dimethylcyclohexane polycondensate: kodel.
3. Terephthalic acid-isophthalic acid ethylene glycol polycondensate: vycron.

The surgical suitability of polyester fibre has been confirmed by various examinations. It seems to meet all requirements of a good surgical suture material and has many additional favourable properties which ensure its advantage over several other widely-applied synthetic fabrics.

The most important properties of the polyester fibre are the following: it is histophilic; resistant to most chemicals; can be autoclaved several times without impairment; owing to its low capillarity it has hardly any draining effect; it is less liable to strain than polyamide fabrics (nylon, perlon) and has an excellent tensile strength. It is identified by selective dissolution.

Tests are most readily carried out on 0.1 g samples in test tube. The reagents should be of analytical purity. Grouping on the basis of selective dissolution is the following:

A. cuoxam-soluble (cuoxam: copper oxide-ammonia)	regenerated cellulose
B. cuoxam-insoluble soluble in cold (25 °C) glacial acetic acid	cellulose-2,5 acetate cellulose triacetate

C. Insoluble in both cuoxam and glacial acetic acid, soluble in 6 *N* HCl at 25 °C	PA6	= polyamide 6
	PA66	= polyamide 66
	PVAc	= polyvinyl acetate
D. Insoluble in cuoxam, glacial acetic acid and 6 *N* HCl; soluble in conc. HNO_3 at 20 °C	PAN	= polyacrylnitril
	PVY	= modified polyacrylnitril
E. Insoluble in cuoxam, glacial acetic acid, 6 *N* HCl and conc. HNO_3; soluble in conc. H_2SO_4 at 25 °C	PE	= polyester

Differentiation of polycondensates of identical primary structure is also to be made by further selective solubility tests.

Differentiation of the three polyesters with dissimilar basic structures is carried out as follows: terylene and vycron dissolve in hot dimethyl formamide, while kodel does not. Vycron is soluble in hot pyridine and cyclohexane, kodel is not.

The synthetic fibres used in the textile industry contain various filling substances. In the case of surgical use these additional components must be removed to prevent any undesirable — pyrogenic, toxic, cancerogenic, etc., — effect on the human or animal organism. The precise technology of extraction is prescribed in detail in the Pharmacopoeias. Polyester fibres are particularly advantageous also in this respect as their noxious components, such as the so-called avivants, antistatics, monomers, etc., can be extracted by cooking in distilled water for 2 hours, under 0.6 atm overpressure and subsequent rinsings in hot and cold water. Some comparative data, calculated in the Textile Research Institute, Budapest, are given below:

Results of extractions

Sample	Substances extractable by distilled water (%)
Nylon 6 (Zehlendorf)	0.066
Nylon 6 Grilon (Emser-Werke)	0.142
Nylon 66 (Emmenbrücke)	0.062
Polypropylene (polymer)	8.840

During boiling, the polyester fibre shrinks to about 10%. In respect of knot retention, polyester is superior to nylon and polypropylene, as its strain is only 2.5—3% as compared to 6 and 15%, respectively. Also, polyester has better staining properties, greater resistance to oxidants and a nearly double tensile strength as compared to polypropylene.

The polyester fibre suture is a braided multifilament, woven of monofilaments of 75 den caliber. Braiding is made so as to ensure a fairly uniform caliber throughout. A substantial advantage of the braided suture is its good flexibility, and the wide variability of its caliber and tensile strength by varying the number of fibres. A disadvantage in comparison to monofilaments is the slight unevenness of its surface. Recently, attempts have been made to eliminate this disadvantage by appropriate coating with various indifferent substances, as otherwise the braided suture has many favourable properties. The braided product is delivered in lengths and forms according to order.

Polyester has already been used in some branches of surgery. The Rico Ltd. (Budapest) uses it regularly for the production of arterial prostheses and surgical meshes. In Hungary, trials of such products have been undertaken by BARTOS et al. (1960, 1964, 1965), who reported good results in both clinical and experimental respect.

The present examinations were carried out to study the behaviour of polyester, catgut and silk suture materials in the tissues of experimental animals, with special regard to the nature of their tissue reactivity.

Experimental

Sterilized polyester (Sizes Nos 0, 2, 5, 7), catgut (Sizes Nos 5/0, 0, 2, 3, 5, 6) and silk (Sizes Nos 0, 2, 4, 7, 9, 11) sutures were implanted in the subcutaneous connective tissue and muscle tissue of horses and dogs.

After appropriate preparation, double loops of 2 cm length, with a knot at both ends, were formed of the sutures and placed in incisions made in subcutaneous connective and muscle tissue. The wounds were then closed by one knotted suture each. On a total 293 catgut, 52 polyester and 50 silk suture loops were implanted into the subcutaneous connective tissue and muscle tissue of 16 horses and 28 dogs, in areas of the neck and trunk.

The implanted sutures and the surrounding tissues were excised at various points of time after implantation, *viz.*, on the 25th, 45th, 75th and 120th days in the case of polyester and silk and on the 5th, 10th, 15th, 20th, 25th, 30th, 45th, 60th, 75th and 120th days in the case of catgut. The specimens were cut transversally to grossly examine the position of the implants and the surrounding tissue reaction. Then the specimens were fixed in Zenker's solution, embedded in paraffin and serial sections were cut over their whole lengths, including the knots, and stained with haematoxylin and eosin or van Gieson's stain.

Results

In the case of horses, the sutures implanted into the subcutaneous and muscle tissues in the cervical, dorsal and inguinal regions were surrounded each by a swelling, 1—1 1/2 cm in diameter, of loose or compact structure, warm, and slightly painful. Wound closure was usually form, the margins being most often covered by a dried crust. No serous or purulent exudation took place in the postoperative period or afterwards. The heat, and tenderness of the swellings tended to disappear by the 3rd—5th day, depending on the thickness of the suture. The lesions tended to become more compact and decreased in size. The dry crust covering the wound margins detached after 6—10 days, leaving a straight line-like scar.

In the case of dogs, the sutures implanted into the subcutaneous and intermuscular connective tissue or in the subcutaneous fat tissue showed essentially the same clinical picture, but the swellings were smaller than in the horses. During the subsequent days the swelling tended to decrease and protruded only slightly or not at all above the skin surface. Regression of the swelling was markedly rapid with polyester sutures. On the 45th postoperation day hardly any perisutural tissue enlargement was palpable in dog and horse alike. On the 75th day, and still more distinctly on the 120th day, the operation scar was the only indication of the implantation of polyester suture.

Catgut and silk sutures implanted into the subcutaneous connective tissue or muscle tissue of the horse were surrounded on the 45th day by compact elastic swellings varying from 6 to 12 mm in diameter, depending on the suture caliber and the depth of the wound. These swellings were still present on the 75th and 120th day; after 120 days the swellings around the sutures implanted into the subcutaneous connective and fat tissue of the dog were usually smaller than in the case of horses. In blocks of tissue excised from the sites of suture implantation on the 5th postoperative day, a halo of hyperaemia

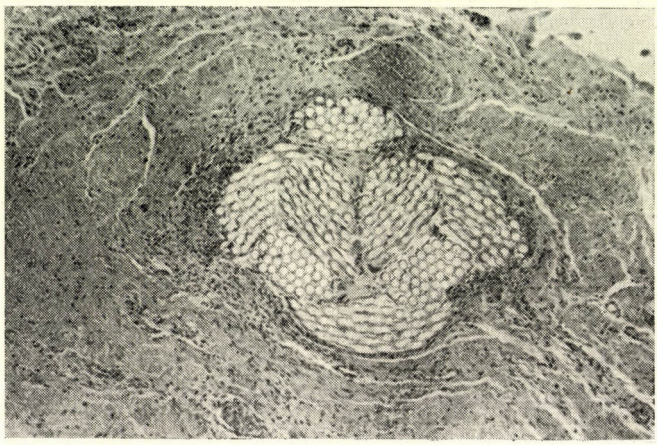

Fig. 1. 25th postoperation day. The polyester suture is demarcated by a thin fibrous connective tissue which is seen to creep here and there in the suture's texture. H. & E. stain. Magnification ×40

and inflammation was seen around the presterilized dry catgut and sterile silk sutures. At this point of time, the implanted sutures were readily identified in the histological sections. The vessels around the sutures had dilated and serum had accumulated in the tissue gaps. In the serum, scattered neutrophils and lymphocytes were seen. In some areas imbibed with serum, erythrocytes appeared.

On the 10th to 15th day following the implantation, catgut sutures were generally surrounded by a zone of reddish-grey, juicy tissue, broader in the horse than in the dog. In this tissue the suture material had a loose grasp and the thicker threads (Sizes Nos 3, 4 and 5) were surrounded by a pasty greyish exudate. In every case, the exudate proved to be sterile. Histologically, in the areas imbibed with serum inflammatory cell reaction was still seen, but the adventitial cells of the vessels showed considerable proliferation and sporadically, newly-formed collagenous fibres were still present.

On the 25th postoperation day, the polyester sutures were surrounded by adherent nodules of connective tissue, whereas the catgut and silk sutures

were enclosed by a sharply demarcated connective tissue capsule. The greyish-white connective tissue was narrower and less conspicuous around the polyester and silk sutures as compared to that surrounding the catgut. Thick catgut sutures and also some thick silk sutures were surrounded by greyish-white, pasty, bacterium-free exudate.

Histologically, on the 25th day the implanted polyester sutures were generally demarcated by circularly arranged, thin fibrous tissue. In the immediate environment of the sutures, angio-fibroblastic tissue infiltrated by

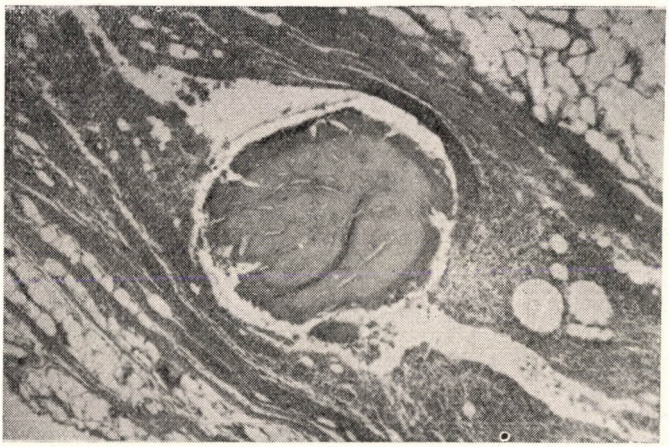

Fig. 2. Cross section of the catgut suture on the 25th postoperation day. Note the many inflammatory cells in the environment. H. & E. stain. Magnification ×40

histiocytes, neutrophils and a few foreign-body giant cells was seen. Here and there the angio-fibroblastic tissue had penetrated the suture material, dividing the multifilaments into bundles or even monofilaments (Fig. 1). At the same time the catgut sutures were either intact or fell asunder into coarse bundles. The intra-sutural angio-fibroblastic tissue contained many neutrophils, lymphocytes, plasma cells, histiocytes and fibroblasts (Fig. 2), with a few foreigh-body giant cells among them. In the peripheral parts of the angio-fibroblastic tissue fibres became separated. By this time the multifilamentous silk sutures appeared looser. Like in the case of catgut, the perisutural demarcation zone was relatively broad, reaching twice the thickness of the thread. The major part of the demarcation zone consisted of angio-fibroblastic tissue infiltrated by many inflammatory cells and foreign-body giant cells and surrounded by newly-developed circular collagenous fibres (Fig. 3).

On the 45th day, the hardly palpable connective tissue nodule surrounding the polyester suture showed microscopically an advanced state of organi-

zation. Strands of angio-fibroblastic tissue, rich in fibroblasts, but poor in motile cells were seen to creep between the bundles or single monofilament components of the multifilamentous polyester suture, surrounding them like sheaths and showing separated fibres. Silk multifilaments also tended to rarify

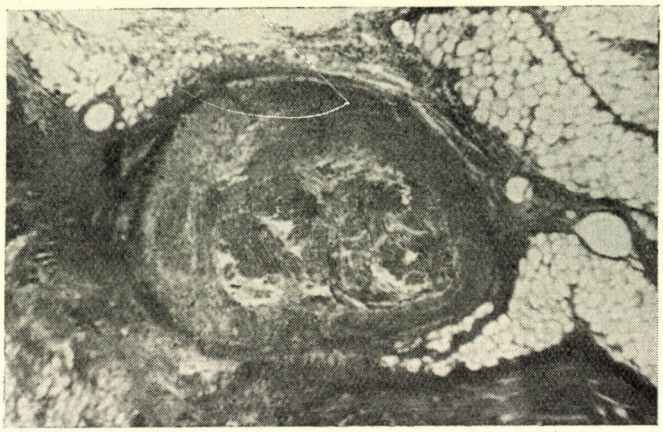

Fig. 3. Angio-fibroblastic tissue with distinct inflammatory cell reaction is seen to surround the silk suture. 25th postoperation day. H. & E. stain. Magnification ×40

Fig. 4. On the 45th postoperation day the bundles constituting the polyester multifilament are in many places ensheathed by fibrous connective tissue. H. & E. stain. Magnification ×80

and the area between the filaments was infiltrated by many granulocytes, histiocytes and foreign-body giant cells (Fig. 5). The proximal layer of the angio-fibroblastic tissue formed around the catgut was still rich in neutrophils, histiocytes and giant cells. As a rule, the texture of the catgut seemed to be

intact, with only superficial unevenness owing to damage by neutrophils (Fig. 6).

On the 75th day, the polyester sutures implanted in connective or muscle tissue were, depending on their sizes, surrounded by variable amounts

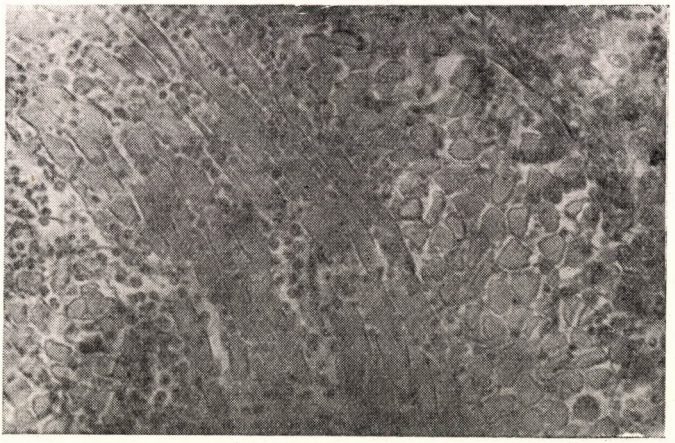

Fig. 5. On the 45th postoperation day the silk suture is falling apart and many leukocytes and histiocytes are seen among its loosened components. H. & E. stain. Magnification ×340

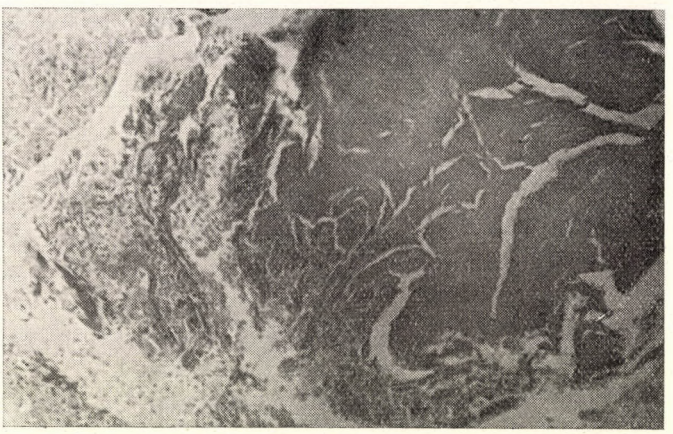

Fig. 6. The texture of the catgut suture is fairly intact. Note the distinct cellular reaction in the surroundings. 45th postoperation day. H. & E. stain. Magnification ×80

of greyish-white cicatricial tissue with which they had irreversibly admixed. Microscopically, in the 75th and 120th days the organization process was found to have markedly advanced and the individual components of the multifilamentous polyester suture seemed to be practically incorporated into the

small scar tissue (Figs 7 and 8). Though the polyester suture was not absorbed by the 120th day, considerable changes had supposedly taken place in its texture as by the 120th day the individual polyester filaments, ensheathed

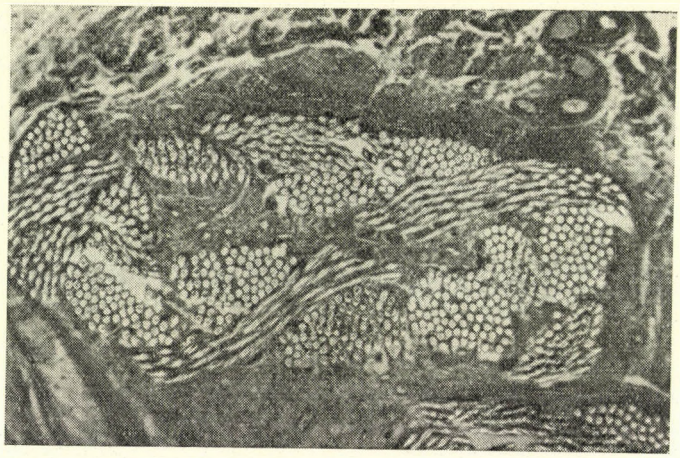

Fig. 7. 75th postoperation day. The polyester suture has admixed with fibrous connective tissue. H. & E. stain. Magnification ×40

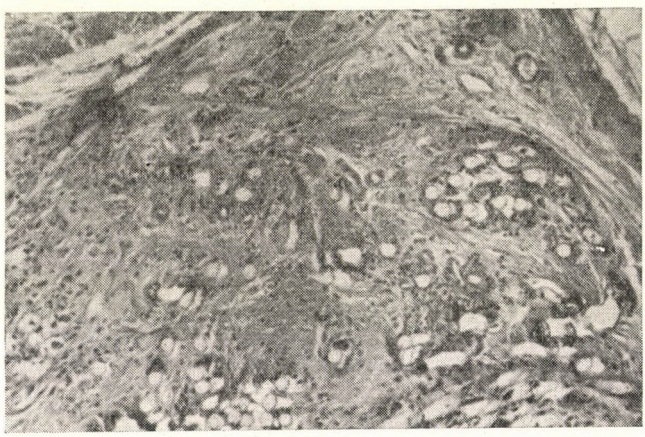

Fig. 8. 120th postoperation day. The divided monofilaments of the polyester suture are surrounded by fibrous connective tissue. H. & E. stain. Magnification ×270

and admixed with connective tissue had deformed, lost the typical structure of their cross-sections, some of them showed split-like stripes and became eosinophilic, in contrast to their original staining properties (Fig. 9).

The catgut and silk sutures were encased by demarcating connective tissue by the 75th and 120th day. The catgut had a somewhat thicker capsule

than the silk suture. Grossly no abscess or fistula was apparent around the sutures. The texture of the catgut showed a variable picture on the 75th and 120th days. In some cases hardly any signs of absorption were seen; only the

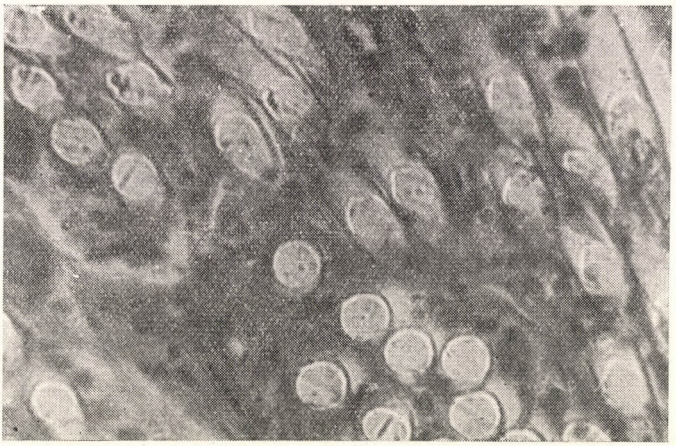

Fig. 9. 120th postoperation day. Note the fibrocytes and fibroblasts among the components of the multifilament. H. & E. stain. Magnification ×400

Fig. 10. A slight decrease of the amount of catgut suture is seen by the 75th postoperation day. The surrounding angio-fibroblastic tissue is rich in moving cells. H. & E. stain. Magnification ×40

surfaces were somewhat loosened. In other cases, absorption had markedly advanced and only fragments of the suture were found. The silk sutures had considerably loosened by the 75th day, falling apart to bundles or single filaments.

In sections obtained from the areas of implantation on the 75th and 120th days, both catgut and silk sutures were surrounded by a fairly broad layer of angio-fibroblastic tissue, rich in neutrophils and other inflammatory cells (Figs 10 and 11). Here and there catgut and silk sutures were surrounded by

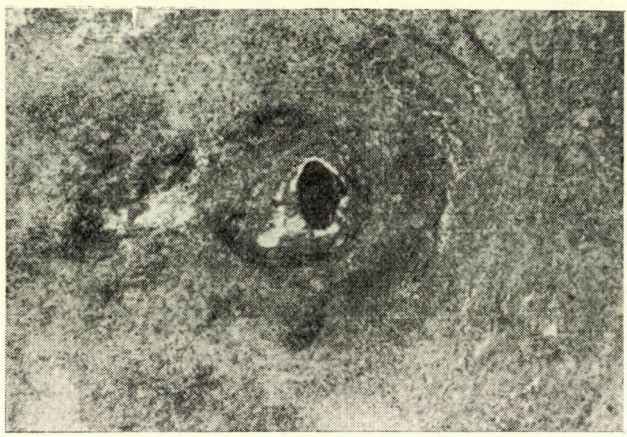

Fig. 11. 120th postoperation day. Note the considerably decreased amount of catgut and the vivid cellular reaction. H. & E. stain. Magnification ×40

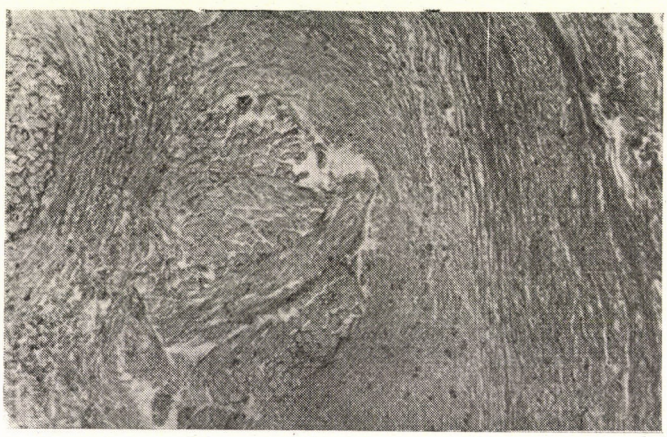

Fig. 12. The loosened silk suture is surrounded by a fairly broad layer of fibrous tissue by the 120th day. Van Gieson stain. Magnification ×40

abscesses of microscopic size — sterile abscesses —. The peripheral parts of the angio-fibroblastic tissue showed a marked separation of fibres, giving rise to a sharply demarcated connective tissue capsule. On the 120th day, the cicatricial tissue was most extensive — 2—3 fold of the suture diameter— around the catgut suture. A smaller amount of cicatricial tissue — 1 1/2—2fold

of the suture diameter — had formed around the silk suture. The polyester suture was not encapsulated by connective tissue, but had admixed with a small amount of connective tissue which contained no inflammatory cells.

Discussion

Comparative studies have revealed notable differences in the behaviour of, and tissue reactivity to, various types of surgical suture material implanted in the subcutaneous connective tissue and muscle tissue of horses and dogs. Over a 120-day period of observation the most favourable results were obtained with the polyester suture. The moderate swelling developing around the implanted suture tended to regress rapidly and was hardly palpable by the 45th postoperation day. At that time as well as on the 75th and 120th day compact nodules were still present around the implants of traditional suture material. The microscopic examination of specimens excised from the areas of implantation has been supportive of the clinical findings. The inflammatory processes which developed around the implants during the initial postoperative period were followed by regeneration phenomena. Naturally, the tissue reactions varied to a certain extent with the type of tissue in which the suture was implanted as well as with the species of the experimental animal. A distinct correlation was found between suture size and tissue reaction. Our results suggest that the behaviour of, and tissue reactivity to, implanted sutures depended primarily on the latter's structural and chemical properties and the mode of their pretreatment.

Out of the three suture materials examined, the local inflammatory reaction subsided earliest in the case of polyester, and was followed by a rapidly progressive regeneration. The angio-fibroblastic tissue formed during the regeneration process grew into the texture of the polyester suture and divided it into bundles by the 25th day. Later on, on the 45th, 75th and 120th days the angio-fibroblastic tissue showed a rapidly progressive fibrous transformation and became more and more admixed with the suture material, leading finally to its organization in the scar tissue. Histologically, the regeneration of the operation field, *viz.*, cicatricial tissue formation, was preceded by only scanty organization phenomena. In the surroundings of the traditional suture materials (silk and catgut), histiocytes and giant cells were still seen up to the 120th day, in spite of progressing encapsulation by connective tissue.

Clinical and histological examinations have both shown that out of the suture materials tested, the polyester multifilament provoked the mildest tissue reaction. After moderate absorption phenomena, the organization of that suture was fairly rapid and uncomplicated. Accordingly, polyester seems to be the suture material of choice for implantation into tissues.

Many authors have analysed the requirements set up for alloplasts (BORNEMISZA, 1960, 1963, 1964; HEINZE, 1955; SIPOS, 1956; HUDEMANN, 1959; PETROVSKY and VENEDIKTOV, 1967; and others). Our own experiences indicate that polyester complies with all requirements of an appropriate material to be implanted. BUCZEK and MICHALSKA (1967) studied the behaviour of the Polish polyester suture product Elana, by implanting it into subcutaneous connective tissue, muscle tissue and stomach and intestinal wall of experimental animals, and obtained excellent results. In Hungary, favourable results have been reported by BARTOS et al. (1960, 1964, 1965) concerning the experimental and clinical trial of arterial prostheses and surgical meshes made of polyester. Good experiences have been made also in the course of their routine clinical use. It should be mentioned that in the present studies, tissue reactions to polyester suture material were similar to those to other good-quality synthetic sutures, *e.g.*, to polyamides like perlon and nylon (BURKHARDT, 1955; FURKA, 1964; GYURKÓ, 1965; GARDÓ et al., 1967), or to polypropylene (GYURKÓ, 1967).

Comparing our findings with the literary data, it may be stated that implanted polyester sutures are distinctly histophilic. Another advantage over the other synthetic sutures is its better knot retention due to a comparatively greater tensile strength and lesser strain.

Acknowledgements. The authors are indebted to Z. HUSZKA, Chief Ingenieur of the Rico Ltd. (Budapest) and I. MOLNÁR, Textile Research Institute (Budapest), for preparing the suture materials used in this study and performing their physical, mechanical and chemical testing.

SUMMARY

Various suture materials (polyester, catgut and silk) were implanted in the subcutaneous connective tissue and muscle tissue of horses and dogs. In the operated region clinical symptoms, behaviour of the sutures and the nature of tissue reaction were followed over a period of 120 days. For histological examination, tissue specimens including the suture were excised from the operation field at different points of time between the 5th and 120th postoperation day. Comparative clinical and histological examinations have yielded the best results with the polyester suture material: the local inflammatory reaction subsided soon after the operation and absorption phenomena were very mild. In sections obtained on the 45th, 75th and 120th days, angio-fibroblastic tissue showing a marked fibrous transformation was seen to creep in the texture of the multifilament, dividing it into bundles and monofilaments and finally admixing with it entirely so that it became organized in the tissue of the operation scar. Unlike the ready organization of the polyester suture, the traditional suture materials (catgut and silk) had been encapsulated by connective tissue. In the internal layer of the connective tissue capsule and intensive absorptional cell activity was apparent up to the 120th day. Out of the suture material tested, the polyester multifilament provoked the mildest tissue reaction.

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THE INFLUENCE OF MATERNAL EGG-WHITE MUCIN-GLOBULIN ON THE RESISTANCE OF OFFSPRING IN THE PERINATAL PERIOD

By

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Recently the effect of the egg reserve proteins on the development of embryo has been observed (MORTON, GILMOUR, MCDERMID and OGDEN, 1965; BUVANENDRAN, 1967). We found that in New Hampshire Breed the mortality of offspring of dams with different genotype depends on the stage of development (PAVEL and PETERSON, 1967).

The present report concerns the association of egg-white globulins with the resistance of offspring in the perinatal period.

Materials and methods

The subject of the study was a New Hampshire population of Kurtna Poultry Breeding Experimental Station. Egg-white globulins were fractionated by using SMITHIES' (1955) slightly modified starch gel electrophoretic method (PÕDER and PAVEL, 1966). In this report the genotypes of mucin-globulins are signed according to MANWELL and BAKER (1962). For simplification the symbol of locus is omitted, for example $G_3^A\ G_3^B\ G_3^C\ G_3^D$ is written ABCD*.

Chicks were experimentally infected by injecting live culture of *S. gallinarum* in a dosage of $1-3\times10^7$ bacterial cells subcutaneously on the first to the third day after hatching. The mortality was registered during 21 post-infection days. For statistical analysis, the percentage data were transformed to the arcsin % scale and subjected to dispersion analysis.

Results and discussion

The pooled data of 5 experiments are presented in Table I. Out of the 6 characters studied (fertilization rate, embryonic mortality, post-embryonic mortality in the first 9 days after infection, post-embryonic mortality in 21 days after infection, perinatal mortality up to the 9th post-infection day and perinatal mortality up to the 21st post-infection day) a statistically significant

* This notation corresponds to Lush's symbol $II^A—II^B\ III^A\ III^B$.

effect of maternal genotypes was found only in embryonic mortality. The dam's genotypes align according to their positive influence in the following order: BBDD, ABDD, AADD, ABCD. Thus, in accordance with our preliminary results (PAVEL and PETERSON, 1967), the embryonic mortality is the highest in diheterozygous birds.

After the experimental infection of newly-hatched chicks with *S.gallinarum*, the differences due to the maternal genotype are gradually diminishing. If there is still observable little, but non-significant, difference during the first 9 days after infection, this will practically disappear by the 21st day after infection.

The survival rates of the offspring of different dams are presented in Table II. It can be seen that the survival percentages in the offsprings of different dams are in the same order, especially in the case when survival rate is calculated from the number of eggs, and not from the fertilized eggs. If one considers also the fertilization process, the diheterozygous birds (ABCD) are characterized by the lowest survival rate of offspring again. If fertilization and embryonic mortality are not taken into consideration, *i.e.* one calculates the survival percentage from the number of hatched chicks, the survival rate of offspring of diheterozygous birds will rise. These data, and those presented in Table I refer to the fact that the effect of the maternal genotype is different in succeeding ontogenic stages.

As to the severe salmonella infection it seems that none of the 4 mucin-globulin genotypes has any selective advantage. The severe infection nevertheless changes the frequencies of genotypes in a population, supporting the view that in certain cases infection has some importance in the persistence of polymorphism. The fact that the differences between maternal effects are not in all hatches significant agrees with the postulation of KIMURA (1966) that the intensity of selection is not constant under different conditions. One must even presume that also in mucin-globulin polymorphism the selection prefers sometimes one, sometimes another, genotype (HALDANE and JAYAKAR, 1963). This opinion is confirmed by the data of SCHMID and THEIN (1968), who found that in some lines of chicks embryonic mortality is associated with allele II^A, i.e., $G_3{}^A$, in the others with allele II^B (i.e. $G_3{}^B$).

It is of some interest to determine the role of heterozygosis in the persistence of mucin-globulin polymorphism. Some information can be given by the comparison of homozygous and heterozygous dams (Tables III and IV). Table III characterizes the effect of homozygous and heterozygous dams on the resistance of offspring. Statistically significant differences between homozygous and heterozygous mothers were noticed only in fertilization and embryonic mortality. The fertilization was higher in hens being heterozygous on locus G_3. Concerning embryonic mortality, however, locus G_2 is associated with the embryonic resistance. Thus the offspring of homozygous dams proved to be

Table I

The offspring mortality of hens with different genotypes

	Stage Number	Maternal genotype			
		ABDD	BBDD	ABCD	AADD
Fertilization	Hens	371	131	76	175
	Eggs	2081	657	404	931
	Fertilized eggs	1585	479	324	651
	Fertility rate	0.7617	0.7291	0.8020	0.6992
Embryonic mortality	Fertilized eggs	1585	429	324	651
	Dead embryos	586	162	158	283
	Mortality	0.3697	0.3382	0.4877	0.4347
Postembryonic mortality in	9 days after injection:				
	Hatched chicks	999	317	166	368
	Dead chicks	740	242	129	276
	Mortality	0.7407	0.7634	0.7771	0.7500
	21 days after infection:				
	Dead chicks	898	286	149	328
	Mortality	0.8989	0.9022	0.8976	0.8913
Perinatal mortality up to	9th post-infection day:				
	Fertilized eggs	1585	479	324	651
	Deaths	1326	404	287	559
	Mortality	0.8366	0.8434	0.8858	0.8587
	21th post-infection day:				
	Deaths	1484	448	307	611
	Mortality	0.9363	0.9353	0.9475	0.9386

more resistant, compared with the offspring of heterozygous ones. It is of interest that statistically a nearly significant difference was noticed in postnatal mortality until the tenth post-infection day on locus G_2. Here again the homozygous dams proved to be more efficient. In Table IV the efficiency of homozygous and heterozygous dams is compared on the basis of calculated deviations. For this purpose the offspring value for each dam was calculated by subtracting it from the mean value calculated for the whole experiment. The genotype values were pooled and divided with the number of genotypes.

As will be seen, the fertilization is mostly influenced by locus G_3. However, in embryonic mortality apart from locus G_2 the influence of G_3 is also noticeable. Both embryonic and post-embryonic mortality of the offspring of homozygous mothers is lower than in heterozygous ones, until the tenth post-infection day. However, concerning the whole postnatal period

Table II

The effect of maternal genotype on the survival of offspring

Survival rate of offspring (%)	Maternal genotype			
	ABDD	BBDD	ABCD	AADD
From the number of:				
a) eggs	4.85	4.72	4.21	4.30
b) fertilized eggs	6.37	6.47	5.25	6.14
c) hatched chicks	10.11	9.78	10.24	10.90

examined, there is no remarkable difference in this respect between homozygous and heterozygous mothers. Accordingly, after the tenth post-infection day there take place some changes in the maternal effect, indicating that this effect is not always unidirectional in various stages of perinatal development.

Since the presented data do not indicate the existence of any over-dominance of mucin-globulin loci, a further study should elucidate what a role is played by the genetic-automatic processes (DUBININ, 1966; DUBININ and GLEMBOZKY, 1967) in the persistence of the polymorphism on mucin-globulin loci.

Table III

Comparison of homozygous and heterozygous dams

Stage	Locus	Expression of character		d^*	p
		homozygotes	heterozygotes		
Fertilization	G_3	0.7199	0.7683	4.84	<0.05
	G_2	0.7400	0.7137	1.47	>0.05
Embryonic mortality	G_3	0.4060	0.3897	0.97	<0.05
	G_2	0.3797	0.4784	4.31	<0.05
Postembryonic mortality (up to 9th post-infection day)	G_3	0.7515	0.7459	0.28	>0.05
	G_2	0.7470	0.7552	0.30	>0.05
Postembryonic mortality (up to 21st post-infection day)	G_3	0.8962	0.8987	0.18	>0.05
	G_2	0.8979	0.8966	0.07	>0.05
Perinatal mortality (up to 9th post-infection day)	G_3	0.8524	0.8449	0.60	>0.05
	G_2	0.8431	0.8723	1.74	~ 0.08
Perinatal mortality (up to 21st post-infection day)	G_3	0.9383	0.9382	0.01	>0.05
	G_2	0.9366	0.9460	0.83	>0.05

* BAILEY, N. T.: Statistical Methods in Biology. Moscow, 1962, p. 58 (in Russian).

Table IV

Comparison of homozygous and heterozygous dams

Character	Locus	Expression of character	
		homozygotes	heterozygotes
Fertilization	G_3	−8.3474	+ 7.9177
	G_2	+3.8801	+10.7808
Embryonic mortality	G_3	−3.5719	+15.6766
	G_2	−2.7417	+32.3944
Postembryonic mortality	G_3	−4.6866	+ 5.5726
(up to 9th post-infection day)	G_2	−2.2969	+ 8.6626
Postembryonic mortality	G_3	−4.7076	− 0.0827
(up to 21st post-infection day)	G_2	−1.7726	− 4.2828

SUMMARY

The effect of the egg-white mucin-globulins on the viability of offspring was studied in New Hampshire chicks. It was found that the maternal mucin-globulin genotype influences the development of the embryo. The highest offspring mortality occurred in the case of diheterozygous dams. Since differences due to the maternal genotype appear only in pooled data and in some experiments, it is concluded that natural selection varies under different conditions.

Pullorum disease as a factor of natural selection levels off the differences due to the maternal genotype. There was found a difference between the homozygous and heterozygous dams in fertilization and embryonic mortality. Fertilization rate was higher in heterozygous hens (locus G_3), while the embryonic mortality was higher also in heterozygous dams (locus G_2).

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ON THE PATHOGENESIS OF DUCKLING HEPATITIS

By

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Viral duckling hepatitis affects primarily day-old ducklings. Birds younger than 10 days usually die of general symptoms within a few hours. In older birds the disease takes a milder course, and losses are less frequent. Ducklings above 4 weeks old are usually resistant.

In 1967 and 1968 a disease with high mortality occurred among 3- to 4-week old ducklings (600—800 g in weight). Death of the birds occurred after a relatively short diseased state and post mortem examination usually revealed an acute liver dystrophy. The liver was enlarged, ochre yellow and friable, and showed haemorrhages of varying extent on its surface and section plane. Histologically, fatty infiltration and extensive necrosis of the parenchymal cells were seen (Fig. 1). Infection experiments have shown that the disease in question was an outbreak of viral hepatitis. To clarify the causes of the unusually severe course in relation to the age of the birds, further examinations were carried out in this laboratory, which are reported below.

Materials and methods

Fifty day-old ducklings were divided into 5 groups. Groups I, II, IIa and III, 9 or 10 birds per group, were kept in the laboratory from day-old age on, whereas the birds of group III/a (10 ducklings) were reared up to 28 days of age in a duck farm. The schedule of experimental infections see under Experimental.

Liver function and the general state of the liver was examined in the living birds. Plasma SGPT and SGOT levels were determined in samples taken at several points of time. Samples were obtained by cardiac puncture and determinations were made by means of the "Fermognost" GPT and GOT tests. The results were expressed in international units. Blood samples for blood sugar assay were taken by cardiac puncture prior to the morning feeding and the blood sugar level was determined by enzyme diagnostic method, using the appropriate "Fermognost" test.

The organs of birds naturally died or killed during the period of experiment were fixed in 8% neutral formalin, formalin-calcium, Susa solution, Zenker's solution or 96% ethanol. Paraffin-embedded or frozen sections were

stained generally with haematoxylin and eosin, or phloxine-tartrazine. Neutral fats were demonstrated with Sudan red or Sudan black stain, cholesterol with the Schultz reaction or polarization method, phosphatides with Baker's staining technique, fatty acids with Fischer's reaction, glycogen with the PAS-reaction, ribonucleoprotein with gallocyanine-chromalaun stain or methyl green-pyronine stain.

Experimental

In the first part of our examinations, infection experiments were carried out with organs from 3- to 4-week old ducklings died of the disease in various flocks, to confirm the diagnosis of duckling hepatitis. The 10 birds belonging to group I were infected with a liver homogenate from 3- to 4-week old ducklings died of acute liver dystrophy in one of the farms.

Of the intraperitoneally infected 10 ducklings 9 died: 4 within 2 days, 2 each on the 3rd and 4th postinfection day, and 1 on the 5th postinfection day. The liver of the birds died on the 1st or 2nd days showed the acute dystrophy typical of rapidly progressive viral hepatitis. In the lobules, extensive fatty infiltration and necrosis of the parenchymal cells was seen. Haemorrhages were apparent in various parts of the lobules. In the liver of the bird died on the 5th day, necrosis and fatty infiltration of the parenchymal cells were only sporadic, but there was a distinct lympho-histiocytic infiltration of the perivascular connective tissue. In the livers of the birds died on the 1st or 2nd day, there was a scanty occurrence of cellular inclusions typical of the disease. Thus, microscopic examination served evidence that the duckling disease observed in the farm in question was actually a viral duckling hepatitis.

In the second part of our experiments an attempt was made to identify the factors responsible for the unusually severe course of the outbreak among the relatively old ducklings. It was postulated that the severe atypical course was due to some dietary damage, primarily of qualitative nature. Accordingly, the remaining 40 ducklings were given different diets up to the time of experimental infection at 3 to 4 weeks of age. Birds belonging to groups II and III, 11 and 9 in number, respectively, received for 21 and 28 days the same raising feed which had been given to the flock where the 3- to 4-week old birds died of duckling hepatitis manifested as an acute liver dystrophy. The 10 birds belonging to the control group II/a were kept for 21 days on a high quality mixed feed (bruised grain feed, meat meal, green feed, skimmed milk, lime supplement), whereas those belonging to the control group III/a, on a high quality granulated feed for 28 days until their transport to our laboratory.

Although the ducklings in group II and III consumed the critical feed *ad libitum* and also their conditions of management were satisfactory, they

grew slowly and their nutritional condition was poor. In group II, individual weights varied between 160 and 300 g at 21 days of age, and in group III between 300 and 450 g at 28 days of age. In groups II/a and III/a, the body weights of the ducklings fed the high-quality mixed feed and starting feed varied between 260 and 340 g and 950 and 1150 g at 21 and 28 days of age, respectively.

The preinfection levels of SGPT, SGOT and blood sugar are summarized in Table I.

Table I

Preinfection levels of SGPT, SGOT and blood sugar in the various experimental groups

Group	Age of birds (days)	Blood levels of		
		GPT (I.U.)	GOT (I.U.)	sugar (mg per 100 ml)
II	21	16.8±6.5	105.7± 4	98.5±23.7
II/a	21	4.6±1.4	13.6± 4.3	153 ±33.4
III	28	16.0±3.9	60.1±39.7	128 ±35.1
III/a	28	6.0±2.5	15.0± 3.9	138 ±35.1

To check liver lesions, 2 of the 11 ducklings belonging to group II were killed when 20 days old and their organs were examined microscopically. In both cases, a severe fatty infiltration was seen, extending either over the

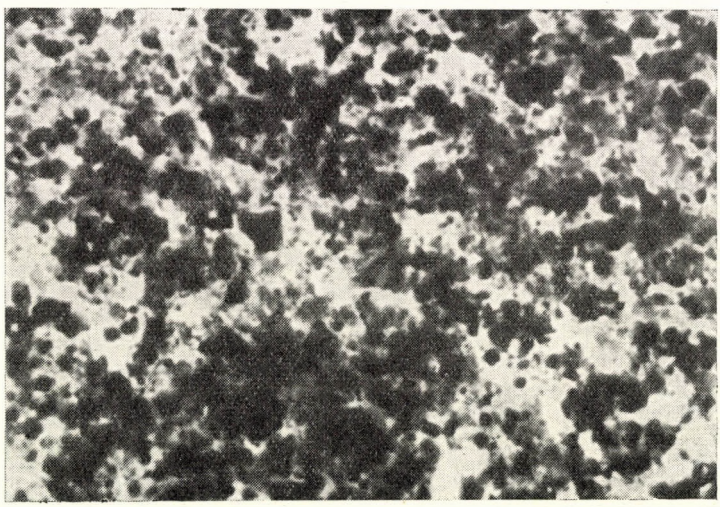

Fig. 1. Extensive necrosis and fatty infiltration of liver cells. Natural infection. Sudan black stain. ×300

entire area of the liver lobules, or over their marginal and central parts. The parenchymal cells were very poor in glycogen and also in ribonucleoprotein. Fat deposition was apparent also in the splenic parenchyma. In contrast, the livers of the two control ducklings from group II/a, also killed on the

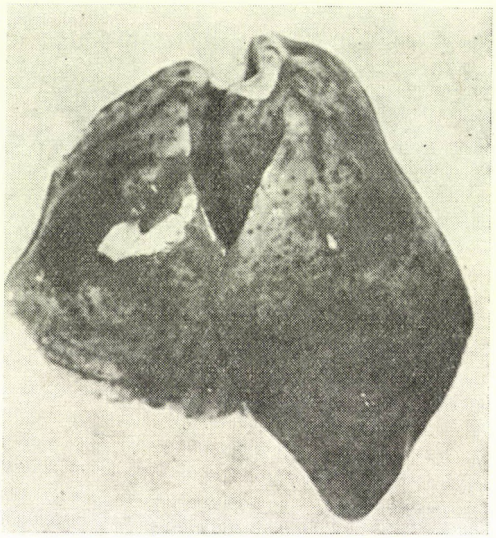

Fig. 2. Acute liver dystrophy with haemorrhages. Experimental infection. Section from the liver of a 21-day duckling died on the 1st day after infection

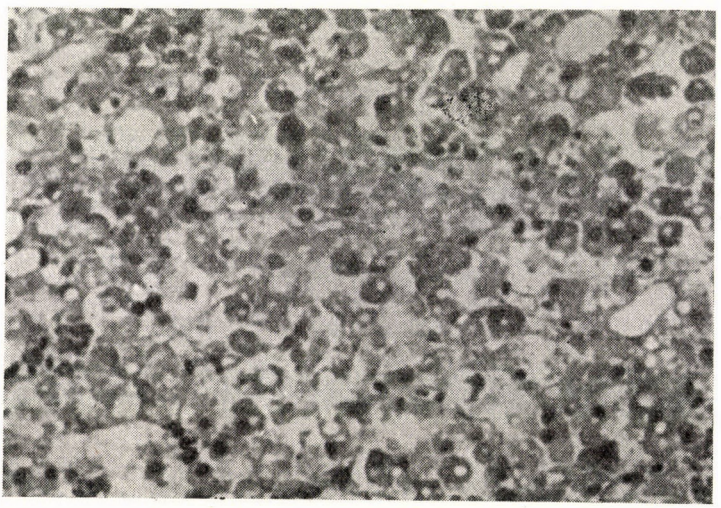

Fig. 3. Diffuse necrosis of liver cells. Liver from a duckling died on the 1st day after infection. Phloxine-tartrazine stain. ×300

20th day, were very rich in glycogen and either no fat at all or only traces of it were seen in the parenchymal cells.

Groups II and II/a were infected with duckling hepatitis virus when 21 days old, whereas groups III and III/a when 28 days old. The experimental

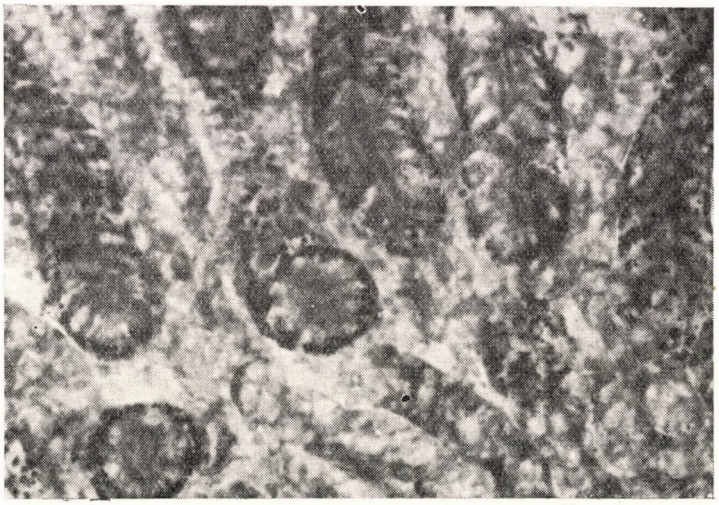

Fig. 4. Lipid droplets in the epithelium of the renal tubules. Kidney from a duckling died on the 1st day after infection. Sudan black stain. ×270

infection was made by the intraperitoneal route, using liver homogenate from day-old ducklings died of infectious hepatitis.

The results of the experimental infection were the following. In group II nine ducklings were infected, further two birds were killed prior to infection as noted above. Of the 9 infected ducklings 8 died, 5 of them on the 1st day, in the 10th, 12th, 14th and 16th postinfection hours, 2 on the 2nd day and 1 on the 3rd day.

Post mortem examination of the 5 ducklings died on the 1st day revealed an acute liver dystrophy. The liver weights varied between 12 and 15 g, the livers showed a conspicuous ochre-yellow colour and many haemorrhages on their surfaces and section planes (Fig. 2). In the lobules a nearly overall necrosis of parenchymal cells characterized by karyorrhexis, pyknosis and nuclear lysis was seen (Fig. 3). In the cytoplasms of the necrotic cells many lipid droplets of varying sizes were apparent and the deteriorating cells were replaced by large fat globules. The globules consisted of neutral fat and very small amounts of cholesterol. In the cytoplasms of the liver cells glycogen was either not demonstrable or only in traces (Fig. 5). Also the ribonucleoprotein content of the liver cells had decreased. In addition to parenchymal

cell lesions many haemorrhages and, in general, an activation of RHS cells were invariably found in various parts of the lobules. The livers of ducklings died late on the 1st postinfection day (in the 14th and 16th hour) showed diffuse infiltration of heterophilic granulocytes and inflammatory cellular reaction of the perivascular connective tissue between the parenchymal columns. In the livers of the birds died on the 2nd day there was still an

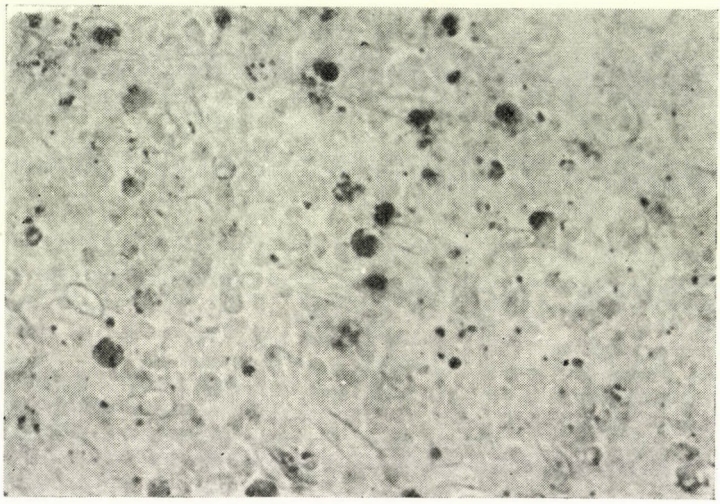

Fig. 5. Conspicuously low glycogen content in the liver from a duckling died on the first day after infection. PAS stain. ×270

Fig. 6. Low glycogen content in the liver of a duckling died on the 3rd day after infection. PAS stain. ×300

extensive fatty infiltration and necrosis of the parenchymal cells, but groups of normal liver cells made already appearance between necrotic areas. The inflammatory cellular reaction of the perivascular connective tissue became very pronounced and there was a sporadic proliferation of the biliary capillaries. Glycogen was still demonstrable in traces and the ribonucleoprotein content was still low.

The spleens of birds died on the 1st or 2nd day were in most cases slightly enlarged. In the Malpighian bodies and in the red pulp many groups of pale cells with high lipid contents were seen. In some spleens numerous heterophilic granulocytes were present. In the cytoplasms of the tubular epithelial cells of the kidneys many fat droplets had accumulated, usually adjacent to the basement membrane (Fig. 4). In another case, infiltration of lymphocytes and heterophilic granulocytes was seen in the interstitial tissue. The other organs showed no notable lesions.

The duckling died on the 3rd day had a serous hepatitis. The parenchymal cells of the liver generally appeared normal, but in most cells the nucleolus was enlarged. Necrotic or rounded cells were scattered in various areas of the lobules. In many parts of the perivascular connective tissue lymphocytic-granulocytic foci had formed and there was a conspicuous proliferation of the biliary capillaries. In the cytoplasms of the parenchymal cells traces of lipids were demonstrable. The glycogen content of the cells was considerably (Fig. 6), and their ribonucleoprotein content moderately, low.

In the spleen, aggregates of lipid-containing cells and, here and there, many heterophilic granulocytes were seen in the Malpighian bodies and in the red pulp. In the pancreatic parenchyma small necrotic foci were apparent. In the necrotic serous glandular epithelial cells occasionally fat deposition and in the interstitial tissue inflammatory cell infiltration was seen. No particular lesions were found in the kidneys.

Birds in group II/a, fed on mixed feed, showed during the postinfection days a slight weariness and inappetence and even lost some weight, but none of them died of the infection.

Of the 9 birds in group III, 28 days old, a weak duckling, 300 g in weight, died on the 2nd day after infection. Post mortem examination revealed a moderate degree of liver dystrophy, typical of viral duckling hepatitis. In the parenchymal cells, there was a sporadic deposition of lipid substances and sporadic necrosis; their glycogen contents were minimal, and also their ribonucleoprotein contents had lowered. Here and there proliferation of the biliary capillaries and mild inflammatory cellular reaction of the perivascular connective tissue were seen. In the splenic parenchyma groups of cells with foamy cytoplasm containing many lipid droplets were apparent.

Out of the survivors, one duckling was exsanguinated on each of the 2nd, 3rd, 4th, 5th and 6th postinfection days to examine their livers.

The duckling killed on the 2nd day, 450 g, was slightly ailing prior to bleeding. In its liver a moderate fatty infiltration was seen in cells located at the marginal parts of the lobules. Most cells had enlarged nucleoli. The cells in the central parts of the lobuli were rich, those in the peripheral parts were poor, in glycogen. In the perivascular connective tissue a marked inflammatory cellular reaction was seen along the blood vessels. An acute inflammatory

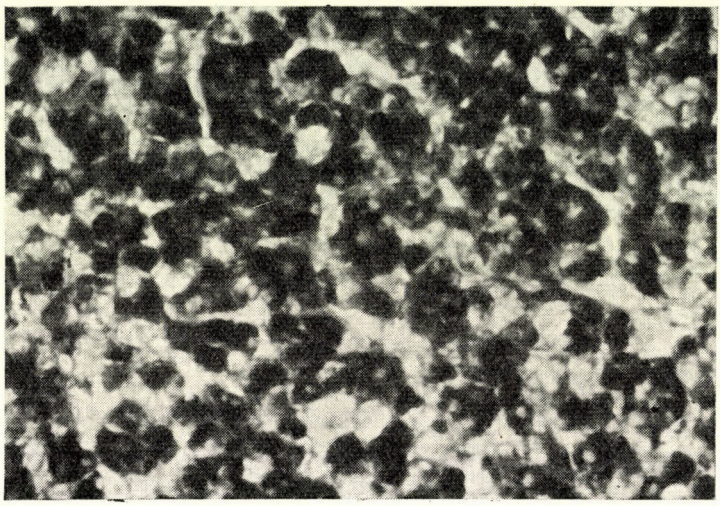

Fig. 7. High glycogen content in the liver of a control duckling killed on the 3rd day after infection. PAS stain. ×300

infiltration was apparent also in the septa of the pancreas and the serous glands showed focal fatty infiltration.

The parenchymal cells of the ducklings killed on the 3rd to 6th day were conspicuously rich in glycogen. They resembled plant cells, with minimal or no lipid content. The cytoplasms of the parenchymal cells contained glycogen at the normal level. In every case, inflammatory cellular reaction of the perivascular connective tissue, with occasional proliferation of the biliary ducts, was found. In the spleen exclusively the cellular elements of RHS had slightly increased. In the epithelial cells of the renal tubules no fatty infiltration was demonstrable. In the pancreas of one duckling a mild inflammatory cellular reaction of the interacinary septa and sporadic necrosis of the glandular tissue were found.

The ten 28-day old ducklings constituting the control group II/a were apparently healthy throughout and none of them died. The livers of the ducklings killed on the 3rd, 5th and 6th postinfection days were rich in glycogen (Fig. 7), and no lipid substances whatever were demonstrable in the parenchy-

mal cells. The liver of the birds killed on the 3rd day and of that killed on the 6th day showed sporadic minor foci of inflammatory cells in the interlobular septa.

Discussion

The first part of the present studies was carried out to clarify the cause of the liver dystrophy which had caused considerable losses among 3- to 4-week old ducklings.

Inoculation of 10-day old ducklings (group I) with liver homogenate from the 3- to 4-week old birds died of liver dystrophy has resulted within 1 or 2 days in fatal infections with typical symptoms of duckling hepatitis. In the livers gross lesions characteristic of viral hepatitis were found. Thus, the experimental infection has evidenced that inconsistently with the general view, viral hepatitis with high mortality may occur not only among day-old, but also among 3- to 4-week old ducklings. According to our observations, outbreaks of this kind take a rapid course and often lead to a fatal outcome in 1 or 2 days. Lesions occur primarily in the liver, most often in the form of liver dystrophy. In such cases, haemorrhages on the surface and section planes of the liver are frequently found.

In the second part of the present studies attempt was made to identify the factor(s) responsible for the unusually severe course and high mortality of the outbreak. In the course of infection experiments with duckling hepatitis virus on 3- to 4-week old birds, losses occurred exclusively in the two groups (Nos II and III) receiving for 3 and 4 weeks, respectively, the poor-quality feed identical in composition with that used in the farm where the outbreak had occurred. Losses were greater in group II, where 8 of the 9 birds died. In this group, the high preinfection plasma transaminase level was indicative of a considerable liver impairment. The preinfection level of the blood sugar was also low. Microscopic examination of organs from two ducklings killed in the preinfection period revealed a markedly disturbed fat and glycogen metabolism. In group III, also fed the deficient feed plasma transaminase activity indicated a lesser degree of liver impairment and only 1 duckling died of the experimental infection. Ducklings from the same flock and of the same age, raised on high-quality feed all survived the experimental infection, responding either with a mild temporary illness or not at all. No reasonable explanation could be found to the question why the ducklings fed the low-value feed one week longer (for 28 days) than their mates in group II showed better liver function tests and lower losses. This might have been due to their age; the liver function of older, 3- to 4-week ducklings is most probably less liable to impairment than that of day-old ducklings with retained yolk sacs,

overburdened by the rapid absorption of the yolk's lipid substances, or that of 2- to 3-week old ducklings being in the transitory phase. These considerations seem to support the earlier observation that ducklings one-month old or older cannot be fatally infected with duckling hepatitis virus.

The results of feeding and infection experiments permit the conclusion that the occurrence and severity of duckling hepatitis among relatively old, 3- to 4-week, ducklings in several farms was promoted by the unsatisfactory conditions of feeding which have led to an impairment of the parenchymal cells prior to infection. This explanation is also supported by the fact that high peroxide and acid values with simultaneous low antioxidant content, as well as sometimes a strong mouldy infection were demonstrable in the feed of the ducklings in the affected flocks. We suppose that the disturbances of hepatic fat and glycogen metabolism were primarily due to the dietary damage. It does not seem to be unlikely, though, that the supply of the birds with lipotropic substances was unsatisfactory.

The role of immunobiological factors in the more or less severe course of the outbreaks of viral hepatitis is, of course, indisputable. But it should be pointed out that the course of viral hepatitis is decisively influenced by the actual state of the parenchymal cells at the time of the infection. In the case of duckling hepatitis, the occurrence of irreversible liver impairment (dystrophy) and consequent fatal outcome is presumably preconditioned by a hepatic damage due to a preceding metabolic disturbance.

In the slowly progressive cases of viral duckling hepatitis the impairment of the parenchymal cells is less distinct. According to our observations, in such cases either serous hepatitis or hepatitis associated with inflammatory cellular reaction of the interlobular septa and proliferation of the biliary ducts develop, involving a notably lower fatality.

Considering that, according to our findings, the actual state of the liver plays an important role in the severity of the course of viral hepatitis, it is recommended that in addition to the usual control measures more attention than hitherto should be paid to dietary factors.

Acknowledgements. The authors are indebted to Mrs. G. PETHES for the preparation of the histological sections and to Mr. B. BORSI for skilful technical assistance in the serological examinations.

SUMMARY

Outbreaks of viral hepatitis causing great losses were observed in flocks of 3- to 4-week old ducklings. The gross and microscopic lesions found on post mortem examination were in every respect similar to those encountered in day-old ducklings died of viral duckling hepatitis. It was supposed that the severe outbreak — quite unusual among ducklings of this age of life — was favoured by liver impairment due to dietary error preceding the infection. This supposition was confirmed experimentally. The low-value feed used in one of the affected flocks was given to two groups of ducklings from day-old age on until experimental infection

at 3 and 4 weeks of age, respectively. The disease could be reproduced in the younger group in a severe form, while in the older group in a mild form. Two groups of control birds of the same ages survived the experimental infection without losses. It was concluded that the severity of the course of viral duckling hepatitis was, in addition to the age of the birds and other factors, considerably influenced by the actual state of the liver cells at the time of infection, with special regard to dietary error. It is, therefore, recommended to strictly observe the dietary factors in the prevention of duckling hepatitis.

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KOLLOIDREAKTIONEN IM LIQUOR CEREBROSPINALIS DES PFERDES

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Einleitung

Der Wert der Liquordiagnostik für die klinische Veterinärmedizin steht heute außer Zweifel, nachdem für alle bedeutsamen Tierarten eine geeignete und gefahrlose Entnahmetechnik erarbeitet wurde und die physiologische Zusammensetzung der Zerebrospinalflüssigkeit bekannt ist. Dennoch haben Liquoruntersuchungen bei Tieren bei weitem nicht annähernd die Bedeutung erlangt wie in der Humanneurologie, obwohl eine Reihe von Erkrankungen des Zentralnervensystems bei Tieren sehr charakteristische Liquorsyndrome erbringen und damit die intravitale Diagnostik wesentlich unterstützen können. Die Gründe dafür sind vielgestaltig und sollen nicht Grundlage dieser Diskussion sein.

Die neurologische Diagnostik mit Hilfe der Liquoruntersuchung wird aber nur dann erleichtert, wenn die vollständigen Syndrome der einzelnen Krankheiten bekannt sind (KAFKA, 1948). Wegen dieser Vollständigkeit der Befunde erscheint es uns gerechtfertigt, die Erfahrungen mitzuteilen, die wir bei der Untersuchung von Pferdeliquoren mit kolloidchemischen Methoden (Goldsol- und Mastixreaktion) gewonnen haben.

Seit der ersten Publikation über die Zusammensetzung des Pferdeliquors durch JOHNE (1897) vor 70 Jahren sind einige grundlegende Arbeiten erschienen, die an einem größeren Material die morphologischen und chemischen Inhaltsstoffe der Zerebrospinalflüssigkeit bei gesunden und neurologisch kranken Pferden quantitativ und qualitativ ermittelten. So haben BEHRENS (1950) und HIEPE (1958, 1960) unsere derzeitigen Kenntnisse über den Liquor gesunder Pferde, unterstützt durch die Angaben von ROEDER und REHM (1942) und FANKHAUSER (1954), begründet. Von neurologisch interessanten Krankheitsbildern beim Pferd sind besonders die Bornasche Krankheit (MÜLLER und SCHULZE, 1953; HIEPE, 1958, 1960; LISCHKE, 1957) und der Dummkoller (Hydrocephalus internus) (FANKHAUSER, 1956) Gegenstand von Liquoruntersuchungen gewesen. Außer Zellzahl und -morphologie wurden besonders die Eiweißkörper diagnostisch verwertet.

Interessant ist dabei die Tatsache, daß in den genannten Publikationen Angaben über den Ausfall von Kolloidreaktionen bis auf eine kurze Andeutung der Kolloidtypen im Pferdeliquor bei FANKHAUSER (1954) fehlen. Lediglich in dieser und in der Arbeit von GYARMATI (1952) konnten Hinweise dafür gefunden werden, daß kolloidchemische Untersuchungen an den Eiweißkörpern des Pferdeliquors bisher durchgeführt wurden. Im Verlauf der letzten Jahre konnten wir bei 146 Pferdeliquoren derartige Reaktionen ausführen, nachdem am Liquor des Schafes zunächst vergleichend mehrere aus der Human-Neurologie bekannte Methoden auf ihre Brauchbarkeit geprüft und die Mastixreaktion (in der Modifikation nach EMANUEL und ROSENFELD, 1927 a, b) als Mastix-Lumbotest und die Goldsolreaktion (nach LANGE, 1912 und BOROWSKAJA, 1934) als für die Verhältnisse des veterinärmedizinisch-klinischen Laboratoriums brauchbarste und diagnostisch sicherste Kolloidreaktionen ermittelt wurden (LIPPMANN, 1960).

Darüber hinaus konnten für einige Krankheiten des Schafes (Listeriose, Bornasche Krankheit) charakteristische Kurventypen festgestellt werden, die eine hinreichend sichere Abgrenzung vom Ausfall der Reaktionen in der Zerebrospinalflüssigkeit gesunder Schafe gestatten und die bekannten Liquorsyndrome beim Schaf ergänzen. In der Zwischenzeit wurden die Ergebnisse unserer diesbezüglichen Untersuchungen am Rinderliquor in Ergänzung zu den von FANKHAUSER (1954) gesammelten Erfahrungen der Berner Schule mitgeteilt (LIPPMANN, 1968).

Methodik

Das Wesen der Kolloidreaktionen besteht darin, daß die Eiweißkörper die disperse Phase in dem als kolloidale Lösung aufzufassenden Liquor bilden. Dieses Kolloidsystem ist normalerweise durch die Verteilung der grobdispersen Globuline und der feindispersen Albumine und ihre gleichsinnige Ladung stabil, auch dann, wenn durch Zusatz lyophober Kolloide in Solform in Gegenwart von Elektrolyten bei Einhaltung einer entsprechenden Konzentration ein Überwiegen der Kolloide eintritt (ROEDER u. REHM, 1942). Erst ein Verschieben im Verhältnis zwischen Albuminen und Globulinen, die bei bestimmten Erkrankungen des Zentralnervensystems in nahezu charakteristischer Weise auftreten, führt zu Ausfällung von Eiweißen in verschiedenen Verdünnungsstufen. Dieser Vorgang wird benutzt, um Eiweißverschiebung im Liquor sichtbar zu machen und sie diagnostisch zu verwerten (SCHMITT, 1932). Ihre Aussagekraft ist damit begrenzt, nach KAFKA (1948) sollten Kolloidreaktionen nur zusammen mit den Eiweißrelationen betrachtet werden und lediglich das Liquorsyndrom ergänzen. Spezifische Befunde können von ihrer gesonderten Betrachtung nicht erwartet werden.

Bei unseren routinemäßig ausgeführten Liquoruntersuchungen kamen die Mastix- und die Goldsolreaktion zur Anwendung.

Die *Mastixreaktion* wurde in der von EMANUEL und ROSENFELD (1927a, b) beschriebenen Modifikation als Mastix-Lumbotest mit handelsüblichen Testlösungen (Hersteller: Fa. Bergmann, KG, Berlin—Zehlendorf) nach Gebrauchsanweisung ausgeführt. In 12 Röhrchen wurde dazu eine progressive Liquor-Verdünnungsreihe (1 : 1 bis 1 : 2000) hergestellt, zu der jeweils 1 ml des stets frisch bereiteten Mastix-Sols gegeben wurde (weitere methodische Einzelheiten siehe bei LIPPMANN (1960), dort auch umfangreiche Literatursammlung über Kolloidreaktionen). Die Ablesung erfolgte nach 24 Stunden, die Ergebnisse wurden jeweils in ein entsprechendes Schema (Abb. 1) eingetragen.

Bei der *Goldsolreaktion* wurde nach der Originalmethodik von LANGE (1912) ebenfalls eine Verdünnungsreihe des Liquors mit physiologischer Kochsalzlösung (12 Röhrchen von 1 : 10 bis 1 : 20 480) hergestellt. Die Bereitung des Goldsols geschah nach den Angaben von BOROWSKAJA (1934), wobei neben einer gewissen Übung auch der ständige Vergleich neu hergestellter Goldsole mit bekannten Solen bzw. bereits geprüften Liquoren notwendig ist. Auch hierbei wurde die Reaktion nach 24 Stunden abgelesen.

Stets kam das Untersuchungsmaterial frisch zur Verarbeitung. Der Liquor wurde 6 min. lang bei 1000 U/min. zentrifugiert. Um eine Verfälschung der Ergebnisse durch Blutbeimengungen zu vermeiden, wurden nur solche Liquoren verarbeitet, die in Anlehnung an die Untersuchungen von WORTMEIER (1940) weniger als 400/3 Erythrozyten je mm^3 (FUCHS-ROSENTHAL-

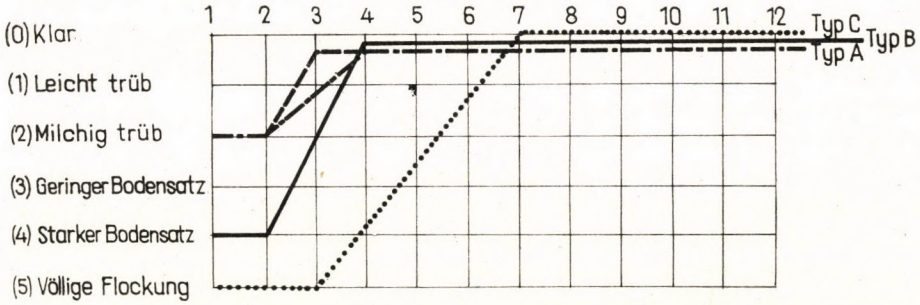

Abb. 1. Kurventypen der Mastix-Reaktion im Liquor cerebrospinalis des Pferdes

Zählkammer) aufwiesen, die überwiegende Zahl der ausgewerteten Liquoren setzt sich aus völlig blutfreiem Liquor zusammen.

Parallel zu den Kolloidreaktionen wurde das übliche Untersuchungsspektrum im Liquor ausgeführt.

Untersuchungsgut

Es gelangten insgesamt 146 postoccipital gewonnene Liquoren von Pferden, die als Patienten oder Versuchstiere stationär in die Klinik aufgenommen worden waren, zur Untersuchung. Das Material setzt sich zusammen aus 32 klinisch gesunden Tieren; 90 Pferden mit Bornascher Krankheit (Polioencephalomyelitis lymphocytaria enzootica equi), bei denen sämtlich postmortal die Diagnose histologisch bestätigt wurde; 5 mit klinischer Diagnose »Dummkoller« (pathol.-anat. Hydrocephalus internus); 6 mit Leberkoller (hepatocerebrales Syndrom) sowie 13 Pferden mit verschiedenen fieberhaften Erkrankungen (Leptospirose-Verdacht, gangränöse Pneumonie, Tetanus, Infektiöse Anämie, Meningitis purulenta, Tuberkulose und Urämie). Diese letztgenannte Gruppe wurde wegen der Heterogenität des Materials bzw. der geringen Tierzahl je Krankheit in die vergleichende Auswertung nicht mit einbezogen. Die übrigen vier Gruppen umfassen noch 133 Pferde. Bei diesen Liquoren kam in jedem Falle die Mastixreaktion zur Ausführung, während mittels Goldsolreaktion wegen technischer Schwierigkeiten nur 65 (25 gesunde Pferde, 37 Bornasche Krankheit, 3 Hydrocephalus internus) von ihnen gleichzeitig untersucht wer-

den konnten. Außerdem sind zehn Liquoren vergleichend mittels Goldsol- und Mastixreaktion sowie der Salzsäure-Collargol-Reaktion (RIEBELING, 1938) und der TAKATA-Probe (TAKATA und GOHR, 1957) im Vorversuch geprüft worden. Dabei erwiesen sich die beiden letztgenannten als weitgehend unspezifisch, wie wir das auch für den Schafliquor feststellen konnten (LIPPMANN, 1960).

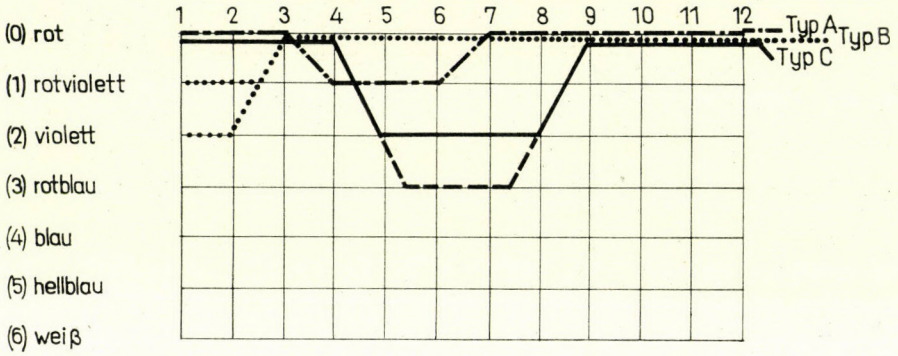

Abb. 2. Kurventypen der Goldsolreaktion im Liquor cerebrospinalis des Pferdes

Besprechung der Ergebnisse

Überblickt man das gesamte Material, so lassen sich bei der *Mastixreaktion* im Pferdeliquor drei Typen unterscheiden, die folgendermaßen gekennzeichnet sind (Abb. 1):

— Typ A: flache Linkskurve bis zum 3. oder 4. Röhrchen, die Veränderungen gehen über eine milchige Trübung nicht hinaus;

— Typ B: mitteltiefe Linkskurve, die ebenfalls bis zur 4. Verdünnungsstufe reicht und in den ersten beiden Röhrchen Bodensatz mit darüberstehender leichter Trübung aufweist;

— Typ C: extrem tiefe Linkskurven mit völliger Ausflockung in den ersten beiden Verdünnungsstufen, graduell abgeschwächt bis zum 7. (6. oder 8.) Röhrchen reichend.

Ausgesprochene Rechtskurven konnten wir in unserem Material niemals finden.

Bei der *Goldsolreaktion* sind ebenfalls drei unterschiedliche Reaktionskurven zu ermitteln (Abb. 2):

— Typ A: Farbumschlag im 4—6. Röhrchen bis rotviolett, übrige Verdünnungsstufen unverändert. Wir möchten diesen Reaktionsausfall noch nicht als »Kurve« bezeichnen, er deutet lediglich einen Ausfall an. Nach SCHMITT (1932) können überdies Zacken in einzelnen Röhrchen beispielsweise als Folge geringfügiger pH-Änderungen eintreten.

— Typ B: flache Linkskurve, beschränkt auf die ersten beiden Verdünnungsstufen, bis violett oder rotviolett reichend;
— Typ C: mitteltiefe Mittelkurve, violette bis rotblaue Verfärbung vom 5. (4.) bis 8. (7.) Röhrchen, während Anfangs- und Endteil der Kurve in gerader Linie (unverändert rot) verlaufen.

Wir haben nun retrospektiv versucht, die Kurventypen den einzelnen Krankheiten zuzuordnen. Als Vergleich geben wir in Tabelle 1 die für die Patientengruppen ermittelten Eiweißwerte und das Verhältnis Globuline : Albumine (Eiweißquotient) an. Die Zahlen stellen jeweils Mittelwert sowie größter und kleinster Einzelwert dar, sie wurden mit der volumetrischen Methode nach KAFKA (1948) bestimmt. Auf eine weitere statistische Bearbeitung dieses Materials haben wir wegen der bekannten Unzulänglichkeit der Methode verzichtet, und die Zahlen zur Orientierung über die Verteilung der Eiweißfraktionen bei den untersuchten Liquoren dem Ausfall der Kolloidreaktionen gegenübergestellt.

Tabelle 1

Gesamt-Eiweiß-Gehalt nach KAFKA (in mg/100 ml) und Eiweiß- (Globulin : Albumin-) Quotient im Liquor cerebrospinalis von Pferden

		Klinisch gesund N = 32	Bornasche Krankheit N = 90	Hepatocerebrales Syndrom N = 6	Hydrocephalus internus N = 5
Gesamt-Eiweiß	$\bar{x}$	54,9	71,8	48,0	49,4
	x_{max}	72,0	216,0	60,0	76,8
	x_{min}	24,0	12,0	28,8	24,0
Quotient	$\bar{x}$	0,51	1,24	1,60	0,32
	x_{max}	1,12	6,46	4,00	0,46
	x_{min}	0,06	0,15	0,53	0,24

Die Häufigkeitsverteilung (jeweils in %) der Kurventypen von Mastix- und Goldsolreaktion (Tab. 2) zeigt zunächst, daß ein sog. negativer Ausfall der geprüften Reaktionen (kein Farbumschlag bzw. keine Trübung in der gesamten Verdünnungsreihe) beim Pferd im Gegensatz zu anderen Tierarten bzw. zum Menschen nicht vorkommt. Wir haben also immer Farbumschläge und Trübungen auch bei gesunden Pferden zu erwarten. Damit finden wir uns in Übereinstimmung mit den Angaben FANKHAUSERS (1954), jedoch fanden wir im physiologischen Pferdeliquor nicht so stark ausgeprägte Mittelkurven bei der Goldsolreaktion bzw. Linkskurven bei der Mastix-Reaktion. Die von GYARMATI (1952) bei an Beschälseuche erkrankten Pferden ermittelten Gold-

Tabelle 2

Häufigkeitsverteilung der Kolloidkurven bei gesunden und neurologisch kranken Pferden

	Klinisch gesund	Bornasche Krankheit	Hepatocerebrales Syndrom	Hydrocephalus
Mastix-Reaktion:	N = 32	N = 90	N = 6	N = 5
Typ A	96,9%	33,3%	50,0%	80,0%
Typ B	3,1%	40,0%	16,7%	20,0%
Typ C	0%	26,7%	33,3%	0%
Goldsol-Reaktion:	N = 25	N = 37	N = 0	N = 3
Typ A	44,0%	37,8%	—	100,0%
Typ B	40,0%	10,8%	—	—
Typ C	16,0%	51,4%	—	—

solkurven entsprechen unserem Typ B und können durchaus noch als zum physiologischen Bereich gehörig gerechnet werden.

Die Verteilung der Kurventypen bei den ausgewerteten Kollektiven zeigt für die *Mastixreaktion* (Tab. 2; Abb. 3) eine sehr deutliche Häufung des Typs A bei gesunden Pferden, nur in einem der 32 untersuchten Fälle war eine mitteltiefe Linkskurve (Typ B) zu ermitteln. Normalerweise gibt also der Pferdeliquor eine flache Linkskurve. Bei Bornascher Krankheit sind ein Drittel der Kurven diesem Normaltyp zuzurechnen, während zwei Drittel mitteltiefe oder

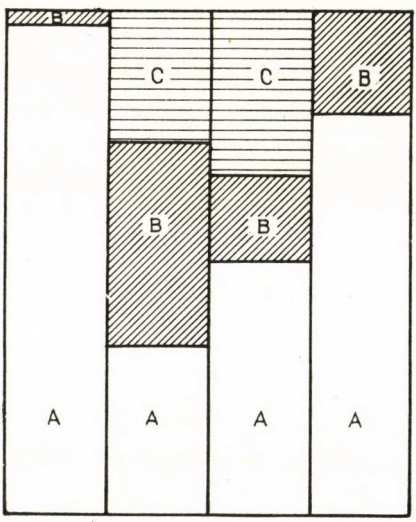

Abb. 3. Häufigkeitsverteilung der Mastix-Kurventypen A, B und C im Pferdeliquor. Säulen von links nach rechts: Klinisch gesunde Pferde (N = 32), Bornasche Krankheit (N = 90), hepatocerebrales Syndrom (N = 6) und Hydrocephalus internus (N = 5). (Relative Häufigkeit, jeweils N = 100 gesetzt)

sehr tiefe Linkskurven von den Typen B und C zeigten. Die Angaben über die Reaktion bei den beiden anderen Krankheitszuständen sind wegen der geringen Patientenzahl als kasuistischer Beitrag aufzufassen, jedoch fällt auf, daß auch hierbei der Typ A überwiegt.

Bei der *Goldsolreaktion* verteilen sich die Typen A und B bei gesunden Pferden (Tab. 2) etwa zu gleichen Teilen, nur in 4 der untersuchten Liquoren wurde eine mitteltiefe Mittelkurve vom Typ C gefunden. Dagegen sind über die Hälfte der Borna-Liquoren diesem Typ C zuzurechnen, während der Typ B (flache Linkskurve) sehr selten vorkommt. Damit läßt sich der Ausfall der Goldsolreaktion bei dieser Krankheit als verschiedengradiger Kurvenverlauf im mittleren Teil der Verdünnungsreihe charakterisieren. Die drei Liquoren von Patienten mit Hydrocephalus internus zeigen übereinstimmend eine Linkszacke vom Typ B.

Es kann in Auswertung des Materials festgestellt werden, daß im Gegensatz zum relativ einheitlichen Ausfall der Mastixreaktion in den einzelnen Gruppen bei der Goldsolreaktion eine erhebliche Streuung der Typen auftritt. Damit wird für den Pferdeliquor ein ähnliches Verhalten wie beim Schaf ermittelt (LIPPMANN, 1960). Beim Vergleich mit den uns aus dem Schrifttum bekannten Kurventypen (FANKHAUSER 1954) fällt auf, daß bei gesunden Pferden die Mastixreaktion nahezu der Kurve FANKHAUSERS entspricht (F. arbeitete allerdings mit der Normomastix-Reaktion), die Goldsolkurven in unserem Material aber wesentlich flacher verlaufen. In der Bereitung des Sols kann dieses unterschiedliche Verhalten schwerlich eine Erklärung finden, denn unsere während des gleichen Zeitraumes mit gleichen Solen erhaltenen Kurven für Schaf- und Rinderliquoren entsprechen weitgehend den von FANKHAUSER angegebenen Goldsolkurven für diese Tierarten. Für Pferde mit Bornascher Krankheit fehlt ein Vergleich, hierüber sind uns im Schrifttum keine Angaben bekannt. Bei mit dem Borna-Virus infizierten Kaninchen fand ZEKI (1934) mit der Normomastixreaktion in den ersten Gläsern eine starke Ausfällung, dieser Reaktionstyp C bzw. B kann gut mit unserem Material in Einklang gebracht werden. Bei Bornascher Krankheit des Schafes fanden wir (LIPPMANN, 1960) für die Goldsolreaktion überwiegend mitteltiefe Linkskurven bis rotblau (entspricht Typ B), für Mastix-Lumbotest mitteltiefe Linkskurven (Typen B und seltener C).

Eine Übereinstimmung zwischen Ausfall der Kolloidreaktionen und Eiweißgehalt bzw. dem Quotienten der Eiweißfraktionen läßt sich kaum beweisen, wie die große Schwankungsbreite dieser Größen darlegt (vergleiche Tabelle 1 und 2). Den Mastixtyp A fanden wir beispielsweise bei einem Eiweißquotienten von 0,15 ebenso wie bei einem solchen von 3,45; ein erhebliches Überwiegen der Globulinfraktion im Gesamteiweißgehalt braucht also nicht unbedingt zu einem bestimmten Kurventyp zu führen. Allerdings muß dabei die relativ hohe methodische Fehlerbreite der volumetrischen Eiweißbestimmung

in Rechnung gestellt werden. Exakte chemisch-photometrische Eiweißbestimmungs-Verfahren lassen Beziehungen zwischen Eiweißquotienten und Kolloidtypen sehr wahrscheinlich zu, wie erste orientierende Unternehmungen mittels Exton-Reagens (KRAUSE, 1968) und einer modifizierten Biuretmethode (MAURER, 1968), über deren Ergebnisse zu gegebener Zeit berichtet wird, zeigen.

In Ergänzung dieser Befunde kann beim Vergleich der Eiweißkonzentration im Liquor gesunder Pferde (KAFKA-Werte) verschiedener Untersucher eine gute Übereinstimmung der Befunde (Tabelle 3) festgestellt werden. Demgegenüber fanden wir einen durchschnittlich höheren Anteil der Globuline im Eiweißgehalt (Mittelwert 0,51 für den Quotienten) als andere Untersucher. Möglicherweise sind die normalerweise im Gegensatz zu anderen Tierarten bei den Kolloidreaktionen auftretenden Farb- und Trübungsänderungen vorwiegend im linken Kurventeil verantwortlich zu machen. Entgültige Klärung hierüber wird erst die Eiweißbestimmung mit verbesserten Methoden erbringen.

Tabelle 3

Eiweißgehalt (Mittelwert, Min., Max., jeweils in mg/100 ml) nach der KAFKA-Methode und Globulin-Albumin-Quotient bei klinisch gesunden Pferden

Autor	Gesamteiweiß $\bar{x}$ (Schwankung)	Quotient $\bar{x}$ (Schwankung)
BEHRENS (1950)	47,58	0,26
	(28,75—71,75)	(0,06—0,69)
HIEPE (1960)	52,44	0,29
	(21,6 —98,2)	(0,02—1,45)
LISCHKE (1957)	48,7	0,29
	(31,2 —73,2)	(0,03—1,19)
THÖRLE (1950)	52,12	0,23
	(33,08—77,20)	—
Eigene Untersuchungen	54,9	0,51
	(24,0 —72,0)	(0,06—1,12)

Eine Vermehrung des Globulinanteils bei Bornascher Krankheit (0,15 bis 6,46; Mittelwert 1,24) ist bei Berücksichtigung der großen Streuung der Einzelwerte besonders augenfällig und gegenüber den von HIEPE (1958, 1960) und LISCHKE (1957) mitgeteilten Verhältniszahlen von 0,02—1,45 ($\bar{x} = 0{,}336$) bzw. 0,1—1,1 zunächst unerwartet. Der Ausfall der Kolloidreaktionen, beson-

ders die starken Flockungen beim Mastix-Lumbotest, können damit eine Erklärung finden. Auch hierbei werden exakte chemische Nachweisverfahren für Globuline eine Beweisführung für diese Hypothese erleichtern.

Schlußfolgerungen

Unter dem Aspekt eines vollständigen Liquorsyndroms ist die Ausführung einer Kolloidreaktion in der Zerebrospinalflüssigkeit des Pferdes für die intravitale Diagnostik zentralnervöser Störungen als Ergänzung zur quantitativen Eiweißbestimmung wertvoll. Bei Beurteilung der Gesamtheit der Befunde einschließlich der eingehenden klinisch-neurologischen Untersuchung lassen sich Schlußfolgerungen aus dem Kurvenverlauf ziehen.

Als technisch einfach und im Ergebnis relativ einheitlich hat sich bei den vorliegenden Untersuchungen die Mastix-Reaktion in Form des handelsüblichen Mastix-Lumbotests erwiesen. Sie ist der Goldsol- und anderen Kolloidreaktionen überlegen und stellt damit die Methode der Wahl bei kolloidchemischen Liquoruntersuchungen dar. Dennoch muß vor einer Überbewertung der Befunde gewarnt werden, aus dem Kurvenverlauf der Mastix-Reaktion allein wird niemals eine Diagnose gestellt werden können. Sie stellt nur eine von vielen Liquoruntersuchungsverfahren dar und muß innerhalb ihrer Grenzen bewertet werden.

ZUSAMMENFASSUNG

Zur Vervollständigung der Liquorsyndrome bei gesunden sowie bei an Bornascher Krankheit, Hydrocephalus internus und hepatocerebralem Syndrom erkrankten Pferden werden die Ergebnisse der Mastix- und der Goldsolreaktion von insgesamt 133 Liquoren mitgeteilt. In der Zerebrospinalflüssigkeit gesunder Pferde ergibt die Mastixreaktion flache Linkskurven, die Goldsolreaktion geringfügige Farbumschläge im Mittelteil der Verdünnungsreihe oder flache Linkskurven. Bei Bornascher Krankheit sind im Liquor mit der Mastixreaktion zum Teil normale Kurventypen, überwiegend aber tiefe Linkskurven zu erzielen, während die Goldsolreaktion vorwiegend flache bis mitteltiefe Mittelkurven ergibt. Bei Hydrocephalus und bei Leberkoller werden im untersuchten Material ähnliche Kurventypen wie bei gesunden Tieren erhalten.

Nach dem Ergebnis der Untersuchungen wird die Mastixreaktion in Form des Mastix-Lumbotest als Methode der Wahl für kolloidchemische Liquoruntersuchungen angesehen. Sie stellt eine wertvolle Ergänzung zur quantitativen Eiweißbestimmung dar.

Vergleichend werden abschließend die bei den untersuchten Liquoren quantitativ ermittelten Eiweißwerte mitgeteilt und diskutiert.

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STUDIES OF PARASITE METABOLISM

I. IN VITRO EXAMINATION OF THE VITAMIN A METABOLISM OF ASCARIS SUUM

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Disturbances of the vitamin A supply of hosts infected with ascarids have been observed for several decades.

HIRAISKI (1927) succeeded in transferring the human roundworm parasite *Ascaris lumbricoides* to swine depleted of vitamin A. ACKERT et al. (1927) demonstrated that in chickens vitamin A deficiency favours the growth of *Ascaridia galli;* in depleted birds the worms grew larger. LENTSKAYA (1963) reported a decrease of the liver's vitamin A content in chickens orally treated with an *A. galli* extract. PANDE and KRISHNAMURTY (1959) measured abnormally low vitamin A contents of the livers from chickens infected with *A. galli.* These phenomena have been tentatively attributed to decreased absorption of vitamin A and carotenoids, owing to impairment of the intestinal epithelium by the presence of the worms.

The above data all deal with the vitamin A depletion of infected hosts; so far no information has been available on the uptake and utilization of vitamin A by the parasites.

In the frame of *in vitro* studies on the metabolism of helminths in this laboratory, the vitamin A metabolism of *Ascaris suum* has been examined first.

The present experiments were undertaken to clarify whether *Ascaris suum* was capable of *in vitro* uptake and utilization of vitamin A and how this was affected by the presence of certain substances and, finally, how vitamin A distributed in the various organs of the parasite.

Materials and methods

Female *Ascaris suum* worms collected from the intestines of freshly slaughtered swine in the Budapest abattoir were used throughout. Prior to the experiments the worms were thoroughly washed several times.

In examinations aimed at determining the distribution of vitamin A in the parasites' organs, the worms were dissected individually as follows: the parasite was suspended at the anterior end, then its caudal end was cut off and the body fluid collected in a conical glass jar. The cuticle and subcuticle were incised longitudinally, the intestine and reproductory organs were separated and then the cuticle was separated from the subcuticle.

During the experiments, the parasites were maintained in the following medium (further on referred to as fluid medium):

Basal medium (Earle's solution):

Components g/1000 ml distilled water:

NaCl	6.9	NaH_2PO_4	0.14
KCl	0.4	$NaHCO_3$	2.2
$CaCl_2$	0.2	Glucose	1.0
$MgSO_4 \cdot 7H_2O$	0.2	Phenol red	0.02

Penicillin	250 000 I.U.
Streptomycin .	0.25 g
Sterogenol	0.01 g
Vitamin A	20 000 I.U.

As vitamin A, the preparation Aquital (Chinoin, Budapest) was used. Composition: axerophtholum aceticum conc. oleosum 2 000 000 I.U., Propylium gallicum 0.05 g., acid. sorbinicum 0.2 g, sorboxaethen, oleinic. 15 g, ad 100 ml.

Previous experiments have shown that the presence of penicillin, streptomycin and Sterogenol in the fluid medium prevents bacterial and fungal growth.

The edible oil used in the experiments was emulsified in a 7% gum-arabic solution by mixing equal amounts in a Turmix homogenizer. From this emulsion 0.2 or 0.4 ml was added to 100 ml fluid medium.

As a carotenoid, the preparation "Carophyll Yellow" (F. Hoffman-La Roche et Co. Ltd., Basel) was used. It was suspended in hot water and added to the fluid medium in a quantity to make an 0.01% concentration.

During the experiments, 10 worms each were maintained in 100 ml fluid medium, which was equal to 6—8 ml medium per 1 g live weight. Both the worms and bottled media were stored at 37 °C. The pH of the fluid medium, changed by the metabolic products, was adjusted daily to 7.2 by the addition of 1 *N* NaOH. The figures in the Tables all represent corrected values of utilized vitamin A. The slight losses of vitamin A due to decomposition during storage had been subtracted. As controls worm-free fluid media were incubated.

From the culture flasks in the incubator, 2 worms each and appropriate amounts of fluid medium were removed for vitamin A assay on the days indicated in Table I. The worms were homogenized and 5 g amounts of the homogenates were used for the assays.

Vitamin A was determined by CARR-PRICE's method (1926).

Experimental

Preliminary experiments had shown that on maintenance in fluid medium for 4 days, the vitamin A content of *Ascaris suum* specimens tended to increase, whereas that of the medium to decrease. Following their recovery in the abattoir, the worms contained no demonstrable amounts of vitamin A.

Table I

Distribution of vitamin A in the organs of *A. suum* on various days of the experiment

Day of experiment	3rd day					7th day					9th day				
Parasite number	8 worms					10 worms					7 worms				
Organ	Cut.	Scut.	Sex	Int.	Flu.	Cut.	Scut.	Sex	Int.	Flu.	Cut.	Scut.	Sex	Int.	Flu.
Vitamin A I.U./g	41	55	90	165	40	48	34.5	61	125	21.5	41	56	85	127	15
% distribution of vitamin A	8.3	36.9	30.5	14.6	9.7	14.1	30.5	28	11.3	16.1	8.3	35.8	31	12.8	12.1

Per cent related to total body weight; Cut., Cuticle 18.7; Scut., Subcuticle 39.0; Sex, Reproductory organs 18.5; Int., Intestine 4,3; Flu., Body fluid 19.5

First of all attempt was made to clear the distribution of vitamin A uptake in the parasites' organs. Five worms each were placed in flasks, in fluid medium corresponding to their weights. The worms were examined on days 3, 7 and 9; after dissecting them individually, the identical organs from 5 worms were pooled and homogenized. The results are shown in Table I and

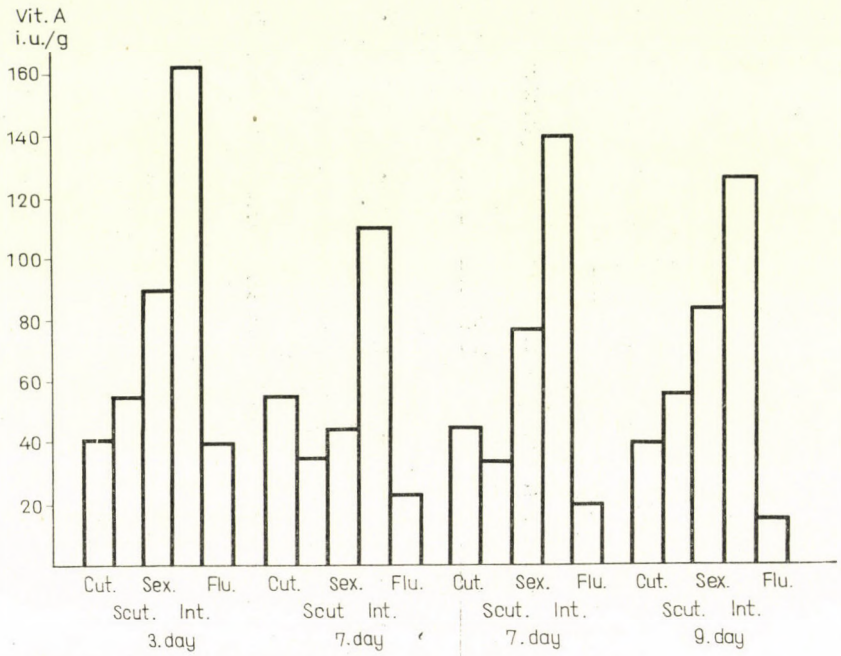

Fig. 1. Distribution of vitamin A in the organs of *Ascaris suum.* Cut., Cuticle; Scut., Subcuticle; Sex., Reproductory organs; Int., Intestine; Flu., Body fluid

Figs 1 and 2. In this context, the question arose whether a correlation did exist between the fat content and vitamin A content of the individual organs, considering the lipid-soluble nature of vitamin A. Fat was determined in a Soxhlet apparatus. The results are shown in Table II.

Table II

Fat content of some organs of *Ascaris suum* (%)

Cuticle	5.3
Subcuticle	1.2
Reproductory organs	5.1
Intestine	3.3
Body fluid	1.1

Comparison of the fat contents of the individual organs with their vitamin A contents listed in Table I has shown that they are unrelated.

In further experiments the quantitative relations of vitamin A uptake were examined, also with respect to the rise of vitamin A concentration in the parasites' bodies and its decrease in the fluid medium.

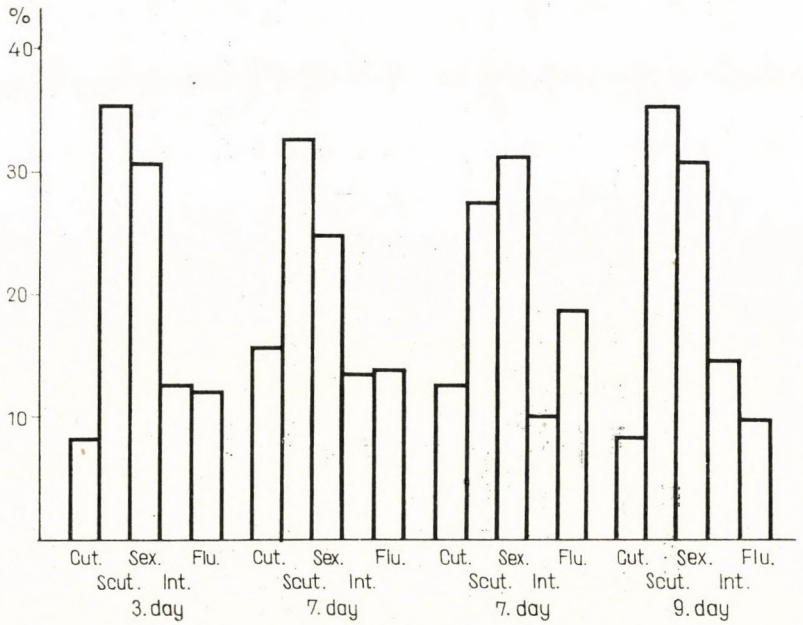

Fig. 2. Percentual distribution of vitamin A in the organs of *A. suum*. Explanation, see legend to Fig. 1

Measurements were made on days 1, 2, 3 and 6. As previous studies had shown a fairly constant percentual distribution of vitamin A in the organs, further determinations were limited to the total vitamin A content of the worm body. Each assay was made on 5 g worm emulsion and from this were calculated the values relative to total worm weight. The slightly uneven decline of the curves representing the vitamin A values are due to individual deviations resulting from the performance of assays on the subsequent days on different worms. Thus, the numerical values given in this paper serve to indicate the tendency rather than the absolute values. The results are shown in Fig. 3 and Table III. In the Figures, the vitamin A contents of the worms have been marked in the sequence of their decrease on the right ordinate, whereas the vitamin A contents of fluid medium corresponding to unit worm weight on the left ordinate.

In subsequent experiments we examined the effects of edible oil as a lipid substance and of carotenoids as precursors on the *in vitro* vitamin metab-

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Table III

Changes in vitamin A contents of the fluid medium and of the worms maintained in it

	Vitamin A content of fluid medium (I.U./ml)					Vitamin A content of fluid medium related to 1 g worm weight (I.U.)					Vitamin A content of worms (I.U./g)			
	Days of experiment													
	0	1	2	3	6	0	1	2	3	6	1	2	3	6
I	20.0	15.5	9.5	7.3	1.6	142.2	110	67.5	52.8	11.4	12.1	16.4	31.8	54.0
II	20.6	19.1	16.7	12.7	7.3	123	115	100	75.2	43.8	4.1	4.1	9.6	13.0
III	20.2	17.9	17.9	17.0	7.3	166	147	147	140	60	11.4	10.0	14.8	8.2
IV	19.9	1.8	0.9	0.7	0	119	10.8	5.4	4.2	0	2.9	0	2.3	1.8
V	20.6	1.4	0.9	0.6	0	140	9.5	6.1	4.1	0	3.4	0	1.8	0
VI	18.2	12.7	8.2	5.9	2.7	109	75.2	49.1	35.3	16.2	7.5	13.7	18.2	42.0

Vitamin A (I.U./100 ml): I, 2000; II, 2000 + 0.4% Tween 80; III, 2000 + 0.8% Tween 80; IV, 2000 + 0.1% edible oil; V, 2000 + + 0.2% edible oil; VI, 2000 + 0.01% Carophyll

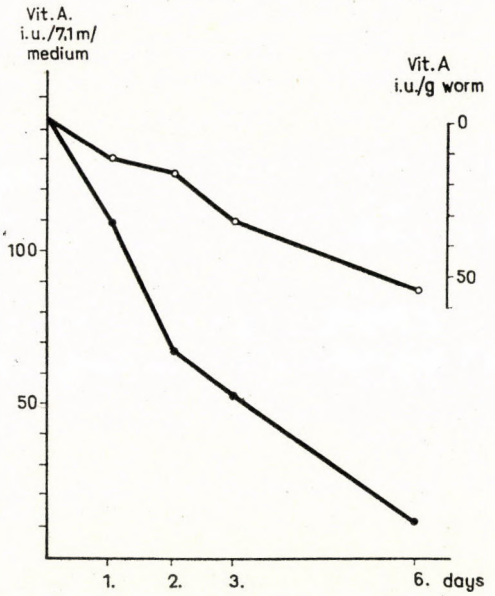

Fig. 3. Changes of vitamin A concentration in fluid medium and parasite body. — ● —: fluid medium; — o —: worm's body

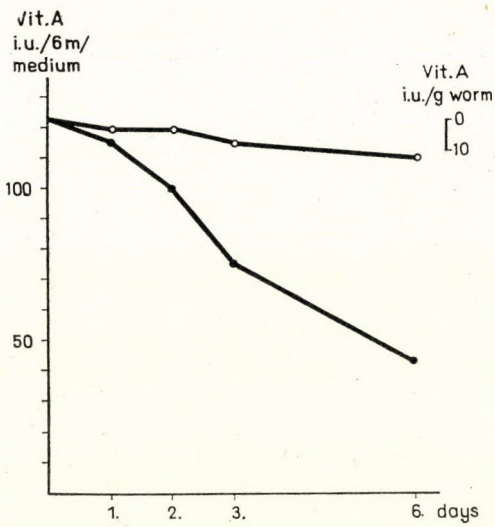

Fig. 4. Changes of vitamin A concentration in fluid medium and parasite body, in the presence of 0.4% Tween 80. — ● —: fluid medium; — o —: worm

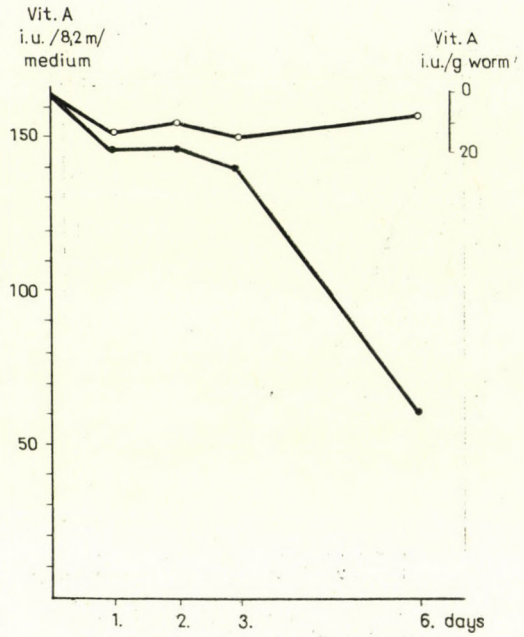

Fig. 5. Changes of vitamin A concentration in fluid medium and parasite body, in the presence of 0.8% Tween 80. — ● —: fluid medium; — ○ —: worm

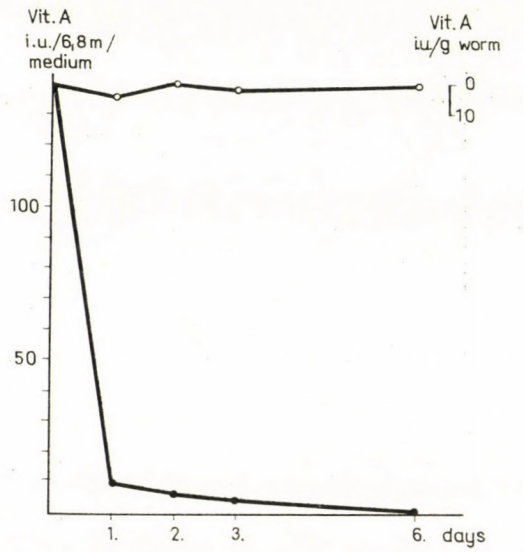

Fig. 6. Changes of vitamin A concentration in fluid medium and parasite body, in the presence of 0.1% edible oil. — ● —: fluid medium; — ○ —: worm

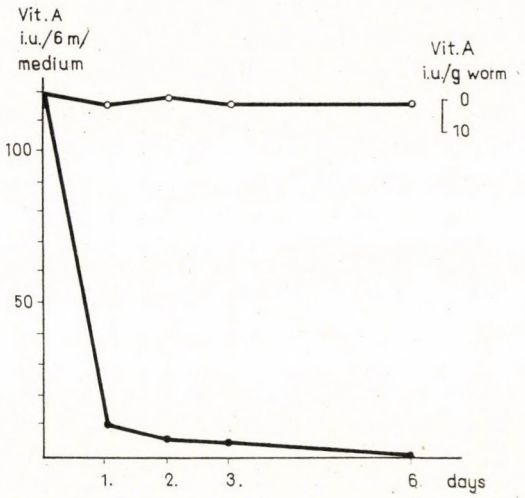

Fig. 7. Changes of vitamin A concentration in fluid medium and parasite body, in the presence of 0.2% edible oil. —●—: fluid medium; — ○ —: worm

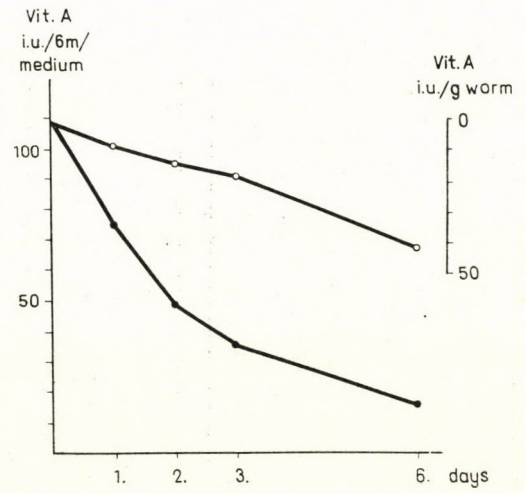

Fig. 8. Changes of vitamin A concentration in fluid medium and parasite body, in the presence of 0.01% Carophyll. —●—: fluid medium; — ○ —: worm

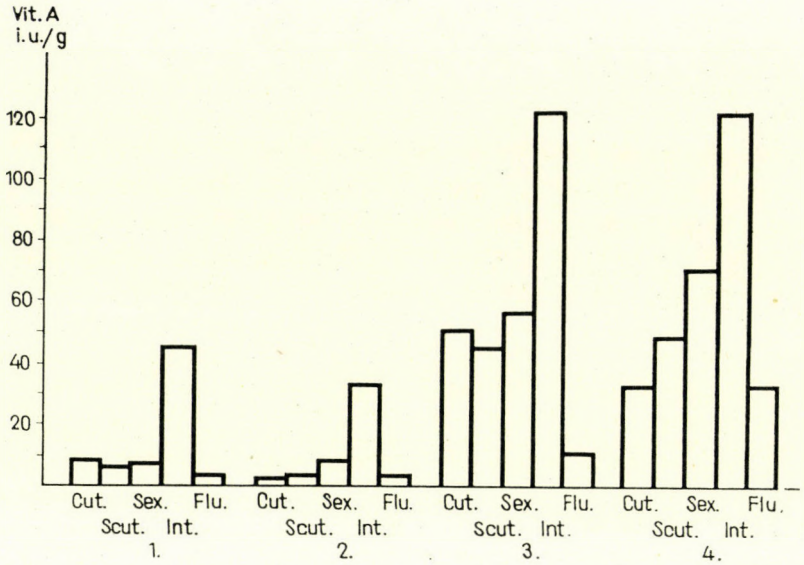

Fig. 9. **Distribution of vitamin A in some organs of *A. suum* in the presence of various substances. 1, 0.8% Tween 80; 2, 0.2% edible oil; 3, 0.14% gum-arabic; 4, 0.01% Carophyll. Further explanation, see legend to Fig. 1**

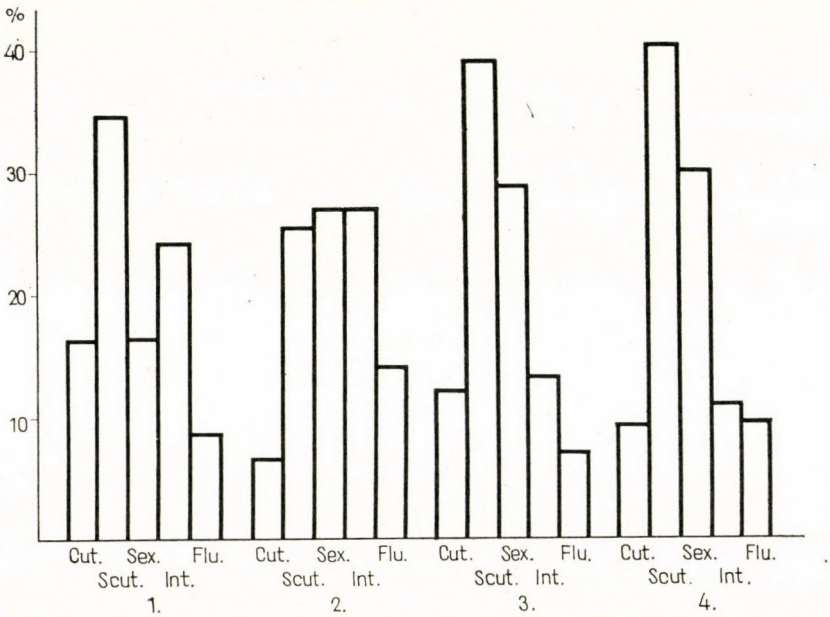

Fig. 10. **Percentual distribution of vitamin A in the organs of *A. suum* in the presence of various substances. Explanation, see legends to Figs 1 and 9**

olism of *Ascaris suum*. Since we had the intention to emulsify the oil either in gum-arabic or in Tween 80, first the influence of the latter two substances was tested *per se*. The presence of 0.14% of gum-arabic in the fluid medium did not affect vitamin A metabolism as compared to that of worms maintained in plain medium, but Tween 80, added in concentrations of 0.4 or 0.8%, was found to slightly inhibit the uptake of vitamin A. Thus, both the oil and carotenoids were emulsified in gum-arabic throughout (Figs 4 and 5; Table III).

The worms were maintained for 1, 2, 3 or 6 days in fluid medium containing 0.1 or 0.2% oil. Changes of the vitamin A concentrations in the media and in the worm bodies are shown in Table III and Figs 6 and 7.

The presence of 0.01% of Carophyll in the fluid medium had no influence on the uptake and utilization of vitamin A by the worms (Table III; Fig. 8).

In the final experiment 0.8% Tween 80, 0.2% edible oil, 0.14% gum-arabic and 0.01% Carophyll were added, each to one lot of medium, to examine on a comparative basis the percentual distribution of vitamin A in the individual organs. Each measurement was made on pooled homogenized organs from 5 worms. The results are summarized in Figs 9 and 10 and Table IV.

Discussion

We are fully aware that the *in vitro* experimental results may not be directly conclusive of *in vivo* conditions. Absence of vitamin A uptake *in vitro* does not imply its absence *in vivo*, since in the host's intestine many circumstances which cannot be provided *in vitro* may favour the process. Nevertheless, it seems to be fairly certain that in case vitamin A is utilized by the worm *in vitro*, it is also utilized *in vivo*, as it seems to be unlikely that a relatively highly organized animal, like a worm, would acquire an entirely new property — utilization of a vitamin — within a few days. The numerical data obtained in the *in vitro* experiments naturally do not apply to parasites harboured in the intestines of hosts, but are certainly of bearing in indicating the tendencies.

The most important conclusion drawn from the experiments carried out under different conditions is that *Ascaris suum* worms are capable of *in vitro* uptake of vitamin A; they store it in dissimilar and fairly constant concentrations in the individual organs and utilize it in their metabolism. The parasite's ability to utilize vitamin A needs no special proof: worms originally not containing it in demonstrable amount showed a 10—14 I.U./1 g level already after maintenance for 1 day in vitamin A containing medium. The parasite's vitamin A content tended to increase until the 6th day, reaching finally a 50—56 I.U./g level, which was maintained up to the 9th day if excess vitamin A was still present in the fluid medium. When, however, the vitamin

Table IV

Organ distribution of vitamin A in the presence of various substances in the fluid medium

Active substance	Organ	Vitamin A I.U./g	Percentual distribution of vitamin A
Tween 80 0.8%	Cuticle	8.6	16.3
	Subcuticle	6.1	34.5
	Reproductory organs	6.4	16.6
	Intestine	45.5	24.2
	Body fluid	3.0	8.4
Edible oil 0.2%	Cuticle	2.4	6.5
	Subcuticle	3.1	25.3
	Reproductory organs	8.6	27.0
	Intestine	30.0	27.0
	Body fluid	3.2	14.2
Gum-arabic 0.14%	Cuticle	51.0	12.1
	Subcuticle	35.0	39.0
	Reproductory organs	57.0	28.8
	Intestine	122	13.2
	Body fluid	10.7	6.9
Carophyll 0.01%	Cuticle	33.2	9.3
	Subcuticle	49.0	40.3
	Reproductory organs	70.5	30.0
	Intestine	122.0	11.0
	Body fluid	33.0	9.4

reserve of the medium became exhausted earlier or the worms were transferred on the 3rd to 6th day into a vitamin-free medium, the vitamin A contents of the worm bodies tended to decrease while no demonstrable vitamin appeared in the medium. This indicates that the vitamin A taken up by the worms was utilized in their metabolism.

The storage of vitamin A in the parasites' organs was not due to binding of diffused vitamin since, vitamin A being fat-soluble, in that case there would have been a correlation between fat and vitamin A in the individual organs.

But no such correlation was found (Table II); apparently, the organ-distribution of vitamin A is related to the parasite's metabolism and is fairly constant. Vitamin concentration related to 1 g organ suspension was highest in the intestine and reproductory organs, decreasing successively in cuticle, subcuticle and body fluid (Table I; Fig. 1). The percentual distribution of vitamin A in the individual organs was fairly constant and independent of the duration of the experiment, vitamin content of the maintenance medium as well as of the presence of substances affecting vitamin A utilization (Tables I and IV; Figs 2 and 10).

Maintaining *Ascaris suum* worms in fluid medium, the latter's vitamin A content tended to decrease and practically disappeared by the 6th day. Simultaneously, the vitamin A content of the parasites tended to increase, though in a lesser degree; the difference between the two values indicated the amount of vitamin metabolized by the worms.

The presence of carotenoids in the fluid medium had no influence on the parasites' vitamin A metabolism. But the addition of 0.1 or 0.2% edible oil effected a conspicuous change: the medium's vitamin A content fell in one day to as low a level as it did in 6 days in oil-free medium. In the same experiment, also the vitamin A contents of the worms were conspicuously low, particularly when 0.2% oil had been added. In the medium used as worm-free control, no particular change of the vitamin content took place. This unequivocally indicates that the presence of oil in the fluid medium enhances both uptake and utilization of vitamin A.

SUMMARY

In vitro studies of the vitamin A metabolism of *Ascaris suum* females have shown that the parasites are capable of taking up the vitamin from their environment, and to store it in a fairly constant percentual distribution in their organs. But the organ distribution is unrelated to the organ's fat content.

The vitamin A taken up from the fluid medium is metabolized by the worms. The carotenoids as precursors have no influence on vitamin A uptake and metabolism, whereas addition of edible oil to the fluid medium considerably enhances both.

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ORALE UND PARENTERALE ÜBERTRAGUNGSVERSUCHE VON KOKZIDIEN AUF NICHT SPEZIFISCHE WIRTE

Von

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Im ersten Teil der Untersuchungen (PELLÉRDY, 1969) über parenterale Infektionen mit *Eimeria stiedai* wurde festgestellt, daß es beim Kaninchen möglich ist, sowohl durch intravenöse als auch intraperitoneale oder intramuskuläre Applikation in vitro excystierter oder unbehandelter, voll sporulierter Oocysten eine mehr oder weniger starke Leberkokzidiose hervorzurufen. Es wurde ferner gefunden, daß nicht nur die Endstadien der endo- und exogenen Entwicklung von *Eimeria stiedai*, d. h. die sporulierten Oocysten, sondern auch jüngere, endogene Entwicklungsstadien (Merozoiten) bei parenteraler Verabreichung invasionsfähig sind.

In den nachfolgend geschilderten Untersuchungen prüften wir die Frage, ob eine Ansiedlung der Kokzidien nach oraler oder parenteraler Applikation nur bei ihren spezifischen Wirten erfolgt oder diese auch bei für die Parasiten artfremden Wirten möglich ist.

Material, Versuchsanordnung und Ergebnisse

Bei unseren Infektionsversuchen versuchten wir ein Gemisch möglichst aller Darmkokzidienarten sowie die Leberkokzidien *(Eimeria stiedai)* des Kaninchens *(Oryctolagus cuniculus)*, auf weiße Ratten *(Rattus rattus)* und Meerschweinchen *(Cavia cobaya)* sowie Kokzidien *(Eimeria tenella)* des Haushuhns *(Gallus gallus domesticus)*, auf Truthühner *(Meleagris gallopavo)* und Perlhühner *(Numida meleagris)* zu übertragen, wobei sowohl bei oraler als auch parenteraler Verabreichung die allgemeine Widerstandsfähigkeit des Wirtes schwächende Verfahren zur Anwendung gelangten.

Soweit angängig, verwendeten wir bei allen Versuchen eine möglichst große Anzahl voll sporulierter oder in vitro excystierter Oocysten, um bei den anschließenden mikroskopischen Untersuchungen die im Organismus verstreuten Kokzidien leichter zu finden.

Vor Verwendung des Infektionsmaterials wuschen wir die Oocysten mehrmals gründlich in physiologischer Kochsalzlösung aus. Für die in vitro-Excystierung wurden die Oocysten nach mechanischer Zerstörung der äußeren Hülle (Mörser, Kugelmühle, Magnetrührer, Homogenisator) entweder in einer

0,25%igen Pankreatin-Lösung oder unter Zusatz von 1—3% Kaninchengalle im Brutschrank bei 37 °C inkubiert. Die parenterale Verabreichung erfolgte in die Schwanz- oder eine Mesenterialvene, die Bauchhöhle oder die Muskulatur des Hinterschenkels der Versuchstiere.

Die übrigen speziellen Versuchsbedingungen, die insbesondere eine Verminderung der Widerstandsfähigkeit des Wirtstieres bezweckten (Hydrocortison-Behandlung, Röntgenbestrahlung, Methionin-Halbmangeldiät mit Äthionin-Zugabe etc.) sind bei den entsprechenden Versuchen angegeben.

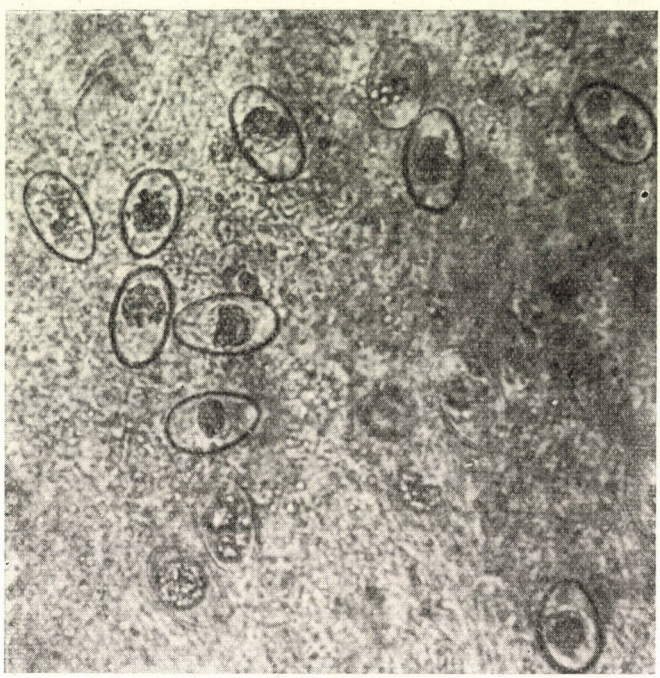

Abb. 1. Nach oraler Infektion mit *Eimeria stiedai* im Rattenkot gefundene Oocysten. Glyzerin-Anreicherung, 500×

Versuch 1

Um herauszufinden, was mit artfremden Oocysten nach oraler Aufnahme bei Ratten geschieht, infizierten wir mit der Schlundsonde zwei junge Ratten von ca. 150 g Körpergewicht mit 5—6 Millionen, etwa zu 70% sporulierter Oocysten von Darmkokzidien des Kaninchens. Die Tiere wurden mit Hibernal betäubt, um eine bessere Einführung der Sonde zu ermöglichen. Die Ratten waren etwa zwei Wochen vorher entmilzt worden.

Am folgenden Tage zeigte die Kotuntersuchung mittels Glyzerin-Flotationsverfahren, daß die vorher kokzidiosefreien Ratten ausschließlich nicht oder inkomplett sporulierte Oocysten mit dem Kot ausschieden (Abb. 1).

Mikroskopisch konnten wir neben völlig intakten Oocysten auch solche beobachten, deren Hüllen mehr oder weniger beschädigt waren. Leere Oocystenhüllen konnten wir nur vereinzelt sehen. In keinem Falle sahen wir infektionsfähige, voll sporulierte Oocysten im Kot, ein Zeichen dafür, daß im Darm dieses für die Kokzidien artfremden Wirtes eine Excystation stattgefunden hatte. Spätere Untersuchungen zeigten, daß in den gebräuchlichen Flotationsmedien wie Glyzerin, konzentrierter Zucker- oder Kochsalzlösung die leeren Oocystenhüllen im allgemeinen nicht an die Oberfläche kommen (SCHRECKE, 1969), ihr Fehlen also methodisch bedingt war.

Mit dem gleichen Resultat haben wir den Versuch mit anderen, teils entmilzten, teils nicht entmilzten Ratten mit und ohne Narkose und mit Küken mit Darm- und Leberkokzidien des Kaninchens wiederholt.

Das weitere Schicksal der aus den Oocysten im Darm der Ratte freigewordenen Sporozoiten wurde histologisch verfolgt. Hierzu wurden Ratten von der ersten Stunde nach der oralen Infektion an bis zum fünften Tag in 24-stündigen Abständen getötet. Bei der mikroskopischen Untersuchung von Darmabstrichen aus 4—5 verschiedenen Darmabschnitten konnten wir nur Oocysten, Oocystenhüllen und freie Sporocysten, aber keine Sporozoiten finden. Bei der histologischen Untersuchung sahen wir in der Darmschleimhaut einer einzigen Ratte, die am ersten Tag nach der Infektion getötet worden war, einige, sich mit Hämatoxylin tiefblau färbende, zwischen den Epithelzellen der Darmzotten liegende Gebilde, die keine besondere Struktur aufwiesen. Für eine von uns vermutete parasitäre Natur dieser Gebilde sprach zusätzlich eine ausgedehnte Eosinophilie an den entsprechenden Stellen in der Darmwand.

In den histologischen Präparaten der Leber von mit *Eimeria stiedai* infizierten Ratten waren keine Hinweise auf einen parasitären Befall zu finden.

Versuch 2

Um die Vermutung zu überprüfen, ob die schon von einer Infektion aus den Oocystenhüllen befreiten Sporozoiten von *Eimeria stiedai* in einem artfremden Wirt leichter vom Darm in die Leber gelangen könnten, infizierten wir vier junge Ratten oral mit in vitro excystierten *Stiedai*-Oocysten. Durch die histologische Untersuchung der am 12., 14., 17. und 18. Tag nach der Infektion getöteten Ratten konnten wir lediglich feststellen, daß die Leberkokzidien des Kaninchens auch mit dieser Methode nicht auf Ratten übertragbar waren.

Versuch 3

In vitro excystierte *Eimeria stiedai*-Oocysten wurden zwei Ratten intraperitoneal eingespritzt. An der Leberoberfläche der am 16. Tag nach der Infektion getöteten Ratten waren nadelstichgroße, manchmal auch etwas

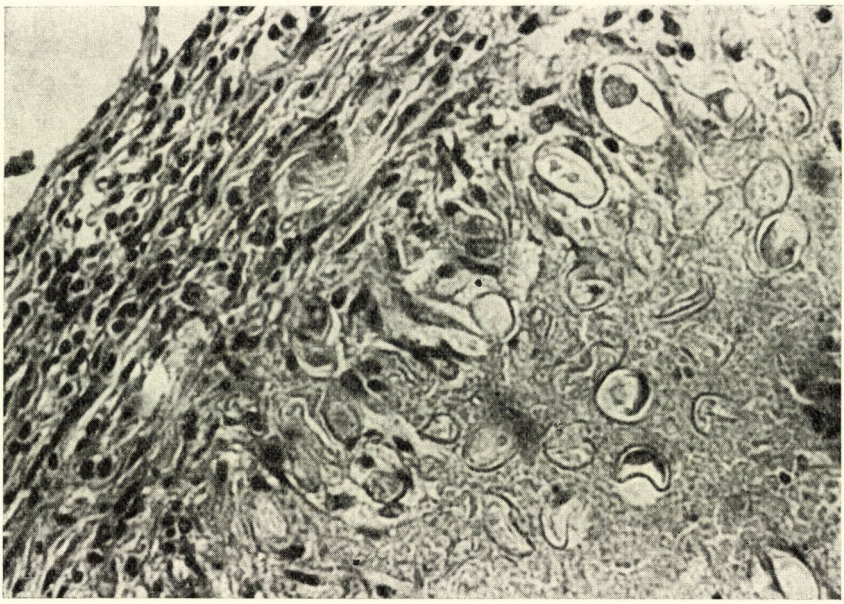

Abb. 2. Rattenleber mit *Eimeria stiedai*-Oocysten unter der Glissonschen Kapsel nach intraperitonealer Infektion. Haem.-Eos., 350 ×

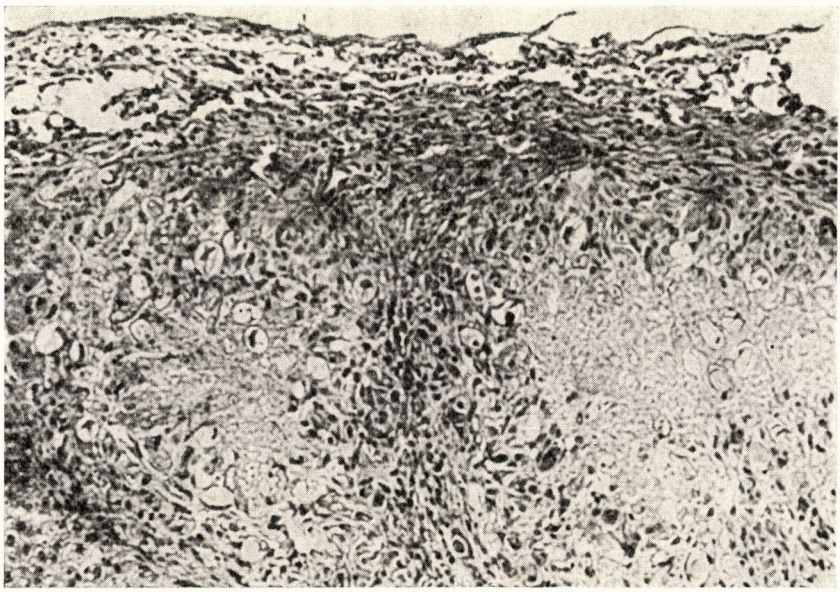

Abb. 3. Oocystenreste und nekrotische Herde in der Leber einer Ratte nach intraperitonealer Infektion. Haem.-Eos., 200 ×

größere, grauweiße Knötchen zu sehen. Bei der histologischen Untersuchung zeigte es sich, daß die Knötchen direkt unter der Glissonschen Kapsel lagen, die an diesen Stellen verdickt war. Sie bestanden aus Haufen sporulierter und nicht sporulierter Oocysten, leeren Oocystenhüllen, sowie aus entzündlichen Zellelementen und Detritus (Abb. 2). Ein Granulozyten enthaltender entzündlicher Hof umgab die Oocysten. In den histologischen Präparaten war keine Andeutung einer Ansiedlung oder Weiterentwicklung der Kokzidien in der Leber zu finden.

Die Tatsache, daß die Oocysten passiv aus der Bauchhöhle in die Leber gelangen können, wurde schon im ersten Teil dieser Untersuchungen nach intraperitonealer Injektion artspezifischer Kokzidienoocysten festgestellt (PELLÉRDY, 1969), jetzt jedoch mit dem Unterschied, daß sie sich im nicht spezifischen Wirt (Ratte) nicht weiterentwickelten. Eine Erklärung dafür, wie die Oocysten aus der Bauchhöhle durch die Glissonsche Kapsel hindurch in die Leber gelangt sind, können wir nicht geben. In anderen Organen (Lunge Milz, Niere) waren keine Kokzidien zu finden.

Bei einer Wiederholung dieses Versuches verwendeten wir zwei Ratten, die am 4. und 18. Tag nach der Infektion getötet wurden. Auch hier konnten wir die oben erwähnten Knötchen in verschiedener Zahl und Ausdehnung sehen. Sie lagen wieder unter der Leberkapsel, und bestanden aus mehr oder weniger unveränderten Oocysten, leeren Oocystenhüllen, Detrituselementen und waren mit einem aus Leukozyten, Lymphozyten, Eosinophilen und, bei der später getöteten Ratte, einem auch aus bindegewebigen Elementen bestehenden Hof umgeben (Abb. 3).

Versuch 4

Zehn junge Ratten wurden intravenös (Schwanzvene) mit excystierten Oocysten von *Eimeria stiedai* infiziert. Die Injektion mußte möglichst langsam erfolgen, um ein, durch die in großer Menge in das Blutgefäßsystem gelangenden Oocysten verursachtes Lungenödem (Abb. 4) zu vermeiden. Trotz aller Vorsicht verendeten einige Ratten während und nach der Injektion. Bei der histologischen Untersuchung stellten wir massive Verlegungen vieler Lungenkapillargefäße durch zahlreiche Kokzidien (excystierte und nicht excystierte Oocysten bzw. -hüllen) fest.

Die überlebenden Tiere wurden zwischen dem 3. und 14. Tag post infectionem (p. i.) getötet. Neben der makroskopischen Untersuchung wurde bei allen Tieren eine histologische Untersuchung unter besonderer Berücksichtigung der parenchymatösen Organe Leber, Lunge, Herz, Milz und Nieren durchgeführt. In den Lungenkapillaren fanden wir regelmäßig steckengebliebene Oocysten und leere Oocystenhüllen, die von einem entzündlichen Hof mit Granulozyten, Riesenzellen etc. umgeben waren. Außerdem sahen wir

größere und kleinere atelektatische und emphysematöse Bezirke im mikroskopischen Bild.

In einem Falle fanden wir eine eingedrückte Oocyste im Blut der linken Herzkammer. Alle anderen Organe waren frei von Oocysten.

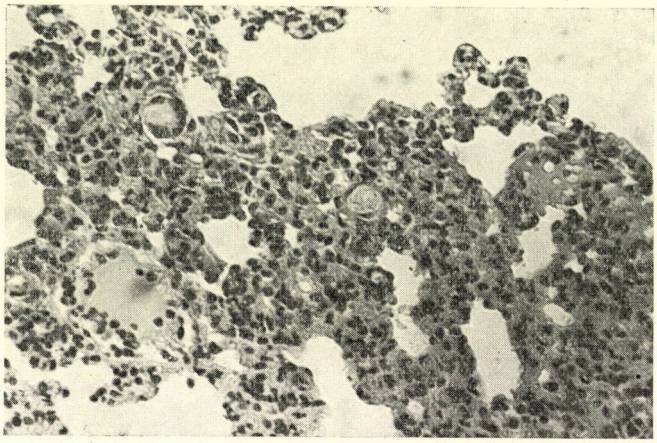

Abb. 4. Lunge einer, während der intravenösen Injektion verendeten Ratte. Kapillarverlegungen durch Oocysten, atelektatische Lungenbezirke, benachbarte Emphyseme, Flüssigkeitsaustritt. Haem.-Eos., 400 ×

Versuch 5

Nach Laparotomie injizierten wir zwei Ratten etwa 1 ml des aus in vitro excystierten *Stiedai*-Oocysten bestehenden Infektionsmaterials auf mehrere Stellen verteilt direkt in die Leber. Bei den am 14. und 17. Tag nach der Operation getöteten Ratten zeigten sich sowohl an der Oberfläche als auch auf den angelegten Schnittflächen im Inneren der Lebern stecknadelkopfgroße weißgraune Knötchen. Die Knötchen bestanden mikroskopisch aus sporulierten und nicht sporulierten Oocysten, leeren Oocystenhüllen, Sporocysten und im Detritus aus Massen von Granulozyten (Abb. 5a). Neben Fibroblasten fielen auch sich mit Hämatoxylin tiefblau anfärbende, meist runde, etwa leberzellkerngroße Gebilde auf, deren Identität wir nicht ganz sicher bestimmen konnten. Möglicherweise handelte es sich bei diesen Gebilden um aus versporten Oocysten in vitro excystierte, injizierte Sporozoiten kurz vor oder nach ihrem Absterben (Abb. 5b). Anzeichen für eine Ansiedlung oder Weiterentwicklung der Kokzidien fehlten auch bei diesen Ratten.

Versuch 6

Zwei junge, splenektomierte Ratten wurden in der zweiten Woche nach der Operation mit Entwicklungsstadien von *Eimeria stiedai* aus der Kaninchenleber infiziert. In Quetschpräparaten der Leber des am 15. Tag p. i.

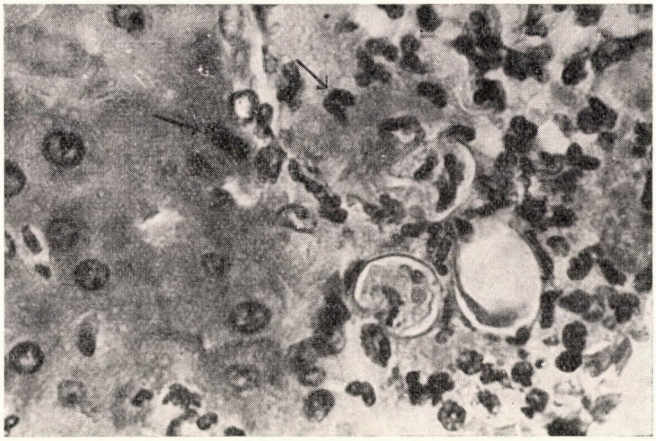

Abb. 5 a. Oocystenreste, Granulozyten, Detritusmasse und nekrotisierendes Lebergewebe in der Leber des gleichen Tieres. Haem.-Eos., 350 ×

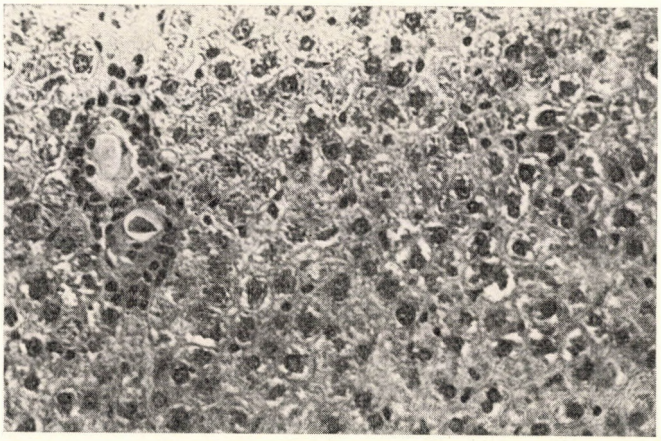

Abb. 5 b. Oocystenreste und parasitenverdächtige Gebilde (Sporozoiten?) in der Rattenleber nach Infektion direkt in die Leber. Haem.-Eos., 1000 ×

getöteten Donor-Kaninchens sahen wir mikroskopisch neben freien Merozoiten zahlreiche, 16—20 Merozoiten enthaltende Schizonten, deren massenhaftes Vorkommen im Lebergewebe auch histologisch bestätigt wurde (Abb. 6). Das Infektionsmaterial wurde durch Abschaben von der Leber und Verdünnen in angewärmter physiologischer Kochsalzlösung mit anschließender Filtration durch Mull gewonnen. Einer Ratte wurde diese Suspension nach Laparotomie in eine Mesenterialvene (Abb. 7), der anderen direkt in das Lebergewebe injiziert. Am 6. Tage nach der zweiten Operation wurden die Tiere getötet. Bei der histologischen Untersuchung fanden wir einige längliche, sich mit Mallory tiefblau färbende Gebilde. Diese Formen, die vermutlich den injizierten

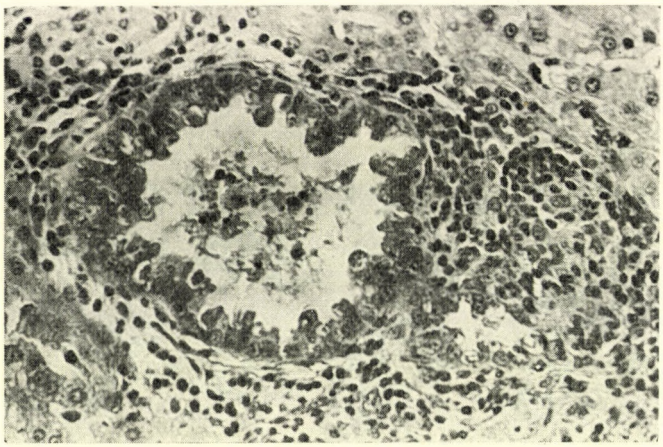

Abb. 6. Leber des zur Merozoiten-Gewinnung benutzten Donor-Kaninchens am 15. Tag nach der Infektion. Haem.-Eos., 300 ×

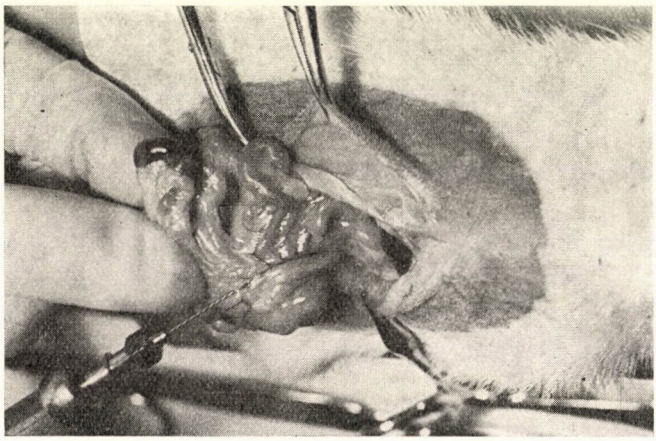

Abb. 7. Injektion intra venam mesenterialem. Geringgradig vergrößert

Merozoiten entsprachen (Abb. 8), wiesen keine besondere Struktur auf und waren nur wenig größer als die Kerne normaler Leberzellen. Wie bei den anderen Fällen beherrschte auch hier eine entzündliche Gewebsreaktion (Abb. 9) das histologische Bild, vor allem an den Stellen, an denen das Infektionsmaterial direkt in das Lebergewebe eingespritzt worden war. Die länglichen, sich mit Mallory dunkelblau färbenden strukturlosen Gebilde, die scheinbar den zugrundegehenden Merozoiten entsprachen, kamen natürlich auch an diesen Stellen in unterschiedlicher Zahl vor.

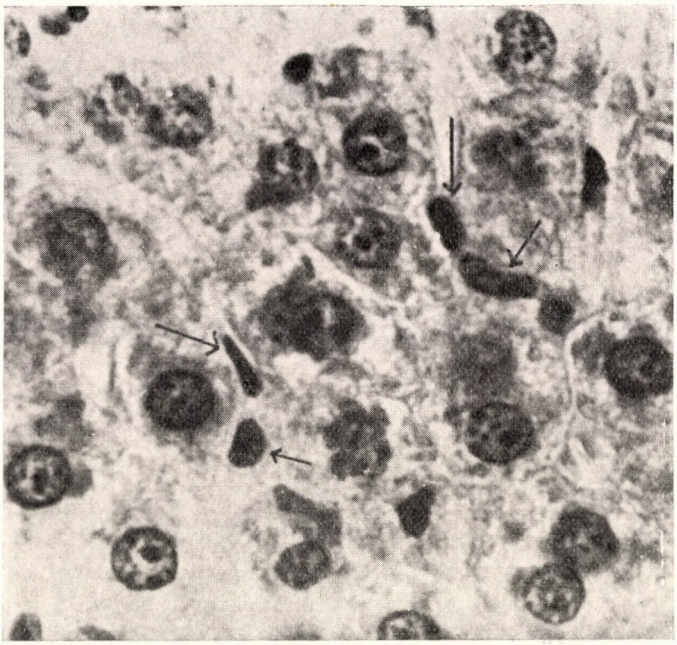

Abb. 8. Merozoiten (?) von *Eimeria stiedai* in einer Rattenleber am 6. Tag nach der intravenösen Infektion mit Entwicklungsstadien von *Eimeria stiedai*. Mallory, 1000 ×

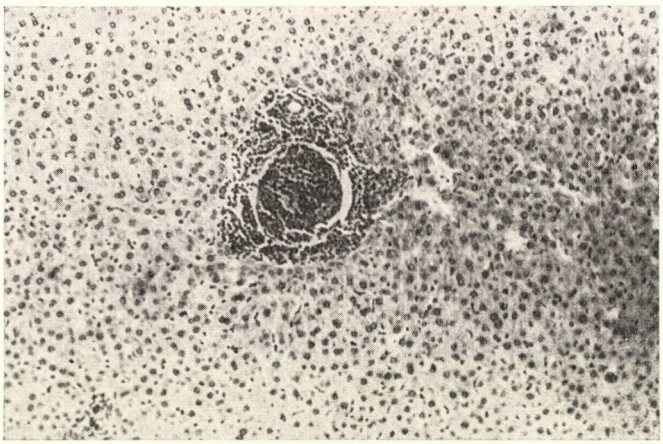

Abb. 9. Entzündungserscheinungen nach direkter Injektion in die Leber. Sechster Tag p. i. Haem.-Eos., 200 ×

Versuch 7

Sechs 150—200 g schweren Ratten wurden 11 Tage lang täglich 3,7 bis 4 mg Hydrocortison subcutan verabreicht. Um eventuellen unerwünschten bakteriellen Infektionen während der Behandlung vorzubeugen, injizierten

wir gleichzeitig jeder Ratte täglich 3000 I. E. Penicillin und 0,02 g Streptomycin subcutan. Anschließend infizierten wir drei Ratten oral mit großen Mengen von Darm- und Leberkokzidienoocysten des Kaninchens. Den drei restlichen Ratten wurden excystierte *Eimeria stiedai*-Oocysten (Oocysten, Sporocysten und Sporozoiten) in die Mesenterialvene injiziert.

Die Tiere der ersten Gruppe wurden am 2., 4. und 6. Tag nach der Infektion getötet, von der zweiten Gruppe starb ein Tier am ersten Tag p. i., die beiden überlebenden Tiere wurden mit Rücksicht auf die längere Entwicklungszeit von *Eimeria stiedai* am 13. und 18. Tag nach der Infektion getötet und untersucht. Im Jejunum der oral infizierten, am zweiten Tag p. i. getöteten Ratte fanden wir bei der histologischen Untersuchung (wie in Versuch 1) vereinzelt Sporozoiten interzellulär zwischen den Epithelzellen liegend. Weder bei dieser Ratte noch in Darm und Leber der beiden anderen oral infizierten Tiere fanden wir Zeichen, die auf eine Kokzidienansiedlung hindeuteten.

Bei den Ratten der intravenös infizierten Gruppe stellten wir bei der Sektion und histologischen Untersuchung im Lebergewebe nur zahlreiche, aus Oocysten, Oocystenhüllen, Sporocysten und Detritusmaterial bestehende Knötchen fest, die von dem schon wiederholt beschriebenen, entzündlichen Hof umgeben waren.

Versuch 8

Um die Widerstandskraft der Ratten mit einem anderen Verfahren zu schwächen, setzten wir zwanzig 10—20 Wochen alte weibliche Ratten der Einwirkung von Röntgenstrahlen aus (480 R; 20 mA, 200 kW, 0,5 cu Filter, 17,93 R/min, $^1/_2$ Stunde). Am Tage nach der Bestrahlung wurden die Ratten in fünf Gruppen zu je vier Tieren eingeteilt.

In Gruppe 1 bekamen die Tiere oral mehrere Millionen sporulierter *Eimeria stiedai*-Oocysten. Die vier Ratten der Gruppe 2 erhielten oral die gleiche Menge eines Gemisches verschiedener Darmkokzidienoocysten des Kaninchens. In Gruppe 3 wurde den Tieren oral ein Gemisch von Leber- und Darmkokzidienoocysten des Kaninchens appliziert. Die Ratten in Gruppe 4 bekamen nach der üblichen Laparotomie in vitro excystierte *Eimeria stiedai*-Oocysten in eine Mesenterialvene injiziert und die vier Tiere der Gruppe 5 blieben als bestrahlte, nicht infizierte Kontrolltiere zurück.

Die Ratten wurden vom 2. bis 18. Tag nach der Infektion getötet. Die Untersuchungsmethoden und erhaltenen Befunde glichen denen der vorhergehenden Versuche, so daß sich eine eingehende Schilderung derselben erübrigt. In den Därmen der Ratten fand keine Kokzidienansiedlung statt. In den Lebern der Tiere aus Gruppe 4 wurden bei den zuerst getöteten Tieren nur die in den Kapillargefäßen (und deren Umgebung) steckengebliebenen

Oocysten, bei den nächsten allmählich die schon beschriebenen Entzündungserscheinungen und bei den zuletzt getöteten Ratten schließlich auch die Zelltypen (Histiocyten, Epitheloidzellen, Riesenzellen), die bei der Entfernung von Fremdkörpern aus dem Organismus eine Rolle spielen, beobachtet.

Zusammenfassend läßt sich sagen, daß wir auch bei den Ratten, deren allgemeine Widerstandskraft entweder durch Hydrocortisongaben oder durch Röntgenbestrahlung vermindert worden war, keine Zeichen feststellen konnten, die auf eine Ansiedlung artfremder Kokzidien hindeuteten.

Versuch 9

Einen anderen Weg, der von den bisher benutzten Verfahren prinzipiell abwich, benutzten wir bei dem Versuch, die allgemeine Widerstandsfähigkeit der Ratten über die Nahrung zu beeinflussen.

Um den endogenen Eiweißstoffwechsel und damit auch die Bildung der Immunglobuline zu beeinflussen, hielten wir Versuchsratten auf einer Methionin-Halbmangeldiät mit Äthionin-Zugabe (62% Kartoffelstärke, 8% Casein, 3% Bierhefe, 46% Puderzucker, 8% Leim, 41% Vitamine und Mineralsalzzusatz, 10% Fette und 0,3% Äthionin). Zwölf etwa 150 g schwere Ratten wurden mindestens zwei Wochen lang auf dieser Diät gehalten. Die Infektion erfolgte zu einem Zeitpunkt, an dem sich die ersten Auswirkungen der Mangeldiät (Mattigkeit, Gleichgültigkeit, Appetitlosigkeit) zeigten. Sechs Ratten wurden mit sporulierten *Eimeria stiedai*-Oocysten, die anderen sechs Tiere mit sporulierten Oocysten von Darmkokzidien des Kaninchens oral infiziert.

Innerhalb von 20 Beobachtungstagen konnten im Kot, abgesehen von den beiden ersten Tagen nach der Infektion, keine Oocysten von Kaninchenkokzidien beobachtet werden. Auch bei der histologischen Untersuchung der zu unterschiedlichen Zeiten nach der Infektion getöteten, von der Diät völlig erschöpften Tiere waren weder im Darm noch in der Leber Zeichen eines Befalls mit diesen Kaninchenkokzidien zu sehen.

Versuch 10

Mehrere Millionen excystierter *Eimeria stiedai*-Oocysten wurden zwei jungen Meerschweinchen intracardial injiziert. Am 15. darauffolgenden Tag konnten wir weder bei der Sektion noch bei der anschließenden histologischen Kontrolle der einzelnen Organe auf eine Kokzidienansiedlung hinweisende Veränderungen feststellen. Lediglich in den Lungen fanden wir an mehreren Stellen durch Oocysten verursachte Gefäßverlegungen mit den Resten einer entzündlichen Gewebsreaktion (Abb. 10). Das Ergebnis dieses Versuches zeigt, daß sich auch beim Meerschweinchen direkt in den Blutkreislauf applizierte Leberkokzidien des Kaninchens nicht weiterentwickeln können.

Versuch 11

Da das Kaninchen (Ordo *Lagomorpha*) mit der Ratte und dem Meerschweinchen (Ordo *Rodentia*) nach der derzeit gültigen zoologischen Systematik von SIMPSON (1945) nur sehr weitläufig verwandt ist, haben wir bei weiteren Versuchen solche Tiere verwendet, die miteinander in zoologisch näherer Verwandtschaft stehen.

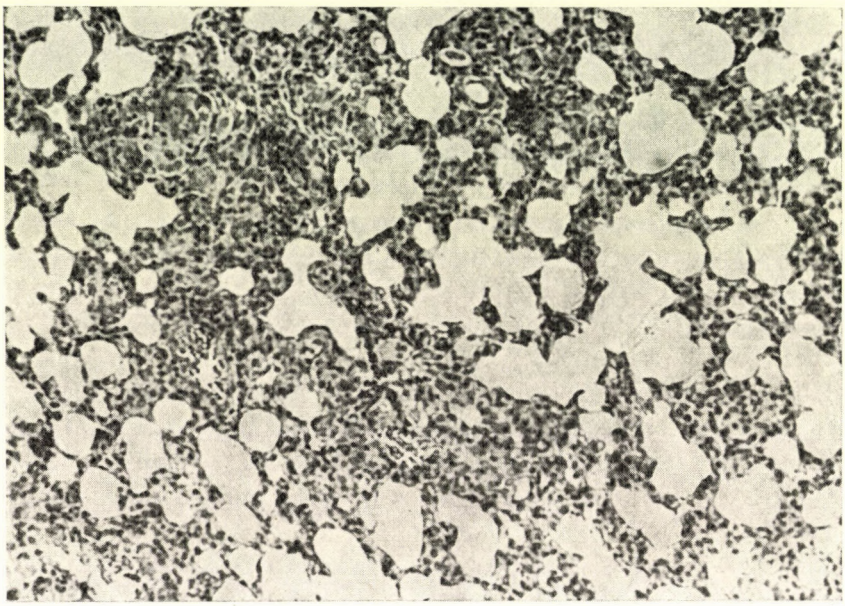

Abb. 10. Eimeria stiedai-Oocysten in der Lunge eines Meerschweinchens am 15. Tag nach intracardialer Injektion. Haem.-Eos. 200×

Eine Gruppe von zwanzig frisch geschlüpften Truthühnern wurde in einer derart sterilen Umgebung gehalten, daß eine interkurrente Kokzidieninfektion ausgeschlossen war. Während der ersten neun Lebenstage erhielten die Tiere täglich 2 mg Hydrocortison subcutan. Am 9. Tag war eine deutliche Wirkung der Cortisongaben festzustellen, der einige Tiere sogar erlagen. Die überlebenden Küken wurden an diesem Tag oral mit einer Million gut sporulierter *Eimeria tenella*-Oocysten, die aus Hühnerküken gewonnen worden waren, infiziert. Bis zum dritten Tag nach der Infektion schieden die Truthühner daraufhin im Kot leere Oocystenhüllen und nicht voll sporulierte *Eimeria tenella*-Oocysten aus. Weitere tägliche Kotuntersuchungen bis zum 9. Tag p. i. zeigten keine Oocysten mehr. Ebenso blieben die späteren Sektionsbefunde und histologischen Kontrollen negativ.

Als Ergebnis bleibt festzuhalten, daß *Eimeria tenella* auf die näher verwandten und durch Hydrocortison-Behandlung geschwächten Truthühner nicht übertragbar ist.

Versuch 12

Bei diesem Versuch handelt es sich im Prinzip um eine Wiederholung des Versuches 11 mit anderen Rezipienten, Perlhühnern. Die sieben Tage alten Vögel wurden ebenfalls neun Tage lang, diesmal mit sich täglich steigernden Dosen von anfangs 2,5 bis schließlich 10 mg Hydrocortison subcutan behandelt. Anschließend bekamen die stark geschwächten Tiere oral je 5 Millionen *Eimeria tenella*-Oocysten. Auch in diesem Versuch traten zum Infektionszeitpunkt die ersten Todesfälle auf. Die Auswertung der Versuchsergebnisse nach den in Versuch 11 geschilderten Kriterien zeigte, daß *Eimeria tenella* unter den verwendeten Versuchsbedingungen auch auf das Perlhuhn nicht übertragbar ist.

Versuch 13

Die Übertragungsversuche bei Methionin-Halbmangeldiät mit 0,36% Äthionin-Zusatz wurden unter denselben Versuchsbedingungen wie in Versuch 9 bei zoologisch näher verwandten Tieren wiederholt.

Je zwanzig junge, kokzidienfreie Perl- und Truthühner wurden nach mehrtägiger Mangeldiät mit einer Million infektionsfähiger *Eimeria tenella*-Oocysten infiziert.

Auch hier zeigten die Versuchsergebnisse an Hand der Sektionen und histologischen Kontrollen, daß der Versuch einer Infektion von in ihrer Widerstandsfähigkeit durch eine Aminosäure-Mangeldiät geschwächten Perlhühnern und Truthühnern mit einer Kokzidienart des Haushuhnes erfolglos geblieben war.

Diskussion

Das Ziel unserer Versuche war, festzustellen, ob sich artfremde Kokzidien bei oraler oder nach parenteraler Applikation in normalen oder in ihrer Widerstandsfähigkeit geschwächten, nicht spezifischen Wirten ansiedeln können.

Die ersten Versuche zeigten, daß im Darm auch die Oocysten der artfremden Kokzidienarten excystieren können. Gleiche Ergebnisse konnten auch bei anderen Tierarten und mit anderen Kokzidienarten erzielt werden (LOTZE

et al., 1961; LOTZE und LEEK, 1963; SPINDLER, 1965; MARQUARDT, 1966). Die in vivo excystierten Sporozoiten entwickeln sich jedoch nach unseren Untersuchungen nicht weiter, sondern können höchstens noch zwischen die Epithelzellen eindringen.

Im Laufe der folgenden Untersuchungen wurden unter verschiedenen Bedingungen in vitro excystierte Oocysten bzw. die aus den Oocysten freigewordenen Endstadien der Sporulation, die Sporozoiten, der Leberkokzidien des Kaninchens in die Schwanzvene oder in eine der Mesenterialvenen, weiterhin in die Bauchhöhle oder direkt in das Lebergewebe junger Ratten injiziert. Diese Bemühungen blieben ebenso erfolglos wie die darauffolgenden Versuche, in denen wir mit endogenen Entwicklungsstadien (Merozoiten und Schizonten) von *Eimeria stiedai* die Indifferenz des nicht spezifischen Wirtes zu durchbrechen suchten. Die stets negativen Ergebnisse bei parenteraler Verabreichung ließen uns zu der Schlußfolgerung kommen, daß die die Invasion verhindernden Barrieren im Falle der Leberkokzidiose des Kaninchens nicht im Darm der Ratte zu suchen sind, sondern daß als Ursache der Spezifität dieser Kokzidien auch andere Faktoren eine entscheidende Rolle spielen.

Im folgenden versuchten wir die allgemeine Widerstandsfähigkeit und damit auch die unbekannten Abwehrmechanismen der Ratten mittels verschiedener Methoden zu vermindern.

Über die Folgen einer *Splenektomie* wissen wir, daß nach der Operation die natürliche Resistenz, also im Grunde genommen die Spezifität, beeinflußt wird. Es ist unter anderem nicht unbekannt, daß sich in entmilzten Menschen der sonst artfremde Parasit *Babesia bovis* ansiedeln und vermehren kann (SKRABALO und DEANOVIĆ, 1957). In einem anderen Fall konnte *Babesia bovis* unter Verlust ihrer Spezifität auf einen entmilzten Schimpansen übertragen werden (GARNHAM und BRAY, 1959). MAYER (1966) berichtet über die erhöhte Empfindlichkeit entmilzter Hamster gegenüber *Trypanosoma equiperdum*. Ähnliches wurde bei serologischen Arbeiten in dem Budapester Institut beobachtet: Zur Antigengewinnung dienende Trypanosomenstämme *(Trypanosoma lewisi)* vermehrten sich in entmilzten Hunden wesentlich besser als in nicht operierten (nicht veröffentlicht). Unsere negativen Übertragungsversuche stehen in gewisser Übereinstimmung mit den erfolglosen Versuchen von ROSE (1968), über die Splenektomie bei Küken in das Immunitätsgeschehen der *Eimeria tenella*-Infektion einzugreifen.

Die Wirkung von Hydrocortison sowie von Röntgenstrahlen auf das Immunitätsgeschehen ist bekannt, so daß auf eine Darstellung der darüber vorliegenden Literatur verzichtet werden kann. In unseren Versuchen zeigte sich weder bei oraler noch bei parenteraler Infektion mit nicht spezifischen Kokzidien eine Abschwächung der vorhandenen Resistenz.

Nicht durch Aktivitätsminderung oder Schädigung der körpereigenen Immunzentren sondern durch eine Verhinderung der Bildung von Immun-

stoffen durch mangelhafte Zufuhr der dazu benötigten Bausteine und zusätzliche Gabe eines Antimetaboliten versuchten wir mit der Methionin-Halbmangeldiät mit Äthioninzusatz in den Immunitätsmechanismus einzugreifen. Äthionin ist als Methionin-Antagonist bekannt. Durch seine Zugabe sollte bei gleichzeitig vermindertem Methionin-Angebot die Eiweißbildung (und damit auch die Bildung der Immunglobuline) beeinflußt werden.

Früher durchgeführte Versuche hatten gezeigt, daß Ratten, die auf einer solchen Diät gehalten wurden, klinisch manifest an normalerweise nur latent vorhandener Leptospirose und Listeriose erkrankten (BERTÓK, 1964). Bei den Kokzidioseübertragungsversuchen zeigte sich jedoch auch hier kein Erfolg.

Alle diese Versuche bestätigten die bisherige Auffassung, daß die artbestimmte Indifferenz eines Wirtstieres gegen einen Parasiten vermutlich in keinem Zusammenhang mit irgendeiner Art der natürlichen oder erworbenen Immunität steht.

Letzten Endes haben die durchgeführten Untersuchungen ausnahmslos — wenn auch von der negativen Seite her — die strenge Artspezifität einiger, in warmblütigen Tieren schmarotzender *Eimeria*-Arten bestätigt. Obzwar wir über die Natur dieser Spezifität nichts Näheres wissen, bekräftigen die negativen Ergebnisse unserer Übertragungsversuche die Anschauung, daß die Spezifität der Kokzidien nicht mit dem Vorhandensein oder dem Mangel einer Aggressivität oder Widerstandsfähigkeit seitens des Parasiten oder des Wirtes zu erklären ist.

Deswegen müssen wir uns auch heute noch mit SANDGROUND's (1929) zwar in den Einzelheiten immer noch nicht geklärten aber trotzdem gültigen Definition begnügen, nach der die natürliche Resistenz »is believed to be simply a matter of species incompatability protecting an animal only against those parasites that require specific conditions for their development differing from those possessed by the naturally non specific host«.

ZUSAMMENFASSUNG

Excystierte und nicht excystierte sporulierte Oocysten von Darm- und Leberkokzidien des Kaninchens wurden oral und parenteral auf Ratten und parenteral auf Meerschweinchen übertragen. Eine Excystierung findet im Darmkanal des nicht spezifischen Wirtes (Ratte) statt. Eine Ansiedlung und Weiterentwicklung konnte jedoch in keinem Falle gefunden werden, auch nicht bei Tieren, deren Widerstandskraft durch Entmilzung, Hydrocortisongabe, Röntgenbestrahlung bzw. Methionin-Halbmangeldiät mit Äthionin-Zusatz vermindert worden war oder nach parenteraler Infektion mit Entwicklungsstadien (Merozoiten) der Kokzidien. Das gleiche Ergebnis beobachteten wir nach der Infektion mit *Eimeria tenella* (Haushuhn) bei Truthühnern und Perlhühnern, denen Hydrocortison verabreicht worden war oder die auf der gleichen Mangeldiät gehalten worden waren wie die Ratten. Die stets negativen Ergebnisse dieser Übetragungsversuche bestätigen einmal die auch heute noch manchmal bezweifelte strenge Spezifizität der Eimerien, zum anderen weisen sie darauf hin, daß diese Spezifizität vermutlich nicht durch immunologische Mechanismen bedingt ist.

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ENZYME-DIAGNOSTIC STUDIES OF BLOOD FROM GEESE AND DUCKS HEALTHY AND WITH LIVER DYSTROPHY

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Enzyme assays have been increasingly used in veterinary medicine, particularly for the diagnosis of hepatic diseases. In the case of poultry the small amount of blood required for enzyme assay presents an additional advantage.

In water fowls dietary error, fattening and certain therapeutic procedures — in ducks also viral hepatitis — may cause liver impairment. Further to this, liver tests may be used also for experimental purposes. But the relevant literary data have been partly unsatisfactory, partly controversial, not only in respect of water fowls, but also for poultry in general. Therefore, it seemed to be worthwhile to compare the results of some enzyme-diagnostic methods in normal and diseased geese and ducks.

In the present study the four blood enzymes most extensively used in the diagnosis of poultry diseases, were examined. Out of them glutamic acid pyruvate transaminase (GPT) is an unilocular enzyme present in the cytoplasms of liver cells. Glutamic acid oxalic acetic acid transaminase (GOT) represents a group of isoenzymes, approximately half of them locating in the cytoplasm, the rest in the mitochondria of liver cells. Aldolase (ALD) is present not only in the liver cells, but, in approximately similar concentration, also in the muscles and kidneys. While the above three enzymes are formed in the liver cells and play a role in their metabolism (cellular enzymes), the fourth enzyme in question, alkaline phosphatase (AP), is an excretory enzyme which is secreted through the liver cells from blood to bile.

Hepatic injury is followed by a rise of the serum levels of cellular enzymes, as the cell membrane becomes permeable, enabling the passage of the enzymes into the interstitial space and therefrom into the blood. Owing to disturbance of the liver's excretory function, also the serum levels of excretory enzymes tend to rise.

With a mild degree of parenchymal cell injury, first of all the cytoplasmic enzyme activity (GPT) tends to rise. Increased activities of mitochondrium-bound (GOT) and still more of excretory (AP) enzymes indicate a severe liver impairment, usually associated with lysis of parenchymal cells. Rise of

the ALD level is less specific, as a similar increase may take place also with muscular and renal diseases.

Based on the available literary data, the results of enzyme assays in the blood of various domestic fowls are summarized as follows. CORNELIUS et al. (1959) give the GOT level of healthy chicks as 370 ± 186 μg/ml of pyruvate and claim that in this particular species no GPT activity was demonstrable. MCDANIEL and CHUTE (1961) measured 154 ± 27 Karmen units of GOT and 14 ± 7 Sigma-Frankel units of GPT in the sera of 10-week old chickens. KOLB (1962) gives the GOT of hens as 270 ± 186 Sigma-Frankel units, whereas their GPT as 0. In chickens infected with Pasteurella, MAGLIONE (1962) noted exclusively rise of GOT. GRÜN, GÜRTLER and KOLB (1963) have described the GOT activities of chickens, geese, ducks and turkeys as 188.2 ± 34, 157.7 ± 54.3, 58.0 ± 17.6 and 377.4 ± 15.9 Karmen units, respectively. For comparison, let us refer to the GOT levels of the horse and dog, which have been found to be 165 ± 33.8 and 26.0 ± 9.5 Karmen units, respectively. GESSLER (1963) has reported a very low, hardly measurable GPT activity in hens. The GOT, AP and ALD activities of blood from 13-week old chickens were given by TETTENBORN (1964) as 56.8 ± 14.6 mU/ml, 32.7 ± 11.0 Bessey-Lowry units and 10.1 ± 1.6 mU/ml, respectively. TRAUTSCHOLD and WERLE (1966) found no demonstrable GPT activity in the blood of hens. HOMBACH (1967) reported a variation of chicken serum GPT between 0.5 and 5.5 Wroblewsky units and noted a considerable rise of activity on poisoning with CCl_4. According to ŠLESINGER (1967), the GOT, GPT and AP values of blood from healthy hens correspond to 2.9 ± 16 mU/ml, 0.45 ± 0.03 KA units and 0.45 ± 0.03 KA units, respectively. According to our own as yet unpublished findings, the GOT, GPT and ALD activities of chicken blood are 96.1 ± 24.7 I.U., 1.6 ± 1.2 I.U. and 6.25 ± 1.17 mU/ml, respectively.

Experimental

Enzyme activity was measured in blood plasma obtained from heparinized blood by centrifugation. Blood samples of 3—4 ml were usually taken from the wing vein in the morning hours; samples from small birds were obtained by cardiac puncture. Enzyme assays were carried out within the first hour after withdrawal of the sample.

Assays were made according to Boehringer's Biochemical Test Combinations, with the modification that the temperature of incubation was adjusted to 41 °C to suit the body temperature of ducks and geese (HOMBACH, 1967).

First of all the normal values were assessed. The GPT and GOT activities were measured in blood samples from 90 healthy geese maintained under different, but satisfactory, conditions of management and feeding. In samples from 30 birds also the ALD and AP activities were determined (Table I).

Birds in group I received after grazing for 6—7 hours maize grains *ad libitum*. Groups II and III had been grazed previously, but as the experiment fell in the period of fattening, by that time they received exclusively maize for 2—4 days.

In group III, assays were repeated a few days before the termination of the fattening period. At that time the GPT activity showed hardly any change (2.2 ± 2.0), whereas the GOT activity fell significantly (2.5 ± 2). It should be noted that only part of the plasma obtained from the fattened geese was suitable for examination, as in many cases the high lipid content made the plasma creamy.

Table I

Normal GOT, GPT, ALD and AP values of geese

Group No.	N	Age (month)	Weight (kg)	GOT I.U./ml	GPT I.U./ml	ALD mU/ml	AP mU/ml
I	30	7	5 —6	15.8± 6.0	3.9±3	4.9±3.1	—
				Limits: 10 —27	1 —8	2.9—8.8	
II	30	6—7	5.5—6.2	13.2± 4.0	3.0±3.0	—	31.3± 9.9
				Limits: 2 —26	0.7		14 —47
III	30	5—6	5 —6.2	14.3± 5	2.4±2.0	—	—
				Limits: 6 —31	1 —6	—	

Finally, samples from a group of 20 geese, with supposed liver impairment owing to inappropriate conditions of management and feeding, were examined and a rise of GPT to an average 20.8±8 was found.

In the second part of the experiments attempt was made to artificially induce liver impairment in 16 geese, 6—7 months old, fed goose feed and maize. Their enzyme activities and blood sugar levels were measured at regular intervals. Seven experimental groups were formed and treated orally on one or more occasions with 0.3, 0.5 or 1.0 ml/kg body weight of CCl_4 or 30.0, 40.0, 7.0 or 4.0 mg of elementary phosphorus. In some cases the smaller doses of phosphorus were given to birds previously treated with CCl_4. CCl_4 was mixed with an equal volume of edible oil and administered through a rubber tube inserted on a syringe, whereas phosphorus was administered in gelatin capsule into the oesophagus or proventriculus of the birds. The weights of the treated birds, dates and doses of CCl_4 and phosphorus treatment, dates of blood sampling, results of enzyme assays and, in some cases, the brief descriptions of gross and microscopic findings are summarized in Table II.

GOT and GPT activities were determined also in the blood plasma from 50 healthy ducklings, 12—30 days old. In this species, the normal mean value of GOT activity was 10.1±4.2 I.U. (6.1—16.7 I.U.), whereas that of GPT activity 5.1±2.8 I.U. (3.8—9.3 I.U.). In viral duckling hepatitis a considerable rise of transaminase activity was found. In a group of 15 birds artificially infected with duckling hepatitis virus the mean values of GPT and GOT were 60.8±24.2 I.U. (28.2—90.8 I.U.) and 108.8±16.4 I.U. (78.2—138.8 I.U.).

Results

Based on assays in plasma from 90 healthy geese, normal GOT was found to be 14.4±5.0 I.U., whereas normal GPT 3.1±2.9 I.U. Normal ALD and AP activities assessed in plasma from 30 healthy birds were in average 4.9±3.1 mU and 31.3±9.0 mU, respectively.

Table II

Data of geese treated with carbon

Goose		Date of treatment	Oral treatment		Fate of bird	Date of testing
No.	Weight		Chemical	Dose		
1.	6.0	1. 10.— 5. 10.	CCl_4	5×1.8 ml		1. 10. 2. 3. 5.
		8. 10	CCl_4 P	1.8 ml 30 mg	Died on 9. 10	
2.	5.0	1. 10 — 5. 10.	CCl_4	5×1.5 ml		1.10 2. 3.
		14. 10.—15. 10.	P	2×7 mg	Died on 16. 10.	5. 15.
3.	5.0	1. 10.— 5. 10.	CCl_4	5×1.5 ml		1. 10. 2. 3. 5.
		8. 10.	CCl_4 P	1.5 ml 40 mg	Died on 9. 10.	
4.	5.0	1. 10.— 5. 10.	CCl_4	5×1.5 ml		1. 10. 2. 3.
		14. 10.—15. 10.	P	2×7 mg	Died on 16. 10.	5. 15.
5.	6.0	1. 10.— 5. 10.	CCl_4	5×3.0 ml		1. 10. 2. 3. 5.
		8. 10.	CCl_4 P	3.0 ml 40 mg	Died on 9. 10.	
6.	6.0	1. 10.— 5. 10.	CCl_4	5×3.0 ml		1. 10. 2. 3.
		14. 10.—15. 10.	P	2×7 mg	Died on 16. 10.	5. 15.
7.	5.0	1. 10.— 5. 10.	CCl_4	5×2.5 ml		1. 10. 2. 3.
		8. 10.	CCl_4 P	2.6 ml 40 mg	Died on 9. 10.	5.
8.	5.0	1. 10.— 5. 10.	CCl_4	5×2.5 ml		1.10. 2. 3. 5.
		8. 10.	CCl_4	2.2 ml	Survived by 1. 11.	16. 17.

tetrachloride and phosphorus

Plasma levels of			Sugar mg/100 ml	Post mortem findings (gross and microscopic)
GOT I.U.	GPT I.U.	Aldolase mU/ml		
10.5	6.5	8.55	138	The pericardium contained 3—4 ml, the abdominal cavity 2—3 ml, of a serous fluid with occasional fibrin filaments. The contents of proventriculus and stomach were dilute and of garlic-like odour. Four birds (Nos 1, 3, 4, 6) had lesions indicative of more or less severe myocardial degeneration and small intestinal catarrh; nearly all birds had lung oedema. The livers, generally hyperaemic and slightly swollen, were friable; livers from birds Nos 2, 4, 5, 6 and 7, contained fat palpable in cross-section. The rest (Nos 1, 3, 4 and 7) had conspicuously hyperaemic livers. (Livers: distinct hyperaemia and sporadic haemorrhages. The parenchymal cells with generally intact nuclei contained fat droplets. In the livers from birds Nos 2 and 5, approximately 30% of the liver cells had broken or shrunken nuclei.)
11.5	8.5	7.87	100	
10.0	9.5		—	
—	6.0	4.74	105	
10.0	—	3.65	121	
9.0	4.5	4.05	99	
9.0	3.0	—	—	
9.5	4.0	4.10	110	
9.0	3.0	2.70	—	
10.5	4.0	4.83	121	
10.5	5.0	2.07	89	
14.5	—	—	—	
9.5	2.0	4.10	97	
6.0	4.5	5.35	121	
8.0	5.5	4.27	105	
7.5	6.5	—	—	
7.5	6.5	2.19	119	
10.5	6.5	2.47	—	
10.5	2.0	3.65	128	
14.5	3.5	2.81	100	
12.0	3.5	—	—	
12.0	1.0	2.36	118	
14.0	2.5	2.47	118	
13.0	3.5	2.47	107	
—	—	—	—	
14.0	2.0	1.85	116	
13.0	3.5	2.58	—	
13.0	1.0	1.99	132	
12.5	2.5	1.18	95	
14.0	4.5	—	—	
10.0	2.0	2.36	118	
13.0	2.5	4.38	123	
10.0	4.5	2.02	119	
14.5	1.5	—	—	
10.5	3.0	4.10	118	
11.0	9.5	—	136	
13.0	3.5	3.80	151	

Table II (continued)

Goose		Date of treatment	Oral treatment		Fate of bird	Date of testing
No.	Weight		Chemical	Dose		
9.	4.5	1. 10.— 4. 10.	CCl_4	4×4.5 ml	Died during heart puncture on 8. 10	1. 10. 2. 3.
10.	5.0	1. 10.— 5. 10. 14. 10.—15. 10.	CCl_4 P	5×5.0 ml 2×7 mg	Died on 18. 10.	1. 10. 2. 3. 5. 16. 17.
11.	5.5	28. 10. 30. 31. 4. 11.	P P P P	4.0 mg 4.0 mg 4.0 mg 4.0 mg	Symptom-free by 31. 1. 1969	28. 10. 1. 11. 6.
12.	5.8	28. 10. 30. 4. 11.	P P P	4.0 mg 4.0 mg 4.0 mg	Symptom-free by 31. 1. 1969	28. 10. 1. 11. 6.
13.	5.0	28. 10. 30. 4. 11.	P P P	4.0 mg 4.0 mg 4.0 mg	Symptom-free by 31. 1. 1969	28. 10. 1. 11. 6.
14.	5.5	4. 11.	CCl_4	5.5 ml	Killed on 5. 11.	28. 10. 5. 11.
15.	6.0	4. 11.	CCl_4	6.0 ml	Symptom-free by 25. 11.	28. 10. 5. 11. 15. 24.
16.	5.0	1. 10.— 8. 10. 28. 10. 29. 10. 31. 10.	CCl_4 P P P	6×2.5 ml 4.0 mg 4.0 mg 4.0 mg	Died on 1. 11.	28. 10. 30. 10.

In several-weeks old ducklings average GOT and GPT activities were 10.1 ± 4.2 and 5.1 ± 2.8, respectively, as calculated from measurements in plasma from 50 healthy birds.

In the group of fattened geese, enzyme activities were not indicative of liver-cell destruction. In contrast, while the GPT activity remained essenti-

Plasma levels of			Sugar mg/100 ml	Post mortem findings (gross and microscopic)
GOT I.U.	GPT I.U.	Aldolase mU/ml		
11.5	2.5	5.28	124	Acute cardiac dilatation, lung oedema, cardiac haemorrhages (suffocation).
14.5	4.5	3.48	101	
10.5	4.5	—	—	
5.5	—	1.85	112	Cardiac dilatation, signs of myocardial and renal degeneration. Liver capsule slightly wrinkled. Cross-section of liver ochre yellow, feels fatty. (The parenchymal columns lost definition: 70—80% of the cells showed lytic or disintegrated nuclei. Many fat droplets of variable size were apparent in the liver cells.)
9.5	4.0	1.46	112	
8.0	4.0	—	—	
12.0	5.5	2.94	102	
115.0	200.0	—	52	
42.5	48.0	21.20	73	
11.5	3.5	1.85	95	
52.5	8.0	—	70	
8.5	1.0	9.90	73	
10.0	5.0	2.47	105	
55.0	13.0	—	107	
7.5	5.5	5.17	69	
9.0	8.0	1.85	95	
90.0	14.0	—	65	
8.5	5.5	8.55	78	
14.5	3.0	—	126	Liver slightly hyperaemic, otherwise negative. (A few large fat droplets are deposited in the intact liver cells.)
18.0	6.0	3.80	67	
3.5	3.5	—	105	
14.0	6.5	3.80	123	
—	—	2.00	102	
2.0	2.0	2.19	—	
2.0	2.5	—	102	Acute cardiac dilatation, lung oedema, myocardial and renal degeneration. Liver: ochre yellow and friable.
95.0	80.0	—	73	(Nucleus shrunken or disappeared in approximately half of the liver cells. Deposition of many fat droplets of variable size in the liver cells.)

ally unchanged, the GOT activity fell considerably, from the initial 14.3 ± 5 I.U. to 2.5 ± 2.0 I.U.

The lack of increase of transaminase activity was supposedly due to the non-necrobiotic nature of the liver changes associated with fattening.

A moderate increase of GPT to 20.8 ± 9 I.U. was found in temporarily

inappropriately fed and managed geese. A considerable rise of activity, sometimes 10—20fold, was measured in ducklings with viral hepatitis.

The 10 geese treated orally for 5 days with 0.3—1.0 ml/kg of CCl_4 showed no manifest clinical symptoms. Accordingly, neither their GOT, nor GPT levels differed significantly from the normal. ALD did not notably change either. There was a slight decrease of the blood-sugar level.

Four (Nos 1, 3, 5 and 7) of the above 10 geese which, in addition to treatment with CCl_4 on 6 occasions received 2 hours after the last dose also 30—40 mg of phosphorus, died in 9—16 hours. On post mortem examination these birds showed lesions indicative of phosphorus poisoning.

The 3 birds (Nos 2, 4 and 6) which received 7 mg phosphorus each on the 14th and 15th days of the experiment died overnight following the administration of the second P-dose. During the 24 hours following the first P-dose no notable change was demonstrable in the blood of these birds by enzyme-diagnostic tests. Microscopic examination of the livers from birds Nos 2 and 4 showed distinct hyperaemia, small haemorrhages and deposition of more or less small fat droplets in the otherwise intact parenchymal cells. But goose No. 10, treated previously on 5 occasions with 1 ml/kg of CCl_5, showed on the day following the administration of 2×7 mg P a considerably increase of both GOT (115 I.U.) and GPT (200 I.U.). On the day preceding death these values fell to approximately one-third or one-fourth, but still were approximately tenfold of the initial values. Simultaneously also the ALD level increased to approximately tenfold (21.2 mU/ml). In this particular bird, post mortem examination revealed, in addition to renal and myocardial degeneration, a necrobiotic fat infiltration involving approximately 70% of the liver cells.

Bird No. 8, given a total dose of 15.0 ml CCl_4 on 6 occasions, survived treatment. Bird No. 9, also treated exclusively with CCl_4, died on the 8th day of the experiment during cardiac puncture, with symptoms of cardiac standstill.

The three birds (Nos 11, 12, 13) given exclusively phosphorus in 4 mg doses on three or four occasions over a period of 8 days, survived treatment and showed no clinical signs for 3 months following the last P dose. In these cases, there was a temporary 5—10fold increase of GOT (on the 5th day) and an approximately 2fold increase of GPT. There was also a simultaneous 2—4fold rise of ALD activity. Blood-sugar level showed no significant alteration.

But goose No. 16, treated 20 days prior to the first phosphorus dose with 0.5 ml/kg of CCl_4 for 6 subsequent days died on the application of 3×4 mg of phosphorus. The GOT and GPT levels measured on the day preceding death were also in this case conspicuously high, about 40fold. Gross lesions of the liver were of the fat-degeneration type; microscopically simple degener-

ation was seen in approximately one half of the liver parenchyma, while necrobiotic fat infiltration in the rest.

To check whether a single large dose (1 ml/kg) of CCl_4 would cause a liver lesion and if it would, of what type, two birds (Nos 14 and 15) were treated accordingly. One of them (No. 14), not showing any clinical symptoms, was killed on the 2nd day after treatment and post mortem findings were essentially negative. Microscopic examination of the liver showed moderate hyperaemia and simple fat infiltration apparent as the deposition of large fat droplets. The other bird (No. 15), observed during the 20-day period following the CCl_4 treatment, did not show any symptom. On the day following the administration of CCl_4, both birds showed a moderate temporary rise of GOT and GPT levels.

The results permit the conclusion that the rise of plasma GOT, GPT and ALD levels is a reliable indicator of the severity of liver lesions also in water fowls.

Acknowledgements. The authors are indebted to Dr. M. GÜNTHER for organizing the blood samplings, and to Mr. B. BORSI, for excellent technical assistance.

SUMMARY

In blood plasma from healthy geese, normal GOT, GPT, aldolase and alkaline phosphatase activities were assessed as 14.4 ± 5.0 I.U., 3.1 ± 2.9 I.U., 4.9 ± 3.1 mU and 31.2 ± 9.0 mU, respectively.

Normal GOT and GPT activities of healthy day-old ducklings were found to be 10.1 ± 4.2 I.U. and 5.1 ± 2.8 I.U., respectively.

No rise of GPT activity, but fall of GOT activity was found in the plasma of geese by the end of the fattening period.

A notable rise of GOT, GPT and aldolase activities, essentially in parallel with the degree of damage, was demonstrated on artificially induced liver impairment. A notable increase of transaminase activity was found in ducklings with viral hepatitis.

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HISTOPATHOLOGICAL LESIONS OF AVIAN ENCEPHALOMYELITIS IN HUNGARY

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Avian encephalomyelitis (AE) is a viral disease characterized by ataxia, incoordination of the movements, paralysis, and tremor, especially of the neck and head (Fig. 1).

The disease occurs in chicks 1 to 6 weeks of age, and pheasants (MATHEY, 1955) are also susceptible. Ducklings, young turkeys, pigeons and guinea-fowls can be infected experimentally only (VAN ROEKEL, 1959; BURTSCHER, 1961; GROTH and GREUEL, 1961; FANKHAUSER and KLINGER, 1961).

The disease was first described in a preliminary report by JONES (1932) in the United States of America as an encephalomyelitis of chicks. Later on (JONES, 1934), a detailed description was given and, because tremor of the head and neck was a prominent symptom, the disease was called epidemic tremor.

In 1938—39, VAN ROEKEL and his coworkers observed ataxia as first symptom, which was followed by tremor in some of the infected birds. Therefore they suggested to call the disease infectious AE. In 1939, the Committee of the Poultry Diseases Nomenclature of the AVMA designated the disease AE.

Histopathological lesions of the disease were studied in detail by JUNGHERR (1939), OLITSKY (1939), JUNGHERR and MINARD (1942) and FRITZSCHE (1952).

AE has been diagnosed in England (MARKSON and BLAXLAND, 1955—58), Sweden (LINDGREN et al., 1957), France (BRION et al., 1959), Denmark (BADSTUE and VELLING, 1959), Austria (BURTSCHER, 1960, 1961), Italy (QUAGLIO and PETEK, 1961) and Poland (MAREK and ROSZKOWSZKY, 1966), and more or less detailed descriptions of the histopathological lesions have been given.

In Hungary, infectious AE had not been observed until 1966 (DERZSY, 1966). The disease was first established by the end of 1967 in the Central Veterinary Institute (CSONTOS et al., 1968). Chickens were submitted from 6 different farms at nearly the same time. The chickens, having been hatched in and distributed from the same hatchery, clinically showed principally ataxia. The central nervous systems (CNS) of the birds were examined histologically. In most of them similar histopathological lesions were found.

In this paper the histopathological lesions characteristic of the disease observed in Hungary are dealt with.

Materials and methods

Forty-seven naturally infected chicks, 2 to 5 weeks of age, were studied. The chicks were partly submitted to this Institute, partly collected from the field.

In addition, 18 chicks were inoculated experimentally at 10 days of age. The CNS and visceral organs of these chicks were investigated at 4 to 5 weeks of age. Chickens were inoculated intracerebrally with the brain suspension of spontaneously infected birds.

Samples were taken from the same regions of different organs of naturally and experimentally infected chickens, which were killed *in extremis*. The organs were fixed in 10% formalin, and after 3 or 4 days the fixative was replaced by a 4% neutral formalin.

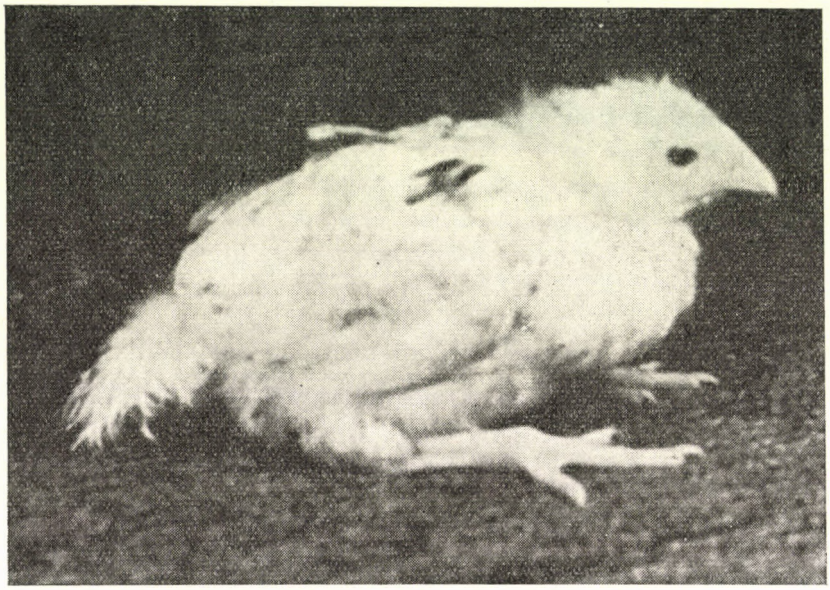

Fig. 1. Infected chicken as sitting on the hip joint

Specimens in frontal planes were excised from the fore-brain in two sites: one from the cerebral hemispheres 5 mm before the chiasma and another at the frontal plane of the chiasma; from the mid-brain the optic lobe and cerebral peduncles; from the hind-brain three or four sites of the medulla and cerebellum. From the spinal cord two specimens were taken from the cervical, and one from the thoracic segments; from the cervical and lumbo-sacral enlargements at three sites each. The schedule of taking specimens is illustrated in Fig. 2. In addition, the brachial and sciatic nerves were examined.

Of the visceral organs specimens taken from the liver, pancreas, gizzard, proventriculus and heart muscle were examined.

The fixed specimens were embedded in paraffin.

The following staining methods were used: P. Mayer's acid haemalaun- or Delafield's haematoxylin-eosin method, Nissl's method modified by Woight'

Woelcke's method, Bielschowsky's and Penfield's silver impregnation methods as well as Cajal's sublimate-gold method, and staining with Sudan III.

Results

No specific lesions recognizable to the naked eye were found at post mortem examinations. Bacteriological results were also negative.

In each of the CNS specimens non-suppurative lymphocytic encephalomyelitis was seen, uniform in character and localization. The lesions confined principally to the grey matter and certain predilection sites of the CNS.

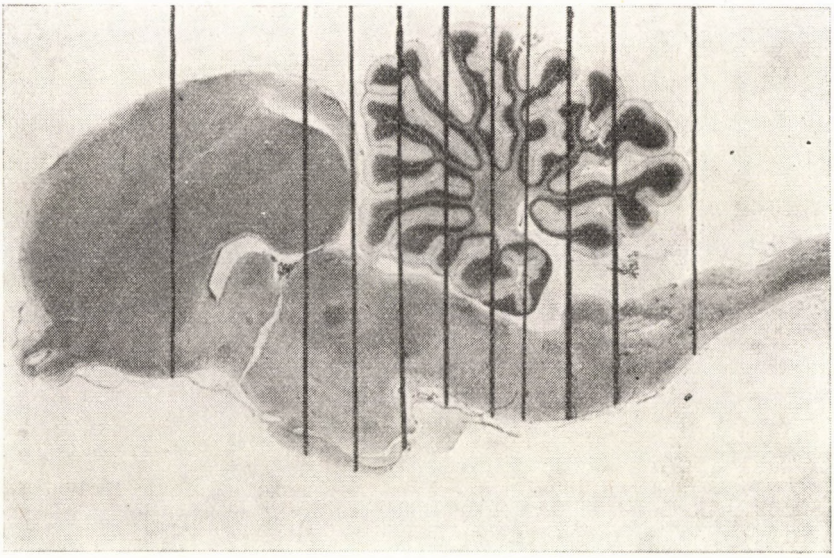

Fig. 2. Sagittal section of a normal chicken brain. Sites investigated are illustrated. ×95

The predilection sites are as follows. (1) In the cerebellum the molecular layer, Purkinje cells' layer and outer and inner cerebellar nuclei. Lesions in the latter ones were more predominant. (2) In the medulla the vestibular nucleus, vagus nucleus, cochlear nucleus, reticular formation, raphe, and motor nuclei of the nervus trigeminus. Sensory nuclei of the trigeminus, vestibular ganglion and the superior oliva nucleus are seldom affected. In the facial nucleus, "corner nucleus" (ECKKERN, KRAUSE, 1922), abducens nucleus and hypoglossal nucleus lesions can be found only occasionally. (3) In the mesencephalon and diencephalon lesions are common in the lateral mesencephalic nucleus, less common in the trochlear nucleus. Dorsal nucleus, oculomotor nucleus and ovoid nucleus are rarely affected. Changes in the ectomammillaris nucleus, red nucleus, isthmus nucleus and optic tract were observed occasionally.

Lesions in the optic lobe confine to the deep medullary zone rather than to corpus posticum. In the intermediate zone changes can be observed only occasionally.

(4) In the cerebral hemispheres lesions of the neostriatum and hyperstriatum predominate, while the archistriatum, paleostriatum, neopallium,

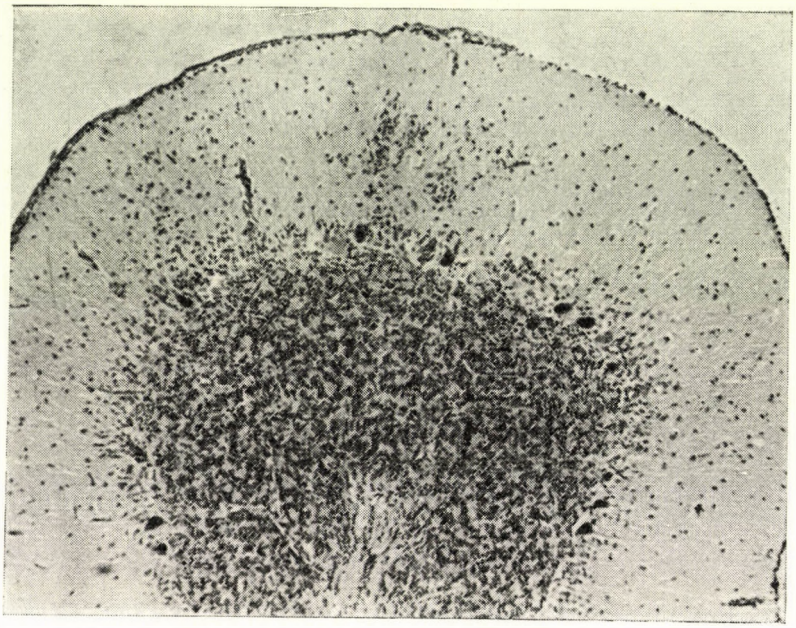

Fig. 3. Part of cerebellar folia. Several degenerated ganglion cells in the Purkinje cell layer, focal glia cell proliferations in both the Purkinje cells'- and molecular layers. Haematoxylin-eosin staining. ×95

hippocampus and parahippocampus are rarely affected. Lesions in the choroid plexus were seen in two cases.

The grey matter of the spinal cord was affected in all cases. Lesions were most prominent in the ventral horns of the cervical and lumbo-sacral enlargements.

The most pronounced lesions in the CNS of chickens infected with AE can be found in the molecular layer and Purkinje cells' layer of the cerebellum (Figs 3, 4, 5).

The lesions of the Purkinje cells are characteristic. Single cells or groups of cells show degeneration of variable degree or necrosis; neuronophagia of Purkinje cells also occurs (Fig. 3). Two types of degenerations in the Purkinje cells have been observed: pyknosis (Figs 3, 4, 7) and central chromatolysis (Figs 5, 6). Pyknosis occurs most commonly. The degenerated cells are shrunk-

en and stained deeply. The nucleus, lying centrally, appears homogeneous or of granular structure, faint in outline. The surrounding cytoplasm shows chromatolysis or has deeply stained, roughly granular Nissl's bodies. In most of the contracted ganglion cells the nuclei cannot be distinguished from the rough Nissl's granules. Such pyknotic Purkinje cells are visible even at low

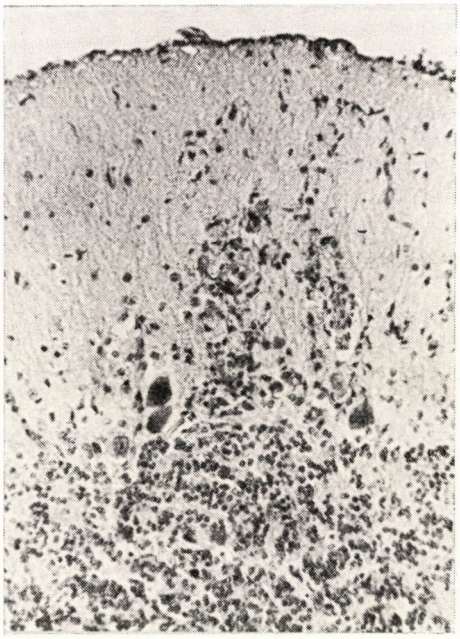

Fig. 4. Part of the cerebellar grey matter. Degeneration of the Purkinje cells and glia proliferations. Haematoxylin-eosin. ×240

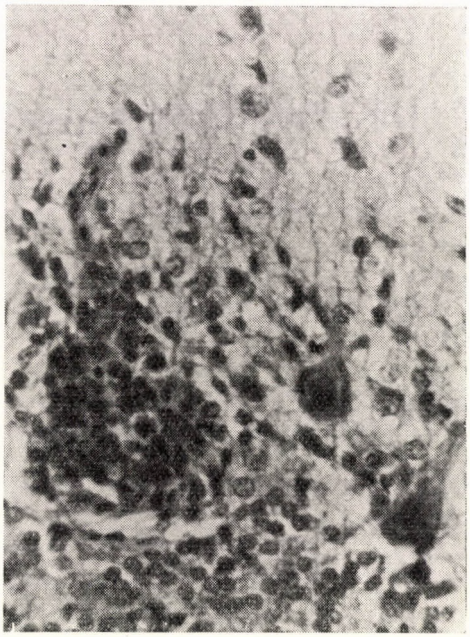

Fig. 5. Part of the Purkinje-cell layer and molecular layer. Degenerated Purkinje cells and focal glia proliferation. Haematoxylin-eosin. ×600

magnification. In the nerve cells showing central chromatolysis the nuclei are enlarged, faint in outline and sometimes eccentric, and the surrounding cytoplasm is free of Nissl's granules, which are displaced to the periphery of the cell. Such Purkinje cells are stained less intensely (Figs 5, 6). The cytoplasm of the necrotized nerve cells is homogeneous, structureless and is stained light acidophil. The nucleus cannot be distinguished.

The climbing fibres, the fibres arising from the basket cells and surrounding the cytoplasm of the Purkinje cells, and the cytoplasmic and axonal pole of the ganglion cells are swollen. This is clearly demonstrated by Bielschowsky's method in both types of degenerations as well as in necrosis (Figs 8, 9). Fragmentation and disintegration of dendrites of the basket cells can also be observed. In the affected Purkinje cells fat granules or droplets cannot

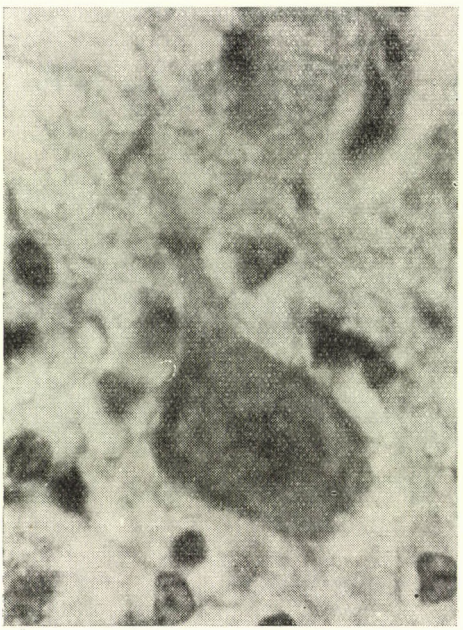

Fig. 6. Part of the cerebellar Purkinje-cell layer. Central chromatolysis. Haematoxylin-eosin. ×1400

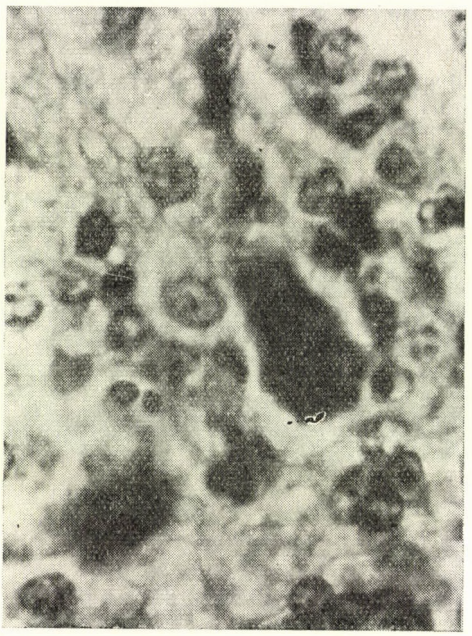

Fig. 7. Part of the cerebellar Purkinje-cell layer. Early neuronophagia around the pyknotic ganglion cells. Haemalaun-eosin staining. ×1400

Fig. 8. Part of the cerebellar grey matter. Swelling of the synaptic nerve fibres around affected Purkinje cells. Bielschowsky's silver impregnation. ×240

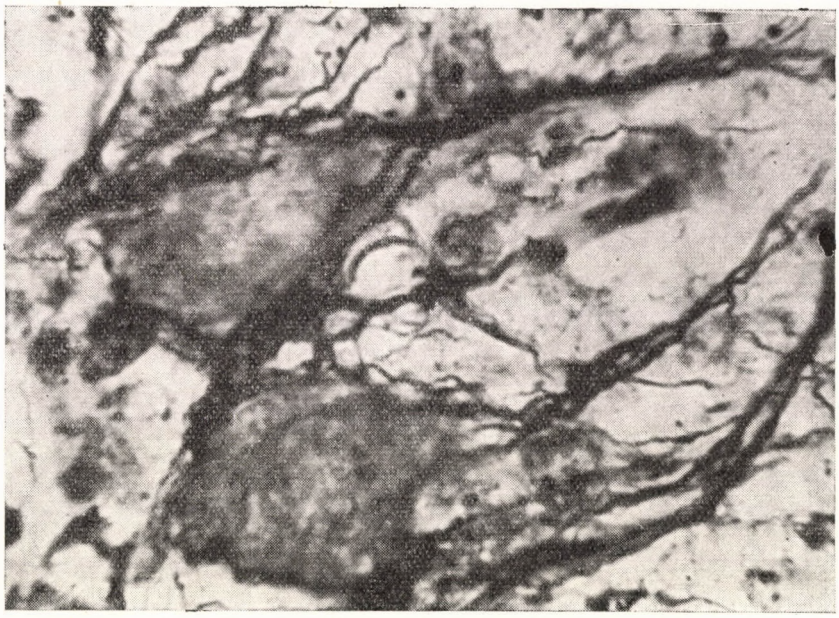

Fig. 9. Part of Fig. 8. ×1400

be demonstrated with Sudan III staining. Nervous tissue around the affected ganglion cells shows vacuolation possibly due to oedema (Figs 4, 5). Neuronophagia occurs commonly. Neuroglia cells enter the cytoplasm of the Purkinje cells (Fig. 7), and Purkinje cells are replaced by foci of glia cells. Foci of glia cells can also be found in the middle part of the molecular layer (Figs 3, 4, 5). In some cases nuclear fragmentation of glia cells can be seen.

As shown by Cajal's sublimate–gold and Penfield's silver impregnation methods glia foci consist principally of fibrous astrocytes; microglia cells (oligodendroglia and Hortega's glia cells) are small in number (Figs 10, 12). Stained by Cajal's method, marked thickening and proliferation of fibres of fibrous astrocytes can be recognized. In some areas fibres project from the granular layer into the molecular layer and usually reach the meninges (fibrous gliosis) (Figs 10, 11).

In the cerebellar grey matter perivascular cellular infiltration of some post- and precapillary vessels can be seen. Usually cuffings are found either between the Purkinje cells or in the molecular layer. In the granular layer or in the pia mater they can be found only occasionally.

The vascular and perivascular infiltrations are usually 2 to 3 layers deep all around the capillaries. The infiltrating cells consist mostly of lymphocytes, while lymphoblasts, histiocytes and plasma cells are small in number.

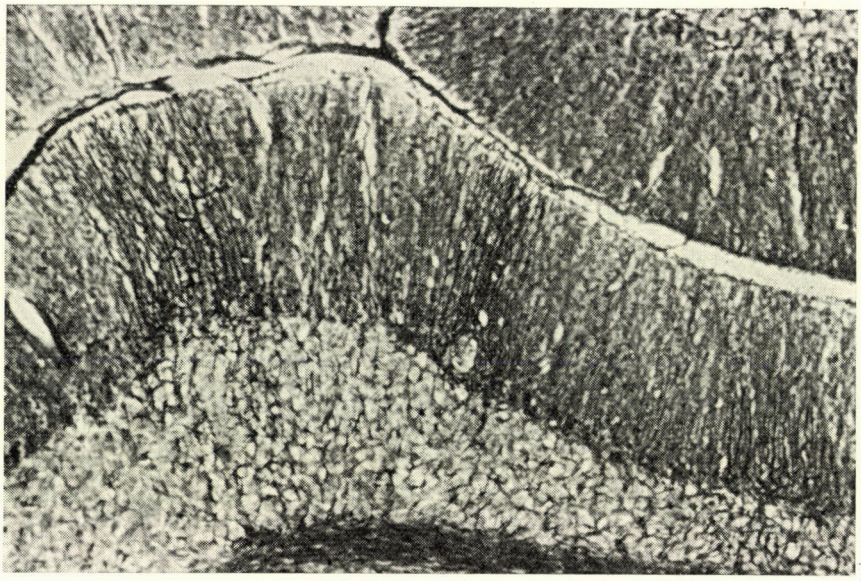

Fig. 10. Part of cerebellar folia. Fibrous gliosis in the cerebellar grey matter. Cajal's subli mate—gold impregnation. ×25

Fig. 11. Part of Fig. 10. Proliferation and swelling of the fibrous astrocyte fibres. Cajal's sublimate-gold impregnation. ×600

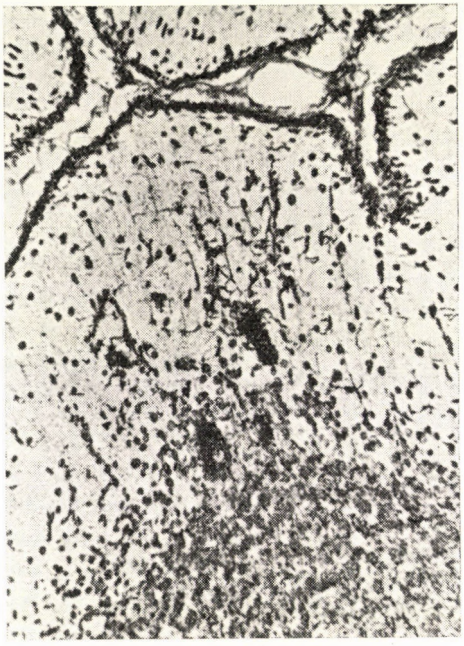

Fig. 12. Part of the cerebellar grey matter. Proliferation of the oligodendroglia and Hortega's glia cells in the affected areas. Penfield's silver impregnation. ×240

Fig. 13. Part of cross-sectioned medulla. Gliosis and nerve-cell lesions in the vagus nuclear group. Haemalaun-eosin staining. ×95

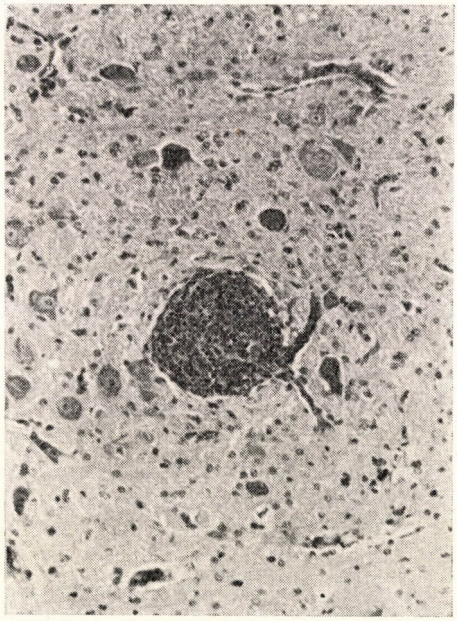

Fig. 14. Part of the medulla. Vascular and perivascular infiltration of a precapillary vessel. Lumen has disappeared. Degenerated nerve cells near to the vessel. Haemalaun-eosin staining. ×240

Irregular focal perivascular infiltration can occasionally be seen. Endothelial cells of the affected capillaries show slight degeneration and moderate proliferation.

Glioses and perivascular infiltrations in the cerebellar grey matter show "flame-like projections" which extend from the granular layer into the molecular layer (Figs 3, 4, 5).

Histological lesions in the nuclei of the cerebellum and those of the medulla and mid-brain show similar features and are discussed together.

Three different kinds of lesions can be found in the mentioned nuclear groups: degeneration or necrosis of varying degree in the ganglion cells, glia proliferation, and vascular and perivascular infiltrations (Figs 13, 14, 15).

Degeneration of the neurons appears mostly as central chromatolysis, and only few pyknotic ganglion cells can be seen (Figs 13, 14, 15).

The cytoplasm of the degenerated nerve cells showing central chromatolysis contain vacuoles of variable size.

Both changes resemble those observed in the Purkinje cells. No fat droplets can be demonstrated in the affected nerve cells.

In the affected nuclei mainly focal glia proliferations develop, replacing the necrotized neurons (neuronophagia) or the affected nervous tissue (Figs 13, 15).

With Cajal's or Penfield's impregnation it was revealed that both macro- and microglia cells take part in the glia proliferation. Of the macroglia cells

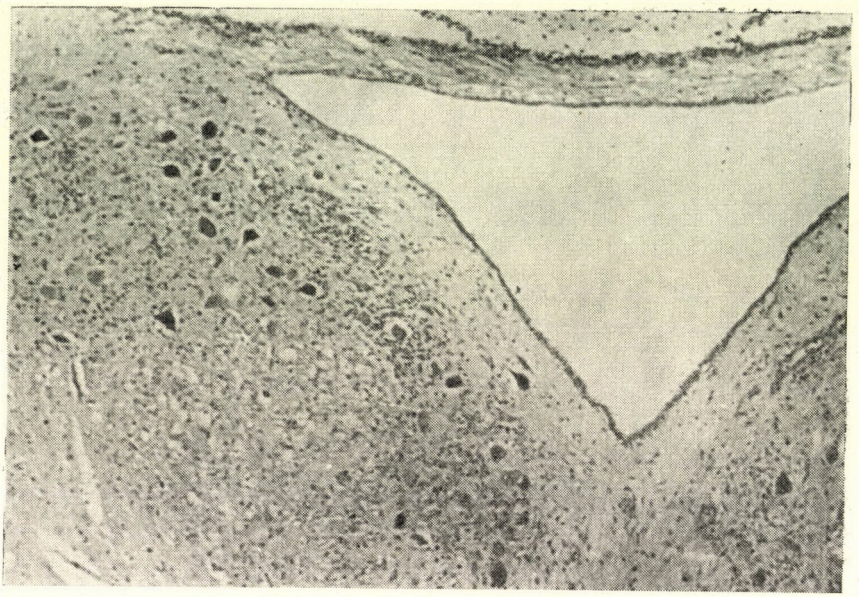

Fig. 15. Part of the mid-brain sectioned frontally. Nerve cell degenerations and glia proliferations in the affected nuclear groups. Haemalaun-eosin staining. ×95

fibrous astrocytes proliferate. The fibres of the astrocytes increase in number and thickness, so in the foci of glia cells a dense network of the fibres of astrocytes can be demonstrated with Cajal's sublimate—gold impregnation method (fibrous gliosis). By using Penfield's method it can be recognized that principally oligodendroglia cells proliferate in these areas of the affected nuclei.

The lesions in the capillaries in the affected groups of nuclei differ from those of the cerebellar grey matter in more frequent occurrence of focal infiltrations of the vessels' wall (Fig. 14), and in that coronary-like thickening of the astrocytes around some capillaries can be observed. Tracts of demyelinated fibres were commonly seen near the affected groups of neurons.

In the optic lobes, at the sites referred to, in addition to the afore-mentioned nerve cell degenerations focal gliosis and an intensive perivascular infiltration can be seen.

At the predilection sites of the cerebral hemispheres histopathological lesions include perivascular infiltrations of varying depth of the layers. Cuff-

ings are usually unilateral and multilayered; in some cases infiltration is uniform all around the vessels. The lumina of the blood vessels with massive cuffings become narrow, fissure-like, even occluded. Blood vessels are sometimes unrecognizable, for red blood cells in the lumina can be seen only occasionally. With Cajal's method fibrous gliosis around several blood vessels

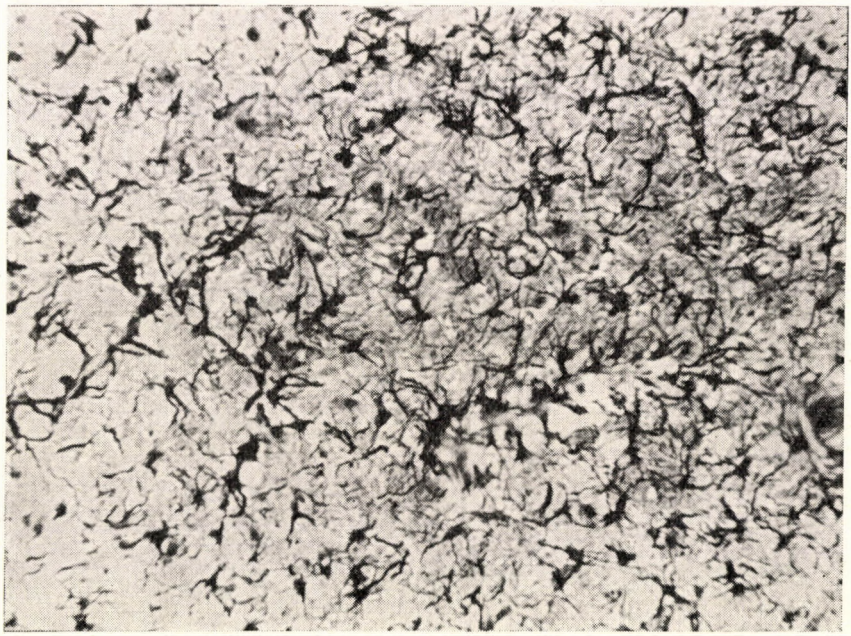

Fig. 16. Focal proliferation of the fibrous astrocytes in the affected areas of the cerebral hemispheres. Coronary-like proliferation around a larger blood vessel. Cajal's sublimate—gold impregnation. ×240

can be demonstrated, similar to those found in the affected nuclei (Fig. 16). The perivascular infiltrations in the cerebral hemispheres are asymmetrical. In addition, moderate proliferation of microglia cells near the degenerated nerve cells are seldom found in the hemispheres (Fig. 17).

In the grey matter of the spinal cord lesions can be seen in almost every area of the different segments. Nevertheles, the cervical and lumbo-sacral enlargements are involved most frequently and most markedly. Lesions in the ventral horns and in areas adjacent to the central canal are more pronounced and more common than those found in the dorsal horns (Figs 18, 19). Changes are usually symmetric, occasionally asymmetric. The lesions along the regions of the cervical and lumbo-sacral enlargements are variable in intensity and distribution.

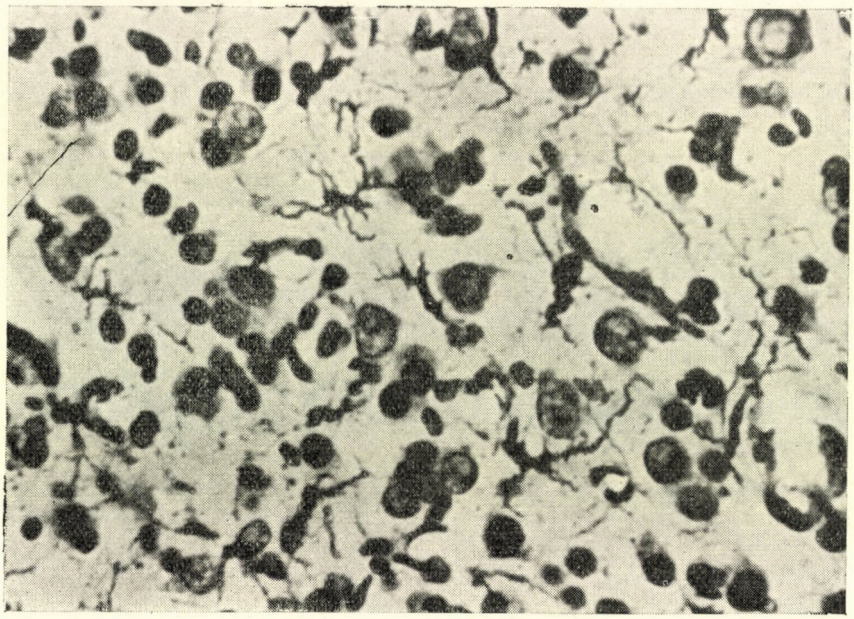

Fig. 17. Proliferation of the microglia cells (Hortega's and oligodendroglia) in the affected areas of the cerebral hemispheres. Penfield's silver impregnation. ×600

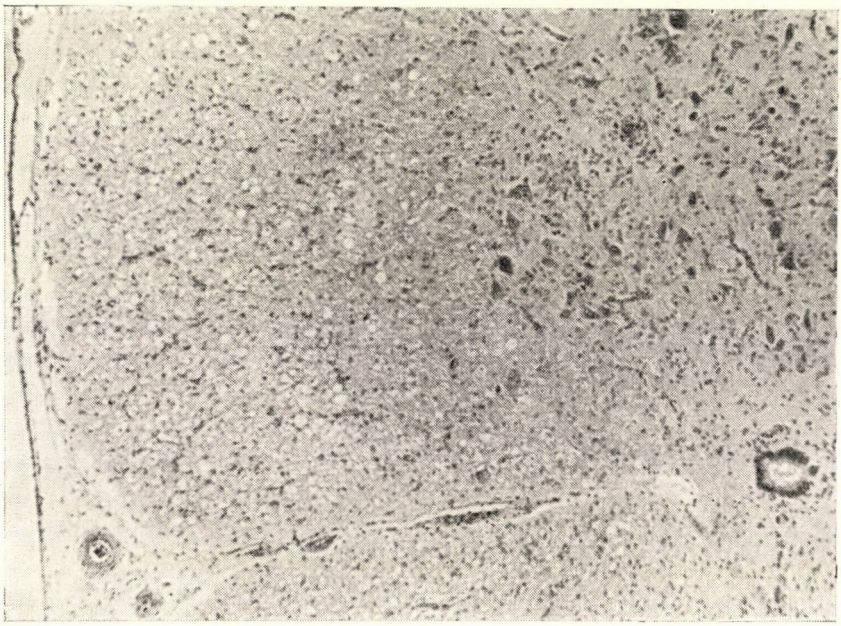

Fig. 18. Part of ventral horns of the cervical enlargement. Degeneration of nerve cells and glia proliferation in the grey matter. Demyelinization of the myelinated nerve fibres in the ventral funiculi. Haematoxylin-eosin. ×240

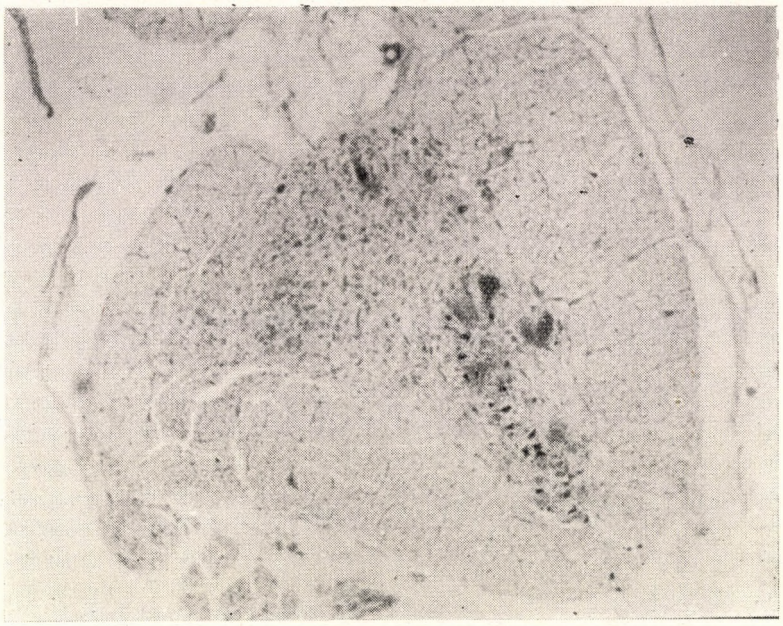

Fig. 19. Left side of the lumbo-sacral enlargement. Cuffing, glia proliferation and degeneration of cells in the ventral horns. Nissl's staining. ×95

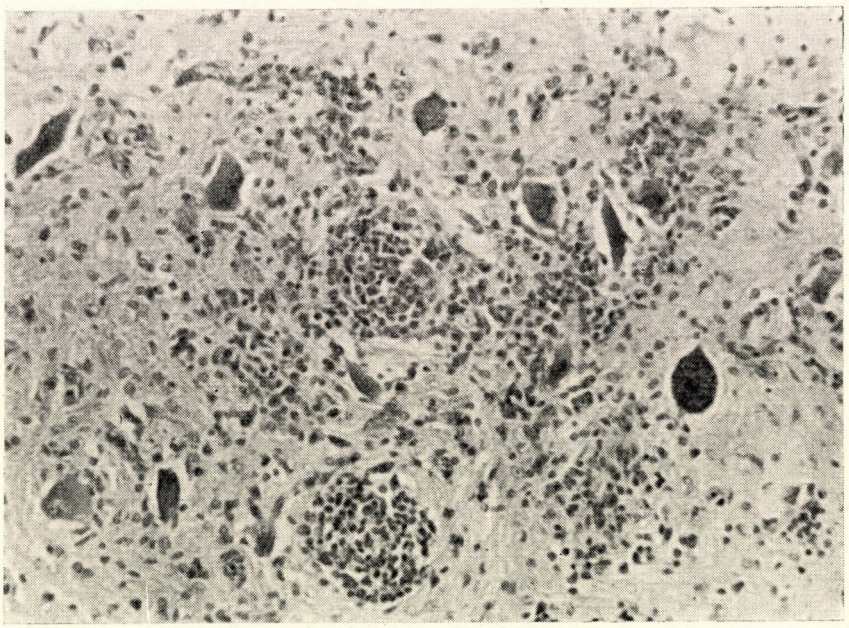

Fig. 20. Part of the ventral horns of the lumbo-sacral enlargement. Lesions seen in Fig. 19. Haemalaun-eosin staining. ×240

The characteristic lesions in the spinal cord are degeneration and necrosis of nerve cells followed by neuronophagia and glia proliferation, and perivascular infiltration. The infiltrating cells are lymphocytes (Figs 19, 20). Degeneration of the nerve cells includes central chromatolysis and vacuolation of the cytoplasm. Shrinkage and pyknosis of neurons are seen less frequently. Both types of degenerations resemble those found in the brain. Large motor nerve cells of the ventral horns are usually affected. Homogeneous, uniformly stained necrotized nerve cells are common. In sections impregnated with Bielschowsky's method different silver-binding capacity of the affected cytoplasm can well be demonstrated. In such cases the degenerated nerve cells change either to homogeneous, structureless, black-stained elements, or the cytoplasm of the necrotized nerve cells become unable to bind silver, therefore the cytoplasm is light, structureless and the nucleus cannot be distinguished (Fig. 21). Marked thickening and fragmentation of the synaptic pericellular nerve fibres can also be demonstrated. As a consequence, neuronophagia varying in severity can also be found. In addition, focal as well as diffuse glia proliferations in the horns are observed. Fibrous astrocytes and microglia cells can be found in approximately equal number. In some birds proliferation of microglia cells is more pronounced.

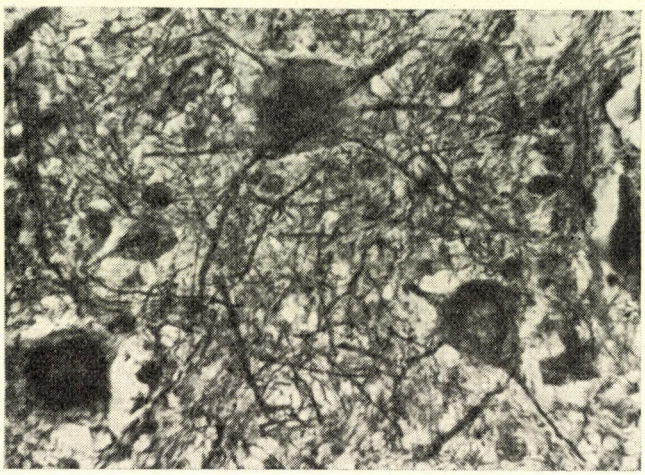

Fig. 21. Part of the ventral horns of the lumbo-sacral enlargement. Normal and affected motor nerve cells. Bielschowsky's silver impregnation. ×600

With Cajal's sublimate—gold method it can be demonstrated that some degenerated nerve cells in the ventral horns both of the cervical and lumbosacral enlargements are surrounded by abundant, brush-like fibres outgrown from the cytoplasm of some of the macroglia cells (fibrous gliosis). In gen-

eral, proliferation and thickening of the macroglia fibres are striking everywhere.

By Woelcke's method marked demyelinization of the myelinated nerve fibres of the ventral funiculi can be demonstrated throughout the spinal white matter (Fig. 18). Such changes in some of the fibres of the lateral and dorsal funiculi can be found occasionally.

In some birds smaller or larger foci consisting of lymphocytes and a few histiocytes are seen in the intervertebral ganglions of the cervical and lumbo-sacral enlargements, mostly at the periphery of the ganglions. In the affected areas degenerated ganglion cells can be recognized. The lesions include central chromatolysis of variable degree. Proliferating satellite cells enter the disintegrated nerve cells and neuronophagia develops.

In the peripheral nerves of the investigated birds cellular infiltrations were not found. In some of the myelinated nerve fibres degenerations varying in severity, demyelinization, axonal degeneration and fragmentation were observed. Neutral fat in the myelin sheath of the demyelinated nerve fibres could never be demonstrated with Sudan III staining.

I have had the opportunity to investigate CNS of four chickens which survived the onset of disease by one and a half to two months. The birds showed either some of the characteristic symptoms: tremor, incoordination of movements or paralysis. In the cerebellum of the infected birds disappearance of the Purkinje cells at some sites was the most striking lesion. Many of the Purkinje cells showed pyknosis. Central chromatolysis of the nerve cells was not a common feature. Cuffings had reduced in number and changed in character. Vascular infiltration was replaced by proliferation of the adventitial glia cells. In some of the birds intensive focal glia proliferation could still be observed. The lesions were observed at the predilection sites of the brain and spinal cord.

Demyelinization in the peripheral nerve fibres was commonly found. In the myelin sheath of the peripheral nerves of some birds also neutral fat could be demonstrated by staining with Sudan III.

Most of the authors (JONES, 1954; JUNGHERR, 1939, 1942, 1953; OLITSKY, 1939; FRITZSCHE, 1952; MARKSON and BLAXLAND, 1955, 1958; LINDGREN et al., 1957; VAN ROEKEL, 1959) dealing with AE have stated that lymphocytic infiltration characteristic of the disease can be found also in some of the organs of the infected birds. Therefore liver, gizzard, proventriculus, pancreas and heart specimens were studied from most of the birds. Lymphoid infiltrations in the liver, muscle layer of the gizzard and proventriculus, and pancreas were found in 82, 41, 78 and 80% of the cases, respectively (Fig. 22). Most of the infiltrating cells were lymphocytes with a few lymphoblasts among them. Usually, the infiltration was of focal character. Two types of infiltrations were observed in the pancreas: (1) round-shaped infiltration demarcated

by a thin layer of fibrous tissue, and (2) infiltration irregular in outlines, without sharp demarcation zones. Infiltration in the heart was found in 61% of the cases. Most of the infiltrating cells were eosinophils (Fig. 23).

The lesions in the CNS of experimental birds inoculated intracerebrally with the infective brain suspension were similar to those found in the sponta-

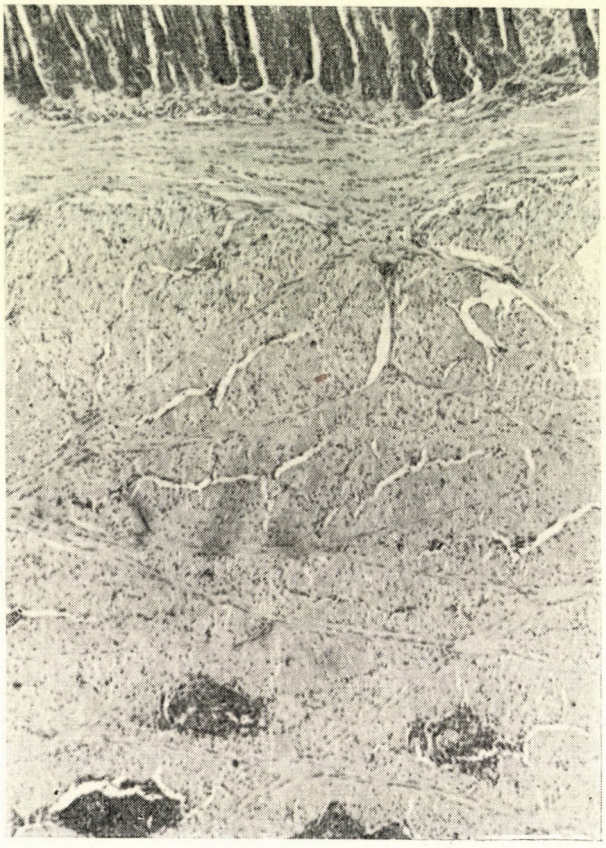

Fig. 22. Part of the gizzard. Focal lymphoid infiltration in the muscular layer. Haematoxylin-eosin. ×95

neous cases. Lesions, more pronounced than in the natural disease, could be demonstrated at the characteristic sites. The perivascular infiltrations were more intensive and more massive. The intensity of the lesions increased with the passage number. Some cuffings penetrated deeply into the white matter. The clinical symptoms of the experimental infection closely resembled those of the spontaneous cases.

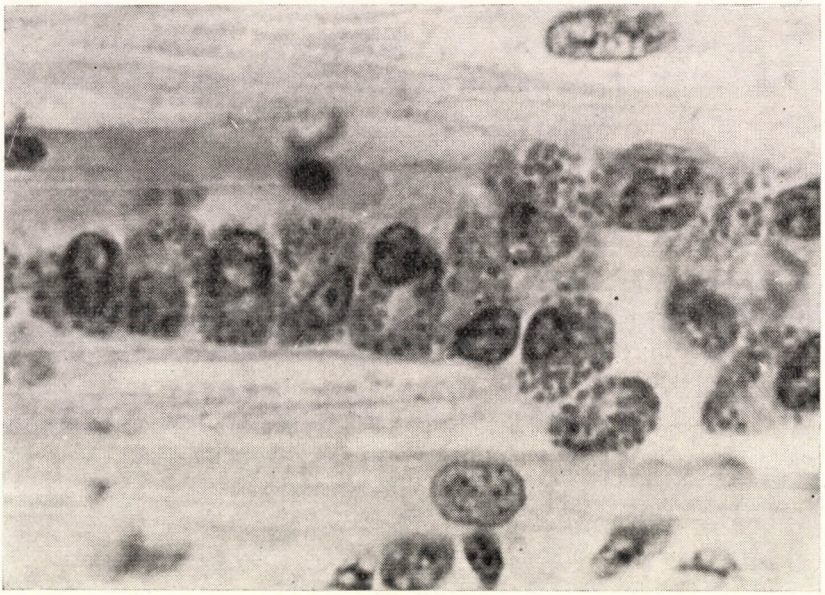

Fig. 23. Part of the myocardium. Infiltrating eosinophil cells between muscle fibres. Haemalaun-eosin. ×1400

Discussion

The virus of AE induces non-suppurative lymphocytic encephalomyelitis in the infected birds. Mostly the grey matter is involved, and the most intensive and characteristic lesions can be found at the predilection sites.

Many authors (JONES, 1934; JUNGHERR, 1953; FEIBEL et al., 1952; FRITZSCHE, 1952; MARKSON and BLAXLAND, 1958; GROTH and GREUEL, 1961; SCHNEIDER, 1964; MAYOR, 1968) have recorded predilection sites which are roughly comparable with those found in the studies described here. Lesions in the cerebellum are emphasized by most of the authors.

As for the most frequently affected cerebral nuclei, eccentric nuclear displacement in the large nerve cells of the reticular nucleus was considered characteristic of the disease by JUNGHERR (1953). MAAS and HELMBOLDT (1962) stated that focal or diffuse gliosis in the dentate and ovoid nuclei are characteristic of AE. MAYOR (1968) called attention to the ovoid nucleus at the most affected site. According to LINDGREN et al. (1957) predilection sites in the brain stem cannot be distinguished.

Based on the studies published here, among nuclei of the cerebellum, medulla oblongata and mid-brain some were affected more frequently, others rarely, or not at all. The most affected groups of nuclei have been listed above.

The most characteristic lesions were found in the cerebellar grey matter. The characteristic lesions in the cerebellum call the attention to the disease.

Flame-like projections from the granular layer into the molecular layer may be considered significant lesions (JUNGHERR, 1953). These are resulted from proliferation mainly of fibrous astrocytes and lymphocytic cuffings that replace disintegrated Purkinje cells.

Unlike my own observations that glioses are resulted from the proliferation mostly of fibrous astrocytes (fibrous gliosis) MARKSON and BLAXLAND (1958) considered them of microglial origin (microgliosis).

Flame-like projections in the molecular layer were first observed by JUNGHERR (1953), and later by others (LINDGREN et al., 1957; BURTSCHER, 1960—61). Degeneration of varying degree and necrosis of the Purkinje cells are also striking. Ataxia as clinical symptom of the disease is ascribable to the affection of these cells. Swelling demonstrable with Bielschowsky's method of the climbing fibres around the affected Purkinje cells, and that of the synaptic fibre network of the basket cells around the nectorized Purkinje cells might be considered characteristic, too (Figs 8, 9).

In FULTON's (1938) opinion, tremor in man is resulted from the affection of the cerebellar nuclei. It is presumed that tremor, one of the clinical symptoms in the examined chickens, might be traced back to the lesions found in the cerebellar nuclei.

Flaccid paralysis of the hind limbs is well explained by the severe lesions in the motor nerve cells of the ventral horns or the lumbo-sacral enlargements (MANNINGER and MÓCSY, 1965).

The lesions in the ganglion cells and in the motor nerve cells of the spinal cord are of similar character. Unlike the Purkinje cells, lesions in these nerve cells are characterized by central chromatolysis and vacuolation rather than pyknosis.

At the mentioned sites fibrous gliosis can be observed. Coronary-like proliferation of the glia cells around some blood vessels described by JUNGHERR and MINARD (1942), LINDGREN et al., (1957) and KÖHLER (1964) has also been confirmed in the present studies. Hortega's microglia cells seemed to have proliferated in the affected areas mainly in the cerebrum.

Affection of the cerebrum is characterized by perivascular and vascular infiltrations. Degeneration of the nerve cells or slight gliosis is seldom observed.

Demyelinization of some peripheral nerve fibres had presumably resulted from the degeneration and necrosis of motor nerve cells. Demyelinization of the sciatic and peripheral nerve fibres, described by JUNGHERR (1939) and MARKSON and BLAXLAND (1958), was also observed.

According to BURTSCHER (1961), who also recorded changes in the peripheral nerves, the lesions are characterized by swelling of the myelin

sheath. OLITSKY (1939) and LINDGREN et al. (1957) did not found demyelinization of the referred nerves.

Iridocyclitis and cataract, observed by FEIBEL et al. (1952), PECKHAM (1957) and BRIDGES et al. (1958), were not seen by me in any of the birds infected with AE.

Lesions in the visceral organs may be considered of diagnostic value if the characteristic lesions in the CNS are present. According to JUNGHERR and MINARD (1942) and JUNGHERR (1953) lymphoid infiltrations in the muscle layer of the gizzard and proventriculus are of diagnostic value, since these are not observed in organs of the normal birds. Infiltrations in the heart muscle were observed by JONES (1934) and BURTSCHER (1960), too, who considered the lesions as chronic myocarditis and focal myocarditis, respectively. BURTSCHER (1960) observed pseudo-eosinophil cell infiltrations in the myocardium. In my cases only eosinophils associated with mononuclear cells were found. The infiltrations between the myocardial muscle fibres consist of this type of cells almost exclusively.

Differential diagnosis

AE should be differentiated from Newcastle disease, encephalomalacia of various origins, Marek's fowl paralysis, ornithosis, toxoplasmosis and mycoplasmosis.

Along with other methods (post mortem examination, serological, bacteriological and virological investigations, and experimental inoculation) histopathological lesions are very useful in differentiating AE from the diseases listed before.

SUMMARY

In the grey matter of the CNS of birds infected with AE non-suppurative lymphocytic encephalomyelitis develops. The lesions are most pronounced at the predilection sites. Of the predilection sites the molecular layer and Purkinje-cell layer of the cerebellum should first be mentioned, then certain groups of nuclei in the cerebellum, medulla oblongata and mid-brain, deep medullary zone of the optic lobe, neostriatum and hyperstriatum of the cerebral hemispheres, and cervical and lumbo-sacral enlargements of the spinal cord.

The lesions include alterative, productive and inflammatory processes. The alterative process consists of pyknotic degeneration, central chromatolysis, vacuolation and necrosis of the affected ganglion cells. The productive process results in focal, sometimes diffuse, proliferation, mostly of fibrous astrocytes and occasionally microglia cells. Inflammatory process includes lymphocytic vascular and perivascular infiltrations. Cuffings are uniform or unilateral and form mono- or multiple layers around blood vessels. Occlusion of the lumina of blood vessels is common.

The three types of processes show varying intensity at the predilection sites. In the cerebellum, alterative and productive processes predominate. In the affected nuclei of the cerebellum, medulla oblongata and mid-brain as well as in the optic lobe alterative, productive and inflammatory processes participate in similar proportions. In the cerebral hemispheres inflammation predominates, other processes can be found only occasionally. Like in

the cerebellum, in the grey matter of the spinal cord alterative and productive processes are prominent. Marked inflammatory processes can be found in some birds after a long persistence of the disease. In the medulla, mid-brain, and ventral horns of the spinal cord the lesions are symmetric, in the cerebral hemispheres they are mostly asymmetric. Cellular infiltrations cannot be found in peripheral nerves.

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EXPERIMENTAL INFECTION OF RHESUS MONKEYS WITH HŐGYES' FIXED STRAIN OF RABIES VIRUS

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Experimental infection with rabies fixed viruses has been extensively dealt with in the literature.

Many data have been available also on HŐGYES' fixed strain of rabies virus (1886, 1888, 1897), which has been used in Hungary as vaccine strain since 1890. Experimental infections with the strain have been performed on rabbits (HŐGYES, 1886, 1888, 1897; AUJESZKY and KERBLER, 1932), sheep (KERBLER, 1930), albino rats (AUJESZKY, CSONTOS and BUZNA, 1924) and albino mice (ALMÁSSY and SZABÓ-SZÜCS, 1956). Nevertheless, relatively little interest has been attached to the pathology of the experimental infections; HŐGYES conducted relevant examinations on rabbits (1886, 1888, 1897) and KERBLER (1930) on sheep.

The present studies of the effect of HŐGYES' fixed rabies virus strain on monkeys were prompted firstly by the need for more detailed information on the strain's pathogenicity for various animal species, secondly by the recent use of monkeys as highly susceptible experimental animals for the safety testing of rabies vaccines (SELIMOV and AKSENOVA, 1966) as well as for the production of rabies antisera (ANDERSON and SGOURIS, 1966), in spite of the fact, that information has been scanty on both natural (SCHMUTTER, 1914; BOULGER, 1966) and artificial rabies infection of monkeys (LEVADITI, NICOLAU and SCHOEN, 1924, 1926; HURST and PAWAN, 1931; NICOLAU, MATHIS and CONSTANTINESCU, 1933).

With these facts in mind it seemed to be worthwhile to closer examine the effect of experimental infection of HŐGYES' fixed strain of rabies virus in monkeys. The present paper is a report of clinical symptoms induced by the intracerebral infection.

Materials and methods

Experimental animals. Rhesus monkeys *(Macaca mulatta)* imported from India were used throughout. Exclusively the animals which proved healthy throughout a more than 6-week observation period were included in the experiment.

Virus. HŐGYES' fixed virus strain from the 2194th passage in rabbit brain was kindly supplied from the Phylaxia State Vaccine Institute, Budapest. The freeze-dried powdered rabbit brain was made up to a 1% suspension in distilled water. 0.2 ml of this suspension was injected into the left thalamus of five rhesus monkeys each. The virus content of the inoculum was 10^6/g as expressed in terms of intracerebral LD_{50} for the mouse.

Inoculation procedure. The monkeys were narcotized with hexobarbital ("Intranarcon"). The top of the skull was depilated and a hole was driven with a dental bur into the skull bone at 3 mm distance to the junction of the coronal and sagittal suture (bregma). Through this hole the virus suspension was injected into the left thalamus, with a 2.5 cm long needle held vertically to the plane of the skull top.

Observation. The inoculated monkeys were initially inspected once daily; on the onset of symptoms they were inspected three times daily and later in every hour.

Results

1. Rhesus monkey No. 66/1967, ♀, 1-year old. Body weight: 2750 g. Basic behaviour characteristic: submissive.

The animal was symptom-free for 4 days following the intracerebral inoculation. On the 5th day it appeared listless and somnolent. On stimulation by blowing and clapping hands it turned aggressive. It assumed a desperate, angry countenance, ground his teeth and cried in a shrill voice. Its eyes were tearful, the pupils dilated, and it shunned light. Its movements were staggering and incoordinated. After crying for a long time it fell asleep. The hind extremities of the sleeping animal assumed a rigid spastic posture and at times a short-wave tremor shook the body. On the 6th day the animal was found lying on its side, with eyes half open, and apparently difficult respiration. On sound stimuli (slamming of the door, clapping of hands) it startled, its respiration became quicker and it made kicking movements with the legs. The same afternoon it was found in a comatous state; respiration was superficial. By the 7th day opisthotonus developed and the animal sunk into deep coma so that it no longer responded even to pain stimuli. It survived in this state for 3 additional days and died on the 10th day after inoculation, without any change of condition.

2. Rhesus monkey No. 67/1967, ♂, 1-year old. Body weight: 2300 g. Basic behaviour characteristic: submissive.

The animal was symptom-free for 3 days following intracerebral inoculation. On the 4th day it was slightly listless. Its original timidity vanished and it showed interest for the surroundings, but its countenance remained scared and its behaviour tended to become aggressive. Its pupils were dilated and prior to closer inspection it lay on one side. Being aroused, it walked around with a staggering gait, with a countenance which seemed extremely sad, queer and distressed. By the fifth day the comatous state had already set in, with sometimes fits of convulsion during which the animal ground its teeth, gave distressed groans and its extremities made kicking forced

movements. On the 6th day it was still unconscious and lay with head bent backward, back incurvated and extremities rigidly stretched. Its respiration was quick and superficial, its eyes were half open and the pupils tended to constrict. On the 7th day its state was unchanged. On the 8th day we succeeded in arousing it from the coma by stimulation: it slowly opened its eyes, its look was clear but it could not move. Shortly afterwards convulsions set in; then the animal fell into deep unconsciousness and died on the next (9th) day.

3. Rhesus monkey No. 68/1967, ♂, 1-year old. Body weight: 2300 g. Basic behaviour characteristic: aggressive.

The animal was symptom-free for three days after intracerebral inoculation. On the 4th day it lay in a queer curled position. On approaching it, it appeared nervous and irritable, breathed quickly and watched angrily. On blowing and clapping of hands it shook its cage and, stretching its anterior extremity through the wire grate, made attempts to grasp our dress and ground its teeth. Then, exhausted, it withdrew into a corner of the cage; in the afternoon we found it still leaning against the cage wire in an apparently dizzy state. On the fifth day in the morning it squatted groaning in one corner of the cage and performed queer kicking forced movements with the right anterior extremity. It breathed quickly and loudly, and with obvious pain. Its look was sad and distressed and its pupils tended to constrict. It held the tail in a straight rigid position. In the afternoon it lay curled, with the head hidden, and groaned with a distorted countenance. All fists were clenched and the anterior extremities made movements. Coma set in by the 6th day. The animal lay in a dizzy state, with the legs rigidly stretched and the arms crossed above the chest. The tail was rigidly stretched backward in a banner-like fashion and occasionally performed spastic twirling movements. On sound stimulus, the animal groaned painfully and kicked convulsively with the legs. On the 7th day we failed to arouse it from the comatous state. It survived for another two days and finally died on the 11th day.

4. Rhesus monkey No. 69/1967, ♀, 1.5-year old. Body weight: 2600 g. Basic behaviour characteristic: submissive.

Three days elapsed without symptoms, but on the 4th day the animal's behaviour changed markedly: its timidity vanished and it became irritable with a tendency for aggressivity. In the afternoon it behaved aggressively without preceding stimulation. Grinding its teeth, it shook the cage and made attempts to bite or scratch the persons coming closer. By the 5th day it became listless and somnolent and in the afternoon it was quite stuporous. It lay unconsciously in comatous state with convulsively clenched fists and sometimes groaned in low tones. It could not be roused by sound stimulus, but on touching it groaned painfully and fell into generalized convulsions. At the morning inspection on the 6th day it had a difficult, stertorous breath,

the eyes were half open, legs stretched and arms bent at the elbow. In the afternoon it was in coma. On the 7th day its state was unchanged, with occasional grinding of teeth. It died on the 8th day.

5. Rhesus monkey No. 70/1967, ♀, 1-year old. Body weight: 2700 g. Basic behaviour characteristic: submissive.

No symptoms occurred through 3 days after intracerebral inoculation. In the morning of the 4th day the originally timid animal assumed a vicious countenance, had an angry look and behaved aggressively. It paced nervously up and down his cage, with a somewhat uncertain gait. By the afternoon it became somnolent and leaned against the cage wire in a squatting position. It was roused by the sound of the opening door, and started to pace to and fro again with an angry, but distressed countenance and dilated pupils. At the morning inspection on the 5th day it was found lying on its side, with the knees drawn up. Its fists were clenched and sometimes spastic convulsions passed through its body. During the convulsive fits it twirled the tail. Its respiration was difficult and painful and it trembled on sound stimuli. By the afternoon it fell in a comatous state. On the morning of the 6th day it was unconscious but salivated profusely. Inspiration of the saliva occasionally elicited a moist cough. Coma persisted during the 7th day and salivation became foamy. Respiration was superficial. The animal was found dead on the 8th day

Summary of clinical symptoms

The disease caused in monkeys by intracerebral infection with fixed rabies virus could be divided similarly to the disease caused by rabies street virus into three fairly well-defineable stages: the preliminary (prodromal) stage, the excitation (furious) stage and the final (paralytic) stage. Symptoms set on after a latency of 3—4 days.

The preliminary stage was marked by the change of the monkeys' personality pattern. Animals of basically submissive behaviour characteristic first showed psychic hyperaesthesia, then turned aggressive, whereas the basically aggressive animals became increasingly irritable, and finally vicious.

The behaviour change was followed by the phase of excitation when the animals appeared particularly scared and distressed (dysthymia). Their countenance was sad but angry, the pupils were dilated and on coming closer to the cage, they immediately attempted a vicious attack: with ruffled hairs, they shook the cage wires and reaching through the cage bars made attempt to scratch the person approaching. The fits of vice associated with desperate crying, grinding of teeth, and dyspnoea; afterwards the animals sank back exhausted into a somnolent state and further stimuli were needed to elicit another fit.

There was a conspicuous increase of reflex excitability. Owing to light sensitivity, the monkeys drew back into the darkest corner of the cage and turned their backs to the window. In spite of this, there was copious lacrimation. On sound stimuli, as loud voice, clapping of hands and closing of the door the animals shrank back (hyperacusia, acuphobia) and a short-wave tremor shook their bodies. They responded with tremor and convulsions also on blowing (hyperalgesia, aerophobia).

In the intervals between the fits of excitation the monkeys were depressed and stuporous (interparoxysmal depression).

The excitation phase involved not only increased vice and irritability, but also a motoric restlessness: the animals paced to and fro in the cage, performed aimless movements with the extremities, walked round, jumped against the cage bars without any obvious reason. Motoric restlessness was soon followed by uncertainty of movements and staggering gait.

The change of respiration rate manifested itself soon: breathing became quicker and obviously painful. With the progression of the process, respiration tended to become more and more superficial with shorter or longer pauses of increasing frequency. In such cases, just like after the paroxysmal fits, the animals showed signs of painful dyspnoea and suffocation: their features distorted, their eyes protruded, the respiratory muscles moved in a dyspnoeal fashion and the monkeys first kept kicking, then lay prostrate.

The phase of excitation lasted only a few hours, then a state of coma set in gradually. During the final stage drousiness, somnolence and coma developed. The animals lay in a posture quite different from that of normal sleeping state: the body was curled up, the knees were drawn up, the hand and foot joints were convulsively bent and the fists clenched. The head bent forward and the tail was convulsively drawn back. In other cases opisthotonus set in: the animals lay with head bent dorsally, back incurvated, extremities rigidly stretched but fists clenched and the tail held rigidly in a straight position like a banner.

Tremor, jerks and convulsions were observed in all cases. Tremor begun at the head and gradually went through the whole body. Muscular fibrillation was seen both in the face and extremities. Convulsive contractions partly of flexor, partly of tensor muscles, resulted in clenching the fists, bent or drawn posture of the extremities, that is, in opisthotonus. Forced movements manifested themselves either by circular gait, forward jerks, or by kicking motions, grinding of teeth, twirling of tail and generalized convulsions. The tonic-clonic seizures were readily elicited by sound or pain stimuli.

The voice of the monkeys first became peculiarly shrill then gradually died down to a painful, low groan. Salivation was observed in only one case and even then only on the sixth postinfection day. The saliva was inspired and caused a painful, moist cough.

The short excitation phase was followed by a comatous state in a conspicuously rapid succession on the 5th to 6th day. Once in this state, the animals were roused with difficulty but responded to pain or sound stimuli with tonic-clonic spasms, grinding of teeth and convulsive twirling of the tail.

The comatous state lasted relatively long, sometimes even for 5 days and the animals died by the 8th to 10th day without regaining consciousness.

Necropsy findings

Post mortem findings revealed in one case hypostatic pneumonia, in two cases signs of urinary-bladder paralysis, and in every case cerebral hyperaemia and petechial haemorrhages in the brain stem.

The brain and spinal cord were reserved for histological examination.

Discussion

Among monkeys, natural rabies seems to be extremely rare; in the literature only two case reports are available (SCHMUTTER, 1914; BOULGER, 1966). Information is also scanty on experimental infection of monkeys with street rabies virus, although their susceptibility was demonstrated by MARX already in 1900. ACTION and HARVEY (1913) maintained the rabies virus in monkey passages. LEVADITI, NICOLAU and SCHOEN studied the susceptibility of various simian species *(Macacus sinicus, M. cynomolgus, M. callithrix, Cercocebus fuliginosus, Papio mormon, Troglodytes niger)*. NICOLAU, MATHIS and CONSTANTINESCU (1933) inoculated monkeys with the Oulo Fato strain, while HURST and PAWAN (1931) with a vampir strain from Trinidad and uniformly demonstrated a greater susceptibility to rabies of monkeys than of rabbits.

Data on monkey experiments with fixed rabies virus are still more scarce. The above-cited authors performed such experiments for comparison to the effect of street rabies virus without giving any details on the clinical manifestation. MARX (1900) inoculated Pasteur's fixed rabies virus strain into the subcutaneous connective tissue and muscles of monkeys. LAMB and MCKENDRICK (1909) reported the decrease of pathogenicity for the rabbit of a fixed rabies virus strain carried through monkey passages. HURST and PAWAN (1931) inoculated rhesus monkeys with fixed rabies virus to compare its effect to that of the vampir strain from Trinidad, but they presented no detailed clinical data.

Thus the results obtained on monkeys with fixed rabies virus strains of various origin, being scanty and inconsistent, are not suited to draw an unequivocal conclusion on the experimental infection of monkeys with fixed rabies virus.

In this laboratory, monkeys were infected intracerebrally with HŐGYES, fixed rabies virus strain with the primary aim to study the associated neurological symptoms.

The disease became manifest on the 4th or 5th postinoculation day. The first sign, the change of "personality pattern" was evaluated in comparison to the category of the animal's basic behaviour characteristic, social and interpersonal behaviour, *etc.*, as established during the pre-experimental observation period. Postulates to this end have been elaborated by NORKINA (1960) and MIRSKY (1960). We have adopted MIRSKY's rules as they seemed more simple, and have accordingly established that the basically submissive animals turned aggressive, whereas the basically aggressive animals vicious, as the first manifestation of the disease. In this respect, HŐGYES' statement (1894) that "the intensity and manifestation of personality change obviously depends largely on the patient's psychic constitution" seems to apply also to monkeys.

The fact that fixed rabies virus caused a typical furious condition on intracerebral inoculation into monkeys was not surprising, considering that the increased sensitivity of monkeys to rabies virus, particularly if administered intracerebrally, had been already noted by LEVADITI et al. (1926) and HURST and PAWAN (1931). Further to this, AUJESZKY and KERBLER (1932) found that in rabbits, furious rabies could be induced by fixed rabies virus if the subdurally administered virus dose was reduced.

In the condition elicited in monkeys by HŐGYES' fixed rabies virus, the phase of excitation lasted only a few hours, but the comatous phase prolonged to 3—5 days. This phenomenon was noticed by HŐGYES himself (1897), who had found that agony had lasted longer on inoculation with fixed than with street rabies virus strain.

The interpretation of the other neurological symptoms observed warrants detailed neurohistological studies.

SUMMARY

Rhesus monkeys were infected intracerebrally with HŐGYES' fixed rabies virus strain which has been regularly used in Hungary for prophylactic and postinfectious vaccination since 1890. Manifest rabies was developed by the monkeys after 3—4 days of latency and the animals died on the 8th—11th postinfection day. During the pathological course the animals underwent all known stages of rabies.

Detailed description is given of the clinical manifestation in all the five infected animals and correlating it with the neurological observations, a comprehensive summary is presented of the symptomatology of the disease caused in monkeys by intracerebral infection with HŐGYES' fixed rabies virus strain.

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ZUM SAUERSTOFFVERBRAUCH DER KOKZIDIEN-OOCYSTEN WÄHREND DER SPORULATION

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Im Zusammenhang mit der Intensivierung biochemischer Forschungsarbeiten auf dem Gebiet der Parasitologie in den vergangenen Jahren ist wieder eine Frage in den Blickpunkt gerückt, die auch für die Praxis von Bedeutung ist: die des Sauerstoffverbrauches von Oocysten während ihrer Sporulation.

Schon PFEIFFER (1892) betonte, daß eine Sporulation von Oocysten der Kaninchenkokzidien nur stattfindet, wenn Luftsauerstoff freien Zutritt hat. CHEISSIN (1935) bestätigte diesen Befund im Rahmen seiner weitreichenden Untersuchungen mit *Eimeria stiedai* und *E. magna* ebenso wie LAPAGE (1940), HENRY (1931) und GOODRICH (1944).

Eingehendere Untersuchungen über die Höhe des Sauerstoffverbrauches nicht sporulierter und sporulierter Oocysten von *Eimeria tenella* führten SMITH und HERRICK (1944) durch, während WILSON und FAIRBAIRN (1961) erstmals den Sauerstoffverbrauch von *Eimeria acervulina*-Oocysten während der Sporulation verfolgten. Neuere Untersuchungen über den Sauerstoffbedarf von Sporozoiten während ihrer Excystation wurden von VETTERLING (1968) durchgeführt. Alle Autoren (bis auf VETTERLING, der auf diese Frage nicht einging) kamen übereinstimmend zu dem Ergebnis, daß eine Sporulation der Kokzidien-Oocysten nur bei Anwesenheit von Sauerstoff möglich ist.

Anders lautende Hinweise haben sich in ihrer Mehrzahl als Irrtümer erwiesen. So zitiert PELLÉRDY (1965), neben der Mitteilung eigener Ergebnisse, Untersuchungen von HENRY (1931) und LAPAGE (1940) über Sporulation unter anaeroben Verhältnissen, was aber auf einer Fehlinterpretation beruht, die durch starke Kürzungen des ursprünglichen Manuskripts entstanden ist. Dem bei DUNCAN (1959) gegebenen Hinweis (p. 195) auf GOODRICH (1944) »GOODRICH (1944) felt that oxygen was not essential for sporulation« dürfte ein Mißverständnis der Worte »but oxygen does not seem to be necessary« — gemeint hat GOODRICH (1944) eine zusätzliche Sauerstoffapplikation — zugrundeliegen.

Bisher nicht widerlegt oder widerrufen sind die Befunde von HENRY (1931), daß *Eimeria scabra* — im Gegensatz zu anderen *Eimeria*-Arten — offensichtlich in der Lage ist »without access to a large amount of oxygen« zu sporulieren, von DUNCAN (1959), daß eine Sporulation der Oocysten von *Eimeria labbeana*, wenn auch nur zu einem geringen Prozentsatz, anaerob erfolgen kann, sowie von PELLÉRDY (1965), der in Anlehnung an HENRYS (1931) Untersuchungsmethoden bei einigen Haustierkokzidien-Oocysten auch eine Sporulation ohne Sauerstoffzufuhr feststellte.

Mit Rücksicht darauf, daß die Angaben der erwähnten Autoren über den Sauerstoffbedarf der sporulierenden Oocysten nicht übereinstimmen sowie auf die Tatsache, daß die Oocysten einiger Kokzidienarten (z. B. ein Teil der bei Fischen vorkommenden Kokzidien) innerhalb des Organismus auch unter vermutlich anaeroben Bedingungen sporulieren können, führten wir Untersuchungen an *Eimeria stiedai* und einem Gemisch verschiedener Darmkokzidien des Kaninchens durch.

Material und Methodik

In einem ersten Versuch wurden in zwei Exsikkatoren je zwei Petrischalen mit Oocysten beider Kokzidienarten in zweiprozentiger Kalium-

bichromatlösung gestellt, eine dritte Petrischale mit Oocysten diente jeweils als Kontrolle und wurde unter den im Labor üblichen Sporulationsbedingungen mit freiem Luftzutritt aufbewahrt.

Ein Exsikkator wurde täglich mit reinem Stickstoff, der andere mit reinem Kohlendioxyd durchspült und danach stets wieder luftdicht verschlossen. Nach 3×24 Stunden wurde der Versuch beendet. An Hand eines von Pellérdy (KOTLÁN und PELLÉRDY, 1934) ausgearbeiteten Sporulationsindexes wurde bei Versuchsende im Falle der *Eimeria stiedai*-Oocystenkultur der Sporulationsgrad exakt bestimmt, während er bei dem Gemisch von Darmkokzidienoocysten nur geschätzt wurde.

Auf Grund der in diesem Versuch erhaltenen Ergebnisse wurde zur Bestimmung der Höhe des Sauerstoffverbrauches einer Reinkultur von *Eimeria stiedai*-Oocysten während der Sporulation dieser in drei weiteren Versuchen mit einem Warburg-Apparat gemessen. Im Anschluß an die Sporulation ließen wir zusätzlich den Keimgehalt der Proben bestimmen.

Die Versuchsdauer betrug in allen Versuchen 3×24 Stunden. Die Oocysten wurden der Gallenblase infizierter Kaninchen entnommen und in zweiprozentiger Kaliumbichromatlösung gehalten. Die Temperatur des Wasserbades betrug in den beiden Hauptversuchen 25 °C. Alle Ergebnisse wurden auf μl verbrauchten Sauerstoffes pro 100 000 Oocysten umgerechnet.*

Ein Vorversuch bei 31 °C mit Material, dessen Herkunft nicht eindeutig definiert war, diente zunächst nur der Ermittlung der zu verwendenden optimalen Oocystenmenge.

Die Oocysten für Versuch 3 stammten alle von einem stark infizierten Versuchstier, das etwa 24 Stunden vor dem Ansetzen des Versuches getötet worden war. Bis Versuchsbeginn wurden die Oocysten nach dem Auswaschen im Kühlschrank bei etwa 4 °C aufbewahrt. Das Versuchsmaterial für Versuch 4 wurde durch Mischen des Gallenblaseninhaltes mehrerer schwächer infizierter Tiere gewonnen und etwa 48 Stunden nach der Schlachtung der Tiere verwendet.

Ergebnisse

Im ersten Versuch betrug der Sporulationsindex (Maximum bei vollständiger Sporulation aller einhundert ausgezählten Oocysten = 400) bei der unter Luftzutritt gehaltenen Probe von *Eimeria stiedai* 321. Die Vergleichswerte für die unter Stickstoff gehaltene Probe waren 11, für die Kohlendioxydprobe 10. Eine ähnliche, wenn auch wegen des Artengemisches nicht auszählbare Relation ließ sich für die Oocysten der Darmkokzidien feststellen. Die anschließende Aufbewahrung der zuvor nur unter Stickstoff und Kohlendioxyd

* Bei allen Auszählungen und der Bestimmung des Sporulationsindexes wurden offensichtlich taube, nicht sporulationsfähige Oocysten nicht berücksichtigt.

gehaltenen Petrischalen unter Luftzutritt ergab innerhalb weiterer drei Tage eine normale Sporulation der in ihnen enthaltenen Oocysten.

Im zweiten Versuch verbrauchten 1 Million Oocysten bei 31 °C etwa 400 μl O_2.

Als Ergebnis des dritten Versuches, in dem zwei verschiedene Oocystenkonzentrationen gemessen wurden (a) 6 Gefäße mit 130 000 Oocysten, b) 6 Gefäße mit 47 000 Oocysten), erhielten wir folgenden Sauerstoffverbrauch:

a)	Gefäß Nr.	1	2	3	4	5	6
	μl O_2	33,5	30,7	31,2	28,3	38,5	40,6
	Mittelwert	$\pm$ s	33,8$\pm$4,8 μl O_2				
b)	Gefäß Nr.	7	8	9	10	11	12
	μl O_2	27,3	31,3	29,5	28,7	30,6	33,8
	Mittelwert	$\pm$ s	30,2$\pm$2,3 μl O_2				

Der Sporulationsindex aus je einem Gefäß beider Meßserien betrug 395. Bei der bakteriologischen Untersuchung wurden in Gefäß Nr 5, 7 und 8 jeweils »einige wenige Kolonien von *E. coli*« festgestellt, die übrigen Gefäße waren keimfrei.

Im vierten Versuch, in dem elf Gefäße mit jeweils 100 000 Oocysten angesetzt worden waren, betrug der Sauerstoffverbrauch:

Gefäß Nr.	1	2	3	4	5	6	7	8	9	10	11
μl O_2	36,9	42,0	43,1	30,3	46,8	40,1	39,3	41,1	38,4	37,3	38,8
Mittelwert	$\pm$ s	39,5$\pm$4,0 μl O_2.									

Der Sporulationsindex aus drei Gefäßen betrug 396, 397 und 399. Bei der bakteriologischen Untersuchung wurden nur in Gefäß Nr. 10 »einige wenige Kolonien von *E. coli*« gefunden, die restlichen Gefäße waren wiederum keimfrei.

Eine Gegenüberstellung der absoluten Meßergebnisse (nicht auf 100 000 Oocysten bezogen) der zwei Meßserien a) und b) in Versuch 3 ergab bei einem Verhältnis der in den beiden Serien verwendeten Oocysten von a : b = 1 : 2,8 eine entsprechende Relation von 1 : 2,5 für den Sauerstoffverbrauch.

Da die Sauerstoffverbrauchswerte für die Gefäße, bei denen anschließend ein geringer Keimgehalt festgestellt worden war, nicht wesentlich über dem allgemeinen Durchschnitt lagen, in drei Fällen sogar darunter, ist dieser Keimgehalt für den Sauerstoffverbrauch offensichtlich ohne Bedeutung, zumal eine nachträgliche Verunreinigung der Proben nicht ganz sicher ausgeschlossen werden kann.

Ob es sich bei dem Unterschied im Sauerstoffverbrauch zwischen Versuch 3 und 4 um eine im Bereich der methodischen Fehlerbreite liegende Differenz handelt oder ob dieser Unterschied durch das andersartige Unter-

suchungsgut (andere Tiere, andere Entnahmezeit post mortem, verschieden lange Aufbewahrungszeiten) bedingt ist, kann auf Grund der bisherigen Befunde noch nicht entschieden werden.

Diskussion

Die erzielten Ergebnisse unserer Untersuchungen stimmen mit denen der meisten oben zitierten Autoren überein. Bei einer nachträglichen Überprüfung der Versuchsbedingungen von HENRY (1931) und PELLÉRDY (1965) können wir annehmen, daß die irrtümlichen Schlußfolgerungen vermutlich dadurch bedingt waren, daß der Sporulationsverlauf seinerzeit unter hermetisch abgeschlossenen Deckgläsern beobachtet wurde, aber auf Grund einer vermutlich zu geringen Oocystenzahl die Flüssigkeit unter dem Deckglas noch genügend Sauerstoff enthielt, um eine Sporulation der Oocysten zu ermöglichen. Ob diese Erklärung allerdings auch für die Ergebnisse von DUNCAN (1959) zutrifft, muß offengelassen werden.

Messungen des während der gesamten Sporulationszeit verbrauchten Sauerstoffes wurden bisher nicht durchgeführt. Eine überschlägige Berechnung dieses Wertes ist nur aus den Angaben bei WILSON und FAIRBAIRN (1961) für *Eimeria acervulina* möglich, wobei sich bei einer angenommenen Meßdauer von 72 Stunden ein Maximalverbrauch von 7,2 μl O_2 für 100 000 Oocysten ergeben würde. Ob diese Abweichung von den eigenen Resultaten auf einem methodischen Fehler beruht oder artbedingt ist, muß den Ergebnissen weiterer Untersuchungen überlassen bleiben.

ZUSAMMENFASSUNG

Eine Sporulation von Darm- und Gallengangskokzidien-Oocysten des Kaninchens findet nur bei Luftzutritt, nicht dagegen bei Begasung mit Stickstoff und Kohlendioxyd statt. Der Sauerstoffbedarf für eine vollständige Sporulation von 100 000 *Eimeria stiedai*-Oocysten liegt zwischen 30,2 $\pm$ 2,3 und 39,5 $\pm$ 4,0 μl O_2.

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ISOLATION AND PROPERTIES OF EQUINE RHINOPNEUMONITIS VIRUS

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In Hungary, the most important horse disease is equine rhinopneumonitis, which has caused severe economic losses among horse stocks including race horse studs.

The viral abortion of mares was described first by DIMOCK and EDWARDS (1933). Subsequently MANNINGER and CSONTOS (1941) and MANNINGER (1949) demonstrated the identity of the causative virus with the agent of the disease known as catarrhal equine influenza. In 1956, SOVINOVA et al. isolated from an acute respiratory disease of horses an agent which proved to be an influenze A virus *(Myxovirus)*. Thenceforth the designation "equine influenza" has been applied to the disease due to influenza virus, and on the suggestion of DOLL (1959) the condition formerly known as catarrhal equine influenza or viral abortion of mares has been named "equine rhinopneumonitis". In the meantime also its agent has been isolated in tissue culture and on the basis of its properties has been classified into the *Herpesvirus* group (RANDALL et al., 1953; MORE and RANDALL, 1958; ARHELGAR, DARLINGTON and RANDALL, 1963). In 1963 PLUMMER and WATERSON isolated another herpesvirus strain (LK virus) from the respiratory tract of a horse with rhinitis. But comparative studies have proved the non-identity of this strain, and of another herpesvirus strain isolated later, with the agent of equine rhinopneumonitis (KARPAS, 1966).

Thus the three respiratory viruses isolated from horses have been, on the basis of their different serological and other properties, classified as serotypes Herpes equi 1 (rhinopneumonitis virus), Herpes equi 2 (PLUMMER and WATERSON's strain LK) and Herpes equi 3 (KARPAS' strain). The present studies are concerned with the occurrence and properties of Herpes equi 1 among horses in Hungary.

Equine rhinopneumonitis (type 1 Herpes equi infection) still presents a great problem in countries with large horse stocks, as *e.g.* in Poland, France, Denmark and in the United States. In Denmark, recent serological screening has shown neutralizing antibodies to rhinopneumonitis virus in the sera of 80% of horses above 2 years old (RASMUSSEN, 1968). Two years earlier the same author (RASMUSSEN, 1966) has reported the isolation of 26 virus strains from the organs of horse foetuses and a few days old foals as well as from the nasal discharge of foals and horses with rhinitis. In Hungary, no recent data have been available on the incidence of the disease. According to earlier studies (BARTHA and FEHÉR, 1954), in a stud 50% of the pregnant mares and 70% of non-pregnant and transposed mares had specific antibodies, as demonstrated by complement fixation (CF). In a race horse stud, where infection was detected by serological screening (CF), but no isolation of the infected mares was possible, 75% of the mares had aborted. Screening by CF initiated by the Central Veterinary Institute (VIZY, 1959—60) revealed a 9—40% incidence of rhinopneumonitis, primarily in south-eastern and northern Hungary.

Virus strains. The strains isolated by us have originated from several stud farms. We succeeded in isolating the agent of both forms of rhinopneumonitis: 4 strains (D, VMh_2, R and Mh) were obtained from the nasal discharge of a foal and from foetal lung. One strain (V) was isolated more than one

year earlier from a freeze-dried antigen prepared from the lung of a foal in the usual way. Strain HU was isolated by us several years ago and has been maintained in tissue culture ever since. For comparison, the reference strains Ky—D and ERPV, and the strain EA/Skovl. isolated in Denmark were used.

Examination of cytopathic effect in tissue culture

Virus isolation was made in monolayer cultures of calf kidney epithelial cells. As an inoculum, the supernatants of a 10% suspension of test material were used after treatment with penicillin, streptomycin and mycostatin. Specific cytopathic effect (CPE) developed by the 5th to 8th day, in the form of foci of variable size. Cell destruction manifested itself by rounding off, degeneration and detachment of the epithelial cells. CPE appeared already in the first passage. Due to the extension of the cytopathic foci, lattices appeared by the 4th to 5th day, their number and size tended to increase.

Cellular damage was best visible at the border of normal and destructed tissue. Coverslip cultures were fixed in methanol and stained with haematoxylin and eosin. The nuclei often swelled even on to the double of the normal size and within them basophilic chromatin granules were seen, which in a later phase arranged chiefly along the nuclear membrane. The nucleoli had either disappeared or fallen apart and assumed a peripheral location. The centre of the nucleus was often free of chromatin; the nucleoplasm was seen in the central part of the nucleus, and toward the cell membrane it was surrounded by a clear zone (halo). Often the nuclear membrane itself had disappeared and chromatin granules in chain-like arrangements were seen in its place. Later on the damaged nucleus shrunk to less than the normal size with sometimes perinuclear condensation, sometimes falling apart of the impaired cytoplasm. In further passages the focal CPE was apparent already on the 3rd day, particularly in the case of the reference strains Ky—D and ERPV, which had been transferred to calf kidney epithelial cell culture to enable comparison.

CPE appeared earlier and in a more severe form with the reference strains and one of our isolates as compared to the rest. The nuclear changes were consistent with those caused by agents belonging to the *Herpesvirus* group.

Neutralization tests. Two reference strains and 4 of our isolates were compared in the virus neutralization test, using hyperimmune ERPV serum prepared in rabbit and sera from 3 aborted mares from one and the same stud. It should be mentioned that strain D was isolated from the aborted foal of one of the three mares. The immune sera were used in dilutions from 1 : 4 to 1 : 128. From the virus strains grown in calf kidney epithelial cells, 100—10000 $TCID_{50}$/ml amounts were added to equal amounts of inactivated serum. The

serum-virus mixture was incubated for 2 hours at 37 °C, and subsequently 0.2—0.3 ml amounts of each mixture were inoculated into 3 or 4 tube cultures of calf kidney epithelial cells. Final reading of the neutralization test was done and the presence or absence of CPE was judged 8—10 days later.

The hyperimmune ERPV rabbit antiserum neutralized not only the homologous strain, but also the strain Ky—D (Table I). Yet it failed to neutralize even in 1 : 4 dilution the calf herpesvirus strain B—53 used as control. Sera from the 3 aborted mares neutralized the homologous D strain and the ERPV reference strain. The mare serum D—3 did not neutralize the calf herpesvirus strain B—53. According to the cross neutralization tests with the reference strain the isolates belonged to serogroup Herpes equi 1.

Virus titres. The strains ERPV, Hu and R inoculated into calf kidney monolayer cultures in a 10^{-1} or 10^{-2} dilution produced total cell destruction by the 7th to 10th day and their titres varied from $10^{-4.0}$ to $10^{-4.5}$. The remaining strains had somewhat lower titres, in the range from $10^{-3.5}$ to $10^{-4.0}$. No notable titre rise occurred after 25—30 passages in calf kidney epithelial cell cultures.

Some physical and chemical properties of the strains. Certain physical and chemical properties of the strains ERPV, EA/Skovl. and our six isolates were determined and compared (Table II). As herpesviruses in general all of them showed sensitivity to chloroform and to pH 3.0, and in $MMgCl_2$ solution they were unstable even at 37 °C. Trypsin resistance varied with the strain. Only the strains EA/Skovl., V and VMh_2 were sensitive to exposure to 0.25% trypsin at 37 °C for one hour, whereas the rest showed a titre decrease of only 1.0 or 0.5 log order as compared to the controls. On exposure to 0.5% trypsin, strains Hu and R were definitely, strain D slightly, resistant while the remaining five strains were distinctly sensitive. Resistance was considerably lower to exposure at 50 °C for one hour. Except for two cultures, one inoculated with undiluted, the other with 1 : 10 diluted Hu strain, no viral growth whatever was demonstrable in the tubes inoculated. Resistance to 30- or 15-minute heat exposure was shown besides Hu also by R, whereas all the other strains were sensitive to short heat exposure as well.

Strains D, V and Mh were inoculated to calf kidney epithelial cell cultures pretreated with 50 μg/ml of 5-iodo-2'-deoxyuridine (IDU). No CPE developed during a 7—10-day period of observation. This implies that IDU inhibited the growth of the examined three virus strains, which accordingly are DNA viruses.

The strains could be stored without loss of activity at —10 to —15 °C for 12—14 months and at +4 °C for 5—7 months.

Table I

Results of virus neutralization test with the isolates and reference rhinopneumonitis virus strains, using rabbit hyperimmune serum and convalescent horse sera

Serum	Virus neutralization test with serum dilutions						Virus (100 – 1000 $TCID_{50}$/ml)	Remark
	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128		
Rabbit hyperimmune	—	—	—	—	—	—	ERPV	Reference strain
ERPV serum	—	—	—	—	—	—	Ky-D	Reference strain (Kentucky, USA), hamster-adapted
	—	—	—	—	—	—	D	Isolate from foetal lung
	—	—	—	—	—	—	V	Freeze-dried antigen from foetal lung
	—	—	—	—	—	○	Mh	Isolate from nasal discharge of foal
	—	—	—	—	—	○	VMh_2	Isolate from foetal lung
	+++	+++	+++	+++	+++	○	B-53	Herpesvirus isolated from calf
D.1*	—	—	—	—	—	○	D	
D.2	—	—	—	—	—	○	D	
Mare serum (strain D isolated from lung of her aborted foal)	—	—	—	—	—	○	ERPV	
D.3	—	—	—	—	—	○	D	
D.3	+++	+++	+++	+++	+++	○	B-53	

Explanation: — Virus neutralized, no CPE apparent; +++ No neutralization, CPE apparent; ○ Not tested; * Sera (D.1, D.2, D.3) from aborted mares (stock D)

Table II

Comparison of some physical and chemical properties of reference and newly-isolated Herpes equi 1 (rhinopneumonitis) virus strains

Strain	Titre	IDU (50γ/ml)	Resistance to								
			Chloroform	Trypsin		50 °C for			pH 3.0	$MMgCl_2$	
				0.25%	0.50%	60	30	15		37 °C	50 °C
						minutes					
ERPV	$10^{-4.0}$	○	○	$10^{-3.0}$	10	—	—	—	—	—	—
EA/Skovl.	$10^{-4.0}$	○	—	—	—	—	—	—	—	—	—
Hu	$10^{-4.5}$	○	—	$10^{-4.0}$	$10^{-3.5}$	10±	10±	10±	—	—	—
R	$10^{-4.5}$	○	—	$10^{-4.0}$	$10^{-3.0}$	—	10±	10±	—	—	—
D	$10^{-4.0}$	DNA	—	$10^{-3.5}$	$10^{-2.0}$	—	—	—	—	—	—
V	$10^{-3.5}$	DNA	—	10	10	—	—	—	—	—	—
Mh	$10^{-4.0}$	DNA	—	$10^{-3.0}$	$10^{-1.5}$	—	—	—	—	—	—
$V.Mh_2$	$10^{-3.5}$	.	—	10	—	—	—	—	—	.	—

Discussion

From both manifestations of enzootic equine rhinopneumonitis (viral equine abortion; catarrhal equine influenza) virus strains were isolated in the first passage in primary monolayer cultures of calf kidney epithelial cells. The isolations were made chiefly from the lungs of aborted foals, but also from the nasal discharge of a foal with rhinitis. Isolation from foetuses was in every case attempted from the lungs as, according to RASMUSSEN (1966), virus concentration is higher in the lungs than in the spleen, liver or tracheal mucosa, in succession. The isolates, like the references strains had moderate or medium high titres ($10^{-3.0}$ to $10^{-4.5}$). RASMUSSEN (1966) has reported somewhat higher titre values in homologous tissue, that is, in primary foal kidney epithelial cell cultures. In rabbit kidney epithelial cell cultures the equine herpesviruses reached titres of $10^{-6.0}$ to $10^{-7.0}$ (TISCHENDORF et al., 1967); titre values of $10^{-7.5}$ and still higher were reported with a hamster-adapted strain (RAC—H) in pig kidney epithelial cell culture (MAYR et al., 1968). In his studies on the strain EA/Skovl., RASMUSSEN (1966) found titres of $10^{-4.5}$ in foal kidney cells, $10^{-5.5}$ in pig kidney cells, and $10^{-3.5}$, the lowest, in calf kidney cells. In the foal and pig cells, the titre remained unchanged during the serial transfers, whereas in the calf cells it fell to $10^{-2.3}$ in the course of 10 passages. In our hands, the same strains reached a $10^{-4.0}$ titre in further transfers in calf cells.

In the calf kidney epithelial cell culture the CPE of the isolates manifested itself first by nuclear changes and subsequently by focal destruction. In general, the CPE resembled that of herpesviruses. The type of nuclear change, growth inhibition by 50 μg/ml of IDU, and the results of cross neutralization tests with the isolates and reference strains have unequivocally shown that our isolates are of the Herpes equi 1 serotype, like the reference strains from the U.S.A. and Denmark.

The examined physical and chemical properties of the reference strains and the isolates were not fully uniform. On 1-hour heat exposure at 50 °C all strains tested, except the Hu strain originally of $10^{-4.5}$ titre, showed a growth ability in part of the cultures to which it was inoculated, either undiluted or in 1 : 10 dilution. Resistance to heat treatment at 50 °C for 15 or 30 minutes was shown in addition to the Hu strain also by the R strain up to 1 : 10 dilution. Resistance to trypsin was in general more pronounced. Sensitivity to 0.25% trypsin was shown only by strains EA/Skovl., V and VMh_2, whereas to 0.5% trypsin only strains Hu and R were fully, and D and Mh partially, resistant.

In the equine abortion form of rhinopneumonitis, diagnosis is based on gross lesions of the foetus, and is complemented by the demonstration of CF antibodies in convalescent serum from the mother. The respiratory mani-

festation of the disease is less readily differentiated, as infection with *Myxovirus influenzae equi* A—1 and A—2, parainfluenza B virus and rhinoviruses cause essentially similar clinical symptoms. Thus the diagnostic differentiation of respiratory rhinopneumonitis is exclusively by virus isolation or by demonstration of a significant rise of specific antibody level in the convalescent serum. As the CF antibodies often disappear from the serum in 3—5 months, screening should be preferentially made by the virus neutralization test, particularly in the case of aged convalescents. This test may enable the demonstration of infection for several years.

SUMMARY

Isolation of equine rhinopneumonitis virus in tissue culture is reported. In cross neutralization tests the isolates were identified as belonging to Herpes equi type 1. Some physical and chemical properties of the examined strains deviated from those typical of the Herpesvirus group. Variation was particularly great in respect of trypsin resistance.

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PROPOSAL FOR SUBGROUPING OF BOVINE ADENOVIRUSES

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(Received April 10, 1969)

In the past decade several authors reported on the isolation of bovine adenoviruses. Six serotypes have been established so far. Serotypes 1 and 2 (KLEIN et al., 1959; 1960), and serotype 3 (DARBYSHIRE et al., 1965) were isolated in bovine kidney cell cultures, whereas serotypes 4 and 5 (BARTHA and ÁLDÁSY, 1966), serotype 6 (RONDHUIS, 1968), the untyped Australian strain designated BIL (COLE, 1967) and the Japanese strain designated Nagano (TANAKA et al., 1968) and Fukuroi (INABA et al., 1968) could be isolated in bovine testicle cell cultures only.

All the adenoviruses isolated from the bovines so far possess the four main properties suggested by the Provisional Committee of Nomenclature of Viruses (Paris, 1965) as characteristic of all adenoviruses, *viz.*, the virion

1. contains DNA,
2. is of cubic symmetry,
3. has no outer envelope (peplos), and the
4. triangulation number is 25, accordingly, the capsid comprises 252 capsomeres.

Several further features earlier considered characteristic of adenoviruses have been found lacking in some adenovirus strains examined recently.

As to the biological and physico-chemical properties, the strain differences among bovine adenoviruses are more pronounced than those among adenoviruses isolated from other hosts. Strains belonging to serotype 1, 2 and 3 possess the common soluble antigen present also in the human strains of adenoviruses. These bovine strains give complete cross reaction in CF test with the antisera prepared with human adenovirus strains and *vice versa*. The bovine strains belonging to types 4 and 5, and strains designated BIL, Nagano and Fukuroi, on the other hand, share only traces of the common soluble antigen of human adenoviruses.

Further differences between types 1, 2 and 3 on the one hand and types 4, 5 and strains BIL, Nagano and Fukuroi on the other exist in respect of heat resistance, formers being completely inactivated, latters only reduced in infectivity, at 56 °C in 30 minutes.

Types 1, 2 and 3 replicate in bovine kidney and testicle cell cultures, and occasionally in kidney cell cultures of other animal species, too. Con-

Table I

Comparison based on some properties of the two subgroups of bovine adenoviruses

Properties	Subgroup 1	Subgroup 2
Cross reaction between		
bovine antigen/human type sera	++++	±
human antigen/bovine type sera	++++	+
Resistance at 56 °C for 30 min.	no	yes
Replication in tissue cultures of		
bovine kidney	yes	no
bovine testicle	yes	yes
Inclusion body	single, irregular in shape	multiple, regular in shape
Adaptability to tissue cultures	readily	after a series of passages

Note: ++++ complete reaction; + weak reaction; ± reaction in traces

versely, types 4, 5 and 6 (and strains BIL, Nagano, Fukuroi) do not multiply in bovine kidney cell cultures, but can readily be propagated in bovine testicle cell cultures and in bovine foetal lung cell cultures. This feature is essential in isolating strains, for isolation of these types of bovine adenoviruses in bovine kidney cell cultures fails.

Characteristic difference can be observed also in the cytopathic effect between the two subgroups. While the members of types 1, 2 and 3 induce usually a single nuclear inclusion irregular in shape, those of types 4, 5 and 6 (and strains Nagano and Fukuroi) form mainly multiple inclusions which are circular in shape and sharp in outline.

Usually strains belonging to the types 1, 2 and 3 can readily be isolated in the first passage, whereas the isolation of the strains of types 4, 5 (and strains isolated in Australia and Japan) require several blind passages in tissue cultures of bovine testicle.

Based on the outlined features dividing of the bovine adenoviruses is suggested. The first subgroup is proposed to include the bovine adenoviruses which (a) possess the soluble antigen common with those of the human strains, (b) are inactivated at 56 °C in 30 minutes, (c) readily replicate in bovine kidney cell cultures, (d) form intranuclear inclusions irregular in shape, and (e) are readily isolated in the first passage. The second subgroup would include strains which (a) have soluble antigen common with the human strains in traces only, (b) are partially inactivated at 56 °C in 30 minutes, (c) fail to propagate in bovine kidney cell cultures, (d) form multiple intranuclear

inclusions regular in shape, and (e) whose isolation in testicle cell cultures usually needs a series of blind passages.

The first subgroup of bovine adenoviruses is in every respect more closely related to human adenoviruses than to members of the second subgroup. Strains proposed to belonging to the second subgroup, again, have certain properties similar to those of avian adenoviruses.

Based on the properties outlined above all strains of bovine adenoviruses isolated so far can be classified into the two proposed subgroups. Thus the strains belonging to types 1, 2 and 3, can be classified into subgroup 1, whereas the members of types 4, 5 and 6 (as well as strains BIL, Nagano and Fukuroi) into subgroup 2.

SUMMARY

Based on some properties, dividing of bovine adenoviruses is suggested. The strains in subgroup 1 possess soluble antigen common with human adenoviruses, are inactivated at 56 °C in 30 minutes, readily replicate in bovine kidney tissue cultures, form single nuclear inclusions irregular in shape and are readily isolated in the first passage.

The strains belonging to subgroup 2 would include strains which have only traces of the soluble antigen common with the human strains, are partially inactivated at 56 °C in 30 minutes, do not replicate in tissue cultures of bovine kidney, form multiple nuclear inclusions regular in shape and usually can be isolated after a series of blind passages only.

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ISOLATION OF BOVINE ADENOVIRUSES FROM TISSUE CULTURES OF CALF TESTICLES

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Since the use of tissue cultures became common in the virology, a variety of viruses, among them adenoviruses, have been isolated from uninoculated tissue cultures. Even the first human adenovirus strain was isolated from uninoculated tissue cultures prepared from surgically removed adenoids and tonsils (ROWE et al., 1956). Isolation of avian adenoviruses from tissue cultures of chick embryo organs was also reported (YATES et al., 1962). More recently, infection with adenovirus of swine kidney tissue cultures was observed (MAHNEL and BIBRACK, 1966). Recently, the isolation of a bovine adenovirus strain from calf testicle cell culture was reported (RONDHUIS, 1968).

In this paper the isolation of type 4 bovine adenovirus from cell cultures of calf testicles is presented.

Materials and methods

Cell cultures were prepared from testicles of calves 4 to 8 weeks of age as described in a previous paper (ÁLDÁSY et al., 1965). Testicles were brought from abattoir. As the cell sheet had become confluent — usually on the 3rd day — the growth medium was replaced by Hanks' solution enriched with 0.5% lactalbumin hydrolysate. Before changing the medium the cultures were checked for complete outgrowth and quality of cells. When cytopathic (CP) changes had appeared, the maintenance fluids of the respective cultures were subinoculated. Chloroform resistance of the isolates was tested according to Bögel and Mayer's method. The isolated strains were typed in neutralization tests, using type-specific sera of types 1, 2, 3, 4 and 5. Reading was made on the 7th day following inoculation.

Results

Since 1960 calf testicle tissue cultures have been prepared in our Institute usually twice a week. In each lot 1 to 5 testicles are used. During this period the CP effect characteristic of adenoviruses (rounding of cells, and nuclear

inclusions) was observed in 5 of the series. Similar CP changes developed following subinoculation of the strains into intact tube cultures.

In one case all tissue cultures of the series showed the characteristic CP changes in 2 to 3 days following the replacement of the growth medium. In two series characteristic CP effect could be observed in 10% of the cultures only, however, an additional 10% of the cultures proved to be infected on the 7th day following replacement, and in some of the apparently intact cell cultures inclusion bodies could be detected in the cells when stained.

In two cases the proportion of the infected tissue cultures amounted to 5% only.

The isolates proved to be chloroform-stable. Based on this property and the characteristic nuclear inclusions, virus neutralization tests were accomplished with the known 5 type-specific antisera. Of the 5 sera no but type 4 neutralized the isolates, indicating that all the 5 strains belonged to type 4 of bovine adenoviruses.

Discussion

Isolation of bovine adenoviruses from uninoculated tissue cultures has not been reported as yet. As our experiences show, infection with adenoviruses of tissue cultures, particularly those prepared from calf testicle, must be taken into account. As it was shown earlier (ÁLDÁSY et al., 1965) adenoviruses could be isolated from various organs of the affected or survived animals for a long period of time. In the viraemic stage the virus may invade the testicles, too. On preparing tissue cultures, the virus retains its infectivity, and multiplies in the susceptible cells. CP changes associated with the viral replication may first be observed as early as on the 5th or 6th day of incubation, but in some cultures not until the 7th to 10th day.

CP changes can usually be detected in unstained cultures. However, detection of the nuclear inclusions need stained preparations. If characteristic changes are revealed even in a small proportion of the tissue cultures, the whole series must be discarded. That is the reason why all the tubes must be checked under microscope before their use, and additional observation of some preparations is strongly advised. Since the first CP changes can be found as late as on the 5th to 10th day of culturing "inborn" infected cells, use of at least 10% of the uninoculated tubes must be left as control. On working with bovine adenoviruses results must not be accepted unless such controls have been used.

No doubt that in the mentioned cases the virus was harboured by the testicles used for the preparation of tissue cultures, for isolation of the virus from any of the media used for culturing testicle tissue failed.

As to our present experiences, strains belonging to serotypes 4 and 5 are difficult to adapt to testicle cells. In turn, adenovirus type 4 could readily by isolated in tissue cultures prepared from infected testicles. It is not surprising that type 4 strains were isolated, because this type of the bovine adenoviruses had proved to be the most distributed in Hungary (ÁLDÁSY et al., 1965).

According to our observations isolation of adenoviruses from a herd must be checked by using double controls. On the one hand, tissue cultures used for isolation must be checked as described afore, on the other hand, virological results must be confirmed by neutralization tests performed with paired sera of the suspected animals.

SUMMARY

Isolation of bovine adenoviruses from uninoculated bovine testicle tissue cultures are reported. The isolates belonged to the serotype 4. On isolating bovine adenoviruses use of proper controls is advised.

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LAUTIÉ, R.: *La maladie d'Aujeszky*. Les maladies animales à virus. Collection de monographies. L'Expansion Éditeur, Paris 6e. 1969. 226 p. 54 fig.

Like the previously appeared three volumes (on African swine fever, rinderpest and infectious anaemia of horses) of this series on virus diseases of animals in French, data on Aujeszky's disease are compiled on the basis of the international literature.

The introduction deals with the nomenclature, importance, history, geographic distribution and epizootiological features of the disease. The material is divided into 5 parts, dealing with (I) the properties of the virus, (II) clinical symptoms and pathological lesions, (III) diagnosis and prognosis, (IV) aetiology and pathogenesis, and (V) prevention, treatment and transmission to man of the disease.

In addition to a well-compiled review on the literature concerning the most important questions of this entity, the author stresses in part IV epizootiological and pathogenetic problems which, despite the huge amount of experimental and empiric information published since the discovery of the disease as an independent entity by AUJESZKY in 1902, are not solved yet. Such questions which wait for a proper answer have to indicate the source of infection in sporadic cases of the disease in seemingly isolated herds, the time of virus shedding by infected animals, the causes of great variability in the epizootic characters of the disease in various countries, *etc.* Anyway an increase of the number of virologically confirmed cases of the disease in countries with rare occurrence of the infection, and the ever increasing importance of it in those countries of Central and South-East Europe, South America, *etc.*, where the disease is prevalent, justifies the author's opinion to qualify it as an emerging disease ("maladie d'avenir"). It is to be welcomed that, despite Aujeszky's disease has not played an important role in France till now, this monography appeared in a more voluminous size than the formerly published volumes of this series. It is a particular pleasure to find that the author used with aim at completeness also the literature published in various small languages of Central Europe. In this way the monography contains a practically complete bibliography (724 references) on the disease with an objective and proper systematical arrangement of the huge material. There are only a few insignificant errors, resulted probably from the use of summaries or abstracts in world languages missing noteworthy data of some papers. It is not the author who has to be blamed for such minute inexactnesses, but it should be reminding for research workers to add to their papers summaries in world languages, equivalent to the well-compiled abstracts containing all important data of the work.

T. SZENT-IVÁNYI

WALKER, Warren, F.: *A Study of the Cat*. 181 pages, 64 figures. W. B. Saunders Co. Philadelphia and London, 1967.

The book has been written for undergraduates in biology and zoology, who have special interest for mammalian morphology and the human organism. The author's aim was to enable a general study of mammalian anatomy on a single model. For this purpose, the cat was chosen because it has many structural and morphological features in common with both man and other mammals and it is easier available for dissection than most useful domestic animals.

The present volume is based on the chapter dealing with mammals in the author's previous book on vertebrate anatomy and contains many additional information. Essentially,

it is a textbook which follows the system of comparative anatomy, with brief reference to the phylogenesis, ontogenesis and functional role of the individual organs. The morphological descriptions conform with the stratigraphic system of dissection.

The book is divided into 9 chapters and Addenda giving instructions in anatomical terminology and dissection techniques. A selected list of references and subject index are appended.

The individual chapters deal with the following topics:

1. Evolution and external anatomy of mammals as a summary of vertebrate evolution. In the second part the structure and appendages of the skin and the anatomical regions are described.

2. The axial and visceral skeleton.

3. Bones of the extremities with indications of their evolution.

4. The muscular system is discussed in a grouping which seems a bit unusual in relation to our school of thought. The description of muscle evolution and general myology is followed by the discussion of the muscles of the posterior trunk in epaxial and hypaxial grouping. Muscles before and behind the hyoid bone are described as "hypobranchial", whereas the masticator muscles as "brachiomeric".

5. The sense organs.

6. The nervous system. Though this chapter is the most detailed part of the book, the reader misses the description of the most important cerebral nuclei.

7. The coelom and the digestive and respiratory systems.

8. The circulatory system.

9. The excretory and reproductive systems.

The most important technical terms are used in phonetic spelling according to the Paris Nomenclature (1955). The book does not disclose novel aspects of comparative anatomy and naturally cannot satisfy the relevant requirements of biological, medical and veterinary research.

GY. FEHÉR

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Feierliche Begehung des zweihundertjährigen Jubiläums

Bei Anlaß des zweihundertjährigen Jubiläums der Tierärztlichen Hochschule Turins (die erste unter den italienischen tierärztlichen Fakultäten und die vierte der Welt), wird seitens des Rektorats der Universität und des Organisations-Komitees der Feierlichkeiten das Programm der festlichen Veranstaltungen vorbereitet, die am 2. Oktober 1969 in Turin stattfinden werden.

Allen tierärztlichen Fakultäten in Italien und im Ausland wird in Kürze die formelle Einladung für die Bestimmung der offiziellen Delegierten sowie das ausführliche Programm der Ceremonien und Veranstaltungen übermittelt.

P.S. Wegen etwaiger Auskünfte wende man sich bitte an den Sekretär des Organisations-Komitees Herrn Prof. Bruno Micheletto, Facoltà di Medicina Veterinaria, via Nizza 52, 10126 Torino.

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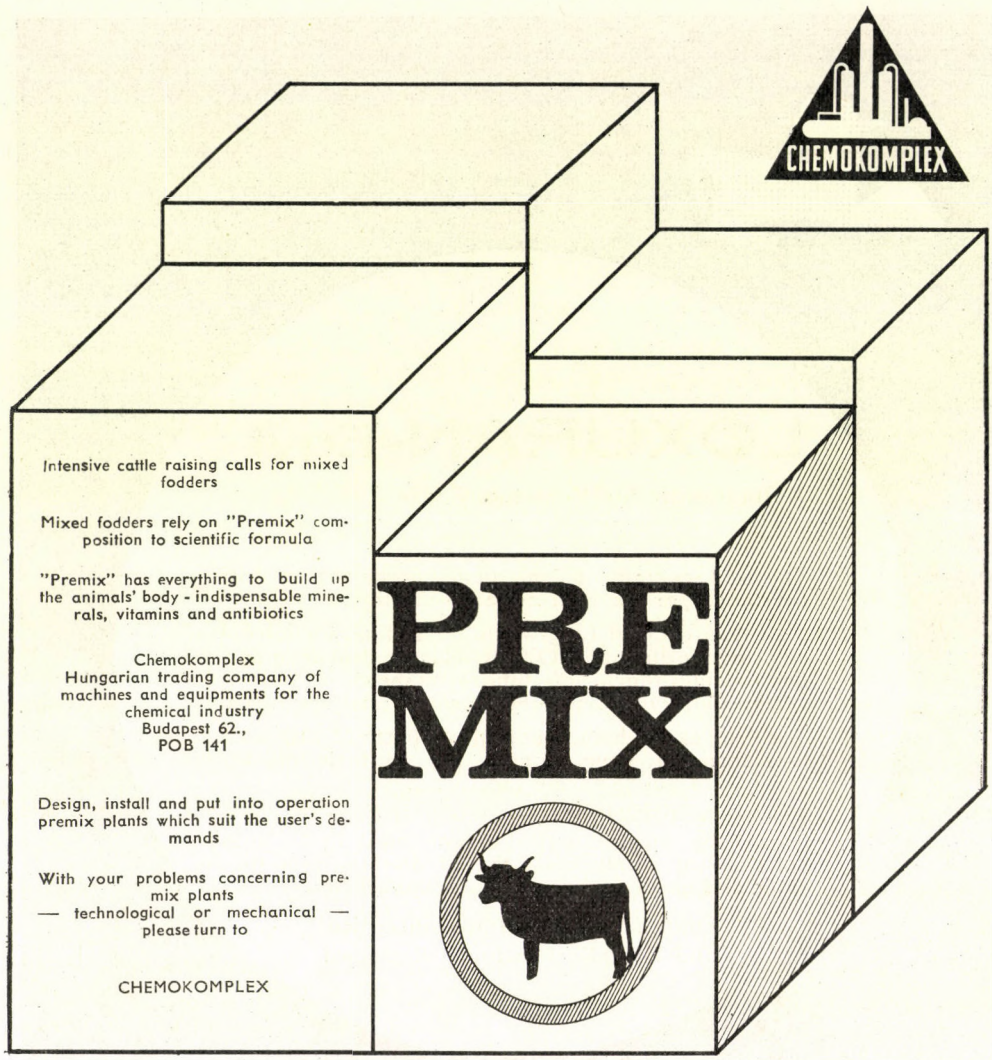

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РЕЗЮМЕ

ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ АНТИГЕННЫХ СВОЙСТВ БИОПЛАСТ-ПРЕПАРАТОВ

И. ХОРВАТ, К. ШОМОДЬВАРИ,

П. ШОМОШ и Я. ШУРЯН

Авторы имплантировали в подкожную клетчатку и брюшную полость кроликов, дальше, в полость костей морской свинки и собаки температурой необработанные биопластовые пластинки из фибрина крупного рогатого скота и человека и температурой обработанные и необработанные биопластовые гвозди. Методами агар-жель-диффузии и анафилаксии изучались антигенные свойства биопластов. Обнаружено, что под воздействием температурой необработанных бычьих и человечьих биопластов появляется в сыворотке кролика и собаки антиген, преципитирующий не только гомологический, но и гетерологический фибриноген млекопитающих (последний не высоким титром). В морской свинке от биопласта из температурой обработанного бычьего фибриногена нет образования антител.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ХИРУРГИЧЕСКОГО ШОВНОГО МАТЕРИАЛА С ОСОБЫМ ВНИМАНИЕМ НА НИТЬ ИЗ ПОЛИЭСТЕРА

Б. КОВАЧ А. и К. ШОМОДЬВАРИ

Во вступлении описываются свойства нити из полиэстера (фабрика Рико), потом детально излагаются результаты данных клиники и гистологии многочисленных сравнительных исследований на разном шовном материале, наложенном на подкожную клетчатку и мышцу у лошади и собаки. Следили за судьбой кетгут, шелковой нити и полиэстеровой нити.

За время наблюдения в 120 дней обнаружено, что около кетгут имелась наиболее энергичная тканевая реакция. Около шелковой нити имелась по качеству подобная, но по размеру меньшая воспалительная зона и хорошо отграниченная соединительнотканевая капсула. Зато полиэстер окружился соединительнотканевыми волокнами незначительной толщины, тесно взаимосвязанными с нитью. Около кетгут и шелковой нити наблюдали иногда в тканевой реакции микроскопического размера абсцессы. Таких изменений около полиэстера никогда не наблюдали.

На основании данных исследований авторы пришли к заключению, что полиэстер не раздражает ткани и он организируется организмом при наличии слабой реакции. Считают возможным, что полиэстер через некоторое время совсем рассосается, поэтому он очень удобен для наложения швов в глубине тканей.

ВЛИЯНИЕ МАТЕРИНСКОГО МУЦИН-ГЛОБУЛИНА ЯИЧНОГО БЕЛКА НА РЕЗИСТЕНТНОСТЬ ПОТОМСТВА ПОСЛЕ ВЫЛУПЛЕНИЯ

Ю. ПАВЕЛ и К. ПЕТЕРСОН

Изучен эффект муцин-глобулинов яичного белка на резистентность потомства у породы Нью-гемпшир. Обнаружено, что генотип материнского муцин-глобулина сказывается на развитии эмбриона. Наибольшая смертность наблюдалась у потомства дигетерозиготных несушек. Поскольку отклонения, вызванные материнским генотипом появляются относительно редко, авторы приходят к заключению, согласно которому естественный отбор под влиянием разных условий изменяется.

Пуллороз, как фактор естественного отбора, выравнивает отклонения, обусловленные материнским генотипом. Обнаружена разница в оплодотворяемости и эмбриональной смертности между гомозиготными и гетерозиготными несушками. Оплодотворяемость у гетерозиготных несушек была выше (локус G_3), при этом и эмбриональная смертность была тоже выше у потомства гетерозиготных матерей (локус G_2).

ДАННЫЕ К ПАТОГЕНЕЗУ ВИРУСНОГО ГЕПАТИТА УТОК

П. КАПП, Ф. КАРШАИ и И. ВЕИНЕР

Среди уток 3—4-недельного возраста авторами наблюдался вирусный гепатит, вызывающий большие потери. Патолого-анатомические и гистологические изменения в павших утках не отличались от изменений, имеющихся в павших от вирусного гепатита дневных утят. Авторы предполагали, что недоброкачественный корм мог вызвать у утят 3—4-недельного возраста повреждения, благодаря которым вирусный гепатит появился в такой тяжелой форме. Свое предположение они доказали экспериментально.

У утят 3-недельного возраста, кормившихся недоброкачественным кормом и экспериментально зараженных вирусом гепатита им удалось вызвать тяжелое заболевание; у утят же 4-недельного возраста доброкачественно протекающее заболевание. Контрольные животные такого же возраста, зараженные вирусом, но не поедавшие недоброкачественного корма, бессимптонно выживали. На основании своих данных авторы приходят к заключению, согласно которому тяжесть течения болезни кроме возраста и других факторов зависит и от состояния печеночных клеток в момент заражения, связанного с условиями кормления. Поэтому подчеркивают значение факторов кормления в профилактике заболевания.

КОЛЛОИДНЫЕ РЕАКЦИИ С ЦЕРЕБРОСПИНАЛЬНОЙ ЖИДКОСТЬЮ ЛОШАДИ

Р. ЛИППМАНН

Ради уточнения ликворного синдрома у здоровых и больных болезнью Борна, внутренным гидрокефаллом и гепатоцеребральным синдромом лошадей, докладывается о результатах 133 мастичных и золотых проб. При изучении ликвора здоровых лошадей мастичной пробой получается от середины влево нисходящая, плоская кривая, золотой пробой — слабое изменение краски в середине ряда разбавления, или от середины нисходящие, плоские кривые. У болеющих болезнью Борна при изучении ликвора мастичной пробой — получены отчасти нормальные кривые, но большей частью глубокие, от середины влево нисходящие кривые, тогда как при изучении золотой пробой — получаются, как правило, плоские, или умеренно глубокие средние кривые. При гидрокефалле и гепатоцеребральном синдроме получены кривые, подобные таковым у здоровых лошадей.

Согласно результатам исследований мастичную пробу в форме мастикс-лумботест можно считать наиболее надежной для коллоидно-химического изучения цереброспинальной жидкости. Она представляет собой ценный дополнительный метод к количественному определению белка.

Докладываются и оцениваются данные по количественному определению белка в церебральной жидкости.

ПРЕДЛОЖЕНИЕ НА ПОДРАЗДЕЛЕНИЕ БЫЧЬИХ АДЕНОВИРУСОВ

А. БАРТА

На основании определенных свойств ставится предложение на подразделение бычьих аденовирусов. Штаммы подгруппы 1 содержат растворимый с человечьими аденовирусами антиген, инактивируются при температуре 56 °С в течение 30 минут, дружно размножаются в тканевых культурах из бычьей почки, образуют единое ядерное включение неправильной формы и легко изолируются в первом пассаже.

Штаммы подгруппы 2 содержат только в следах растворимого, общего с человечьими аденовирусами антигена, только частью инактивируются при температуре 56 °С, не размножаются в тканевых культурах из бычьей почки, образуют множественные, правильной формы ядерные включения и изолируются только после серии слепых пассажей.

ИЗОЛЯЦИЯ БЫЧЬИХ АДЕНОВИРУСОВ ИЗ ТКАНЕВЫХ КУЛЬТУР ИЗ ТЕЛЯЧЬИХ СЕМЕННИКОВ

А. БАРТА и Л. ЧОНТОШ

Докладывается о изоляции бычьих аденовирусов из инокулированной тканевой культуры из телячьего семенника. Изоляты принадлежали к серотипу 4. При изоляции бычьих аденовирусов рекомендуется применение и других контрольных мероприятий.

ИЗУЧЕНИЕ ОБМЕНА ВЕЩЕСТВ У ГЕЛЬМИНТОВ I. ИЗУЧЕНИЕ ОБМЕНА ВИТАМИНА А У ASCARIS SUUM

Ш. ЮХАС и Ш. БАБОШ

Данные исследований in vitro на самках A. suum разрешают заключить о следующем.

Нематоды принимают из среды витамин А.

Поступивший в организм нематоды витамин хранится в органах гельминта в довольно постоянном процентном распределении.

Нет взаимосвязи между содержанием витамина А и содержанием жира в отдельных органах.

Поступивший в организм нематоды витамин А в обмене веществ используется червьем.

Каротиноиды не сказываются на обмене витамина А червья. Оливковое масло в значительной мере ускоряет поступление в нематоду и использование ею витамина А.

ЭКСПЕРИМЕНТЫ ПО ЗАРАЖЕНИЮ КОКЦИДИЯМИ НЕСПЕЦИФИЧЕСКИХ ХОЗЯЕВ ЧЕРЕЗ РОТ И ПАРЭНТЕРАЛЬНЫМ ПУТЕМ

Л. ПЕЛЛЕРДИ и У. ДЮРР

Эксцистированными и нетронутыми спорулированными ооцистами кишечных и печеночных кокцидий через рот и парэнтерально заражались крысы, парэнтерально же заражались морские свинки. В кишечнике неспецифического хозяина наступает эксцистирование ооцист. Но поселения паразита после введения через рот и его дальнейшего развития никогда не наблюдались, даже у животных, сопротивляемость которых снизили путем удаления селезенки, дачи гидрокортизона, облучения рэнтгеном, содержания на полуголодной диете метионина с добавкой этионина, или после парэнтерального заражения стадиями развития (мерозоиты) кокцидий. Такие же наблюдения сделаны при заражении индеек и цесарок видом **Eimeria tenella**, которых тоже обрабатывали гидрокортизоном или содержали на такой же дефицитной диете, как и крыс. Эти неудачи перекрестного заражения являются новым доказательством еще и в настоящее время оспариваемой строгой специфичности аймерий к хозяевам с одной стороны и указывают на то, что эта специфичность вероятно не связана с иммунологическими механизмами с другой.

ЭНЗИМОДИАГНОСТИЧЕСКИЕ ИССЛЕДОВАНИЯ С КРОВЬЮ ЗДОРОВЫХ И СТРАДАЮЩИХ ДИСТРОФИЕЙ ПЕЧЕНИ ГУСЕЙ И УТОК

Й. БОКОРИ и Ф. КАРШАИ

Авторами изучена энзимная активность сыворотки здоровых гусей на разные энзимы. Получены следующие показатели: глутаминовокислая-щавелевокислая трансаминаза (ГЩТ) — 14,4 $\pm$ 5,0 МЕ, глутаминовокислыйпируват-трансаминаза (ГПТ) — 3,1 $\pm$ 2,9 МЕ, алдолаза (АЛД) — 4,9 $\pm$ 3,1 μE, щелочная фосфатаза (ЩФ) — 31,2 $\pm$ 9,0 μE.

У суточных утят изучение энзимной активности крови дало следующие результаты: ГЩТ — 10,1 $\pm$ 4,2 МЕ, ГПТ — 5,1 $\pm$ 2,8 МЕ.

Не обнаружено повышения активности ГПТ у гусей к концу форсированного откорма, больше того, имелось понижение активности ГЩТ.

При поражениях печени соответственно тяжести процесса наблюдалось существенное повышение активности ГПТ, ГЩТ и АЛД. Сильно повысилась трансаминазная активность у уток при вирусном гепатите.

ПАТОГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ПРИ ЭНКЕФАЛО-МИЭЛИТЕ ЦЫПЛЯТ В ВЕНГРИИ

Г. МАЛИК

В сером веществе центральной нервной системы при энкефало-миэлите цыплят возникает негнойное, лимфоцитарное воспаление. Изменения наиболее выражены на некоторых, так наз. предилекционных местах. Среди этих мест на первом месте стоят слой клеток Пуркине и молекулярный слой мозжечка, дальше, некоторые ядерные скопления мозжечка, продолговатого и среднего мозгов, глубокий слой зрительной доли, неостриатум и гипостриатум мозга и шейное и крестцовое вздутия спинного мозга.

В изменениях наблюдаются алтеративные, продуктивные и воспалительные процессы. Алтеративные изменения характеризуются пикнотическим перерождением ганглионных клеток, центральным тигролизом, вакуольным перерождением или некрозом ганглионных клеток. Для продуктивных процессов характерно разрастание особенно волокнистых астроцитов и отчасти микроглии. Воспалительные изменения представлены в виде клеточной инфильтрации лимфоцитами стенки и окресности мелких кровеносных сосудов. Муфты около кровеносных сосудов или симметрические, или односторонние, одно- или несколькоядерные, часто закупорывают просвет кровеносного сосуда.

На указанных местах эти три формы изменений представлены не в одинаковой степени. В мозжечке превалируют алтеративные и продуктивные процессы, воспалительные очаги имеются только в незначительном количестве. В затронутых ядрах мозжечка, продолговатого и среднего мозгов, дальше, зрительной доли эти три формы изменений имеются в равной степени. В большом мозге доминируют воспалительные процессы. В сером веществе спинного мозга, подобно мозжечку, имеют место главным образом алтеративные и продуктивные процессы. Здесь воспалительные процессы наблюдаются у животных с длительным течением болезни. В продолговатом и среднем мозгах и рогах спинного мозга изменения имеются обычно симметрически, но в полушариях они ассиметричны. В периферических нервах клеточной инфильтрации нет.

ИЗУЧЕНИЕ ЭКСПЕРИМЕНТАЛЬНОГО ЗАРАЖЕНИЯ ВИРУСОМ-ФИКС (ХЕДЕШ) ОБЕЗЬЯН РЕЗУС

Д. КАРАССОН

Автором заражались в мозг обезьяны резус вирусом-фикс бешенства (Хедеш), применяющимся для профилактических и после-инфекционных прививок. Обезьяны после 3—4 дней инкубации стали показывать клинические признаки болезни и к 8. — 11. дням после инфекции погибли. Заболевшие обезьяны показывали все стадии бешенства.

Детально описывается клиническое течение болезни и, резюмируя нейрологические наблюдения на пяти животных, дается синтез клинической картины бешенства обезьян резус, зараженных в мозг вирусом-фикс Хёдеш.

К ПОТРЕБНОСТИ В КИСЛОРОДЕ ООЦИСТ КОКЦИДИЙ ПРИ СПОРУЛЯЦИИ

У. ДЮРР и Л. ПЕЛЛЕРДИ

Ооцисты кишечных и печеночных кокцидий кролика спорулируют только при наличии воздуха. Нет споруляции в атмосфере азота и двуокиси углерода. Потребность в кислороде до полной споруляции 100 000 ооцист Eimeria stiedai колеблется в пределах $30{,}2 \pm 2{,}3$ и $39{,}5 \pm 4{,}0$ μl O_2.

ИЗОЛИРОВАНИЕ ВОЗБУДИТЕЛЯ РИНОПНЕЙМОНИТА ЛОШАДЕЙ И ИЗУЧЕНИЕ СВОЙСТВ ИЗОЛИРОВАННЫХ ШТАММОВ

Й. РОМВАРИ, Б. ТОТ и Л. ВИЗИ

Описывается изолирование вируса ринопнеймонита лошадей в тканевой культуре. Изолированные вирусные штаммы сравнивались в перекрестной-нейтрализационной пробе с референтными штаммами и антисывороткой. Доказано, что изолированные штаммы тождественны с серотипом equi herpes-1. Часть изолированных штаммов по некоторым физико-химическим свойствам отличалась от таковых из группы херпес. Бросается в глаза разница в резистентности к трипсину между штаммами.

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STUDIES ON THE THYMUS OF THE NILE TELEOST FISH, CLARIAS LAZERA C. AND V.

By

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(Received July 6, 1967)

Historical

STANNIUS (1850) was the first to make a survey upon the thymus of fishes. Later on MAURER (1886) studied the histogenesis and growth of the thymus. BEARD (1903) elaborated a theory that the thymus is the original source of lymphocytes in the body of fish. This view has been abandoned, since subsequent authors proved that lymphocytes appear in the embryo before the thymus is differentiated.

HAMAR (1909) published an extensive work on the developmental anatomy of the thymus of different species of teleost fishes. ROMIEU and JULLIEN (1941) and JULLIEN (1942) gave short notes on the relation between the thymus and the lymphoid head kidney in teleost fishes. AL HUSSAINI and RIZKALLA (1957) described the topographical anatomy, histology and age involution of the thymus in the cichlid fish, *Tilapia nilotica*.

Materials and methods

Specimens of *Clarias lazera* ranging from 100—1050 mm in length were killed, the thymus was carefully removed and fixed. Bouin's, 5% formol, 10% formol, Carnoy's and Maximow-Zenker's formol fluids were used as fixative. Serial sections of 6 μ thickness were prepared after the material had been dehydrated, cleared and embedded in the usual way. The sections were stained with Delafield's, Heidenhain's, Harris' haematoxylin and counterstained with eosin or orange G. For verifying the connective tissue fibres the sections were stained with Verhoeff's haematoxylin- van Gieson and Mallory's tripple stain. The test for mucin was carried out by staining with Mayer's mucicarmine, 0.2% aqueous toluidine blue and aqueous solutions of toluidine blue 1: 25 000 or 1: 28 000 (HEMPLEMANN, 1940).

The topography of the thymus and the relation between it and the lymphoid head kidney were studied in specimens ranging from 9—47 mm in length. The specimens were fixed in Bouin's fluid, dehydrated and cleared as usual. Paraffin embedding took place *in vacuo*. Serial transverse, frontal and longitudinal sections of 6—7 μ thickness were prepared and stained with Delafield's haematoxylin-eosin.

Observations

I. Topographical anatomy

Like in most other vertebrates, in *Clarias lazera* the thymus is a paired symmetrical structure. According to the dorso-ventral flattening and expansion of the head region of the fish, the branchial cavity* is very much extended. Inside the skull, on each side, and behind the true gills, an air-chamber is

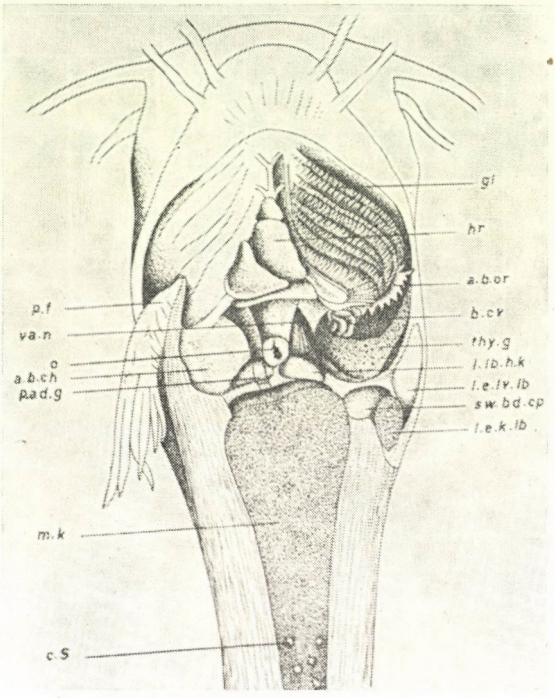

Fig. 1. Ventral view of a dissected *Clarias lazera* 50 mm long, showing the position of the thymus in relation to the neighbouring organs. ×5. Symbols: a.b.ch, air-breathing chamber; a.b.or, air-breathing organ; b.cp, blood capillary; b.cv, branchial cavity; b.v, blood vessel; c.dé, cell débris; c.S, corpuscle of Stannius; c.t, connective tissue; c.t.c, connective tissue cell; c.t.cp, connective tissue capsule; c.t.f, connective tissue fibres; ct, cortex; cy.s, cystic space; dg.a, degenerating area; gl, gill; hr, heart; in. mu, intracellular mucus; l.e.k.lb, left embedded kidney lobe; l.e.lv.lb, left lobe of head kidney; lym, lymphocytes; m.k, median kidney; mc, macrophage; md, medulla; mi.d, mitotic division; mu.cy, mucous cyst; my.c, myoid cell; o,oesophagus; p.ad.g, position of adrenal gland; p.f, pectoral fin; p.t, pronephric tubule; ph.rt.c, phagocytic reticular cell; pig.c, pigment cell; py.a, pyknotic area; py.thm, pyknotic thymocyte; r.b.c, red blood corpuscle; r.lb.h.k, right lobe of head kidney; rt. c, reticular cell; st.m, striated muscle; sw. bd, swim bladder; sw.bd.cp, swim bladder capsule; thb, thymoblast; thm, thymocyte; thy.g, thymus gland; v.zn, ventral zone; va.g, vagus ganglion; va. n, vagus nerve; vc, vacuole

* For convenience of descriptive purposes the "branchial cavity" is used here to designate the whole cavity containing both true gills and accessory air-breathing arborescent organs, the former being located in the "gill-chamber" and the latter in the "air-chamber".

located in which two highly vascular accessory air-breathing arborescent organs are found. The thymus is somewhat shifted backwards from its common position, which is directly behind the gills, and comes to lie behind the well-developed accessory air-breathing arborescent organs (Fig. 1). In our best knowledge, such a peculiar position of the thymus has not been described in teleost fishes.

On each side, the thymus is located in the posterior concavity of the air-chamber, and acquires its general outline from the position it occupies in relation to the adjacent organs. In general outline, the thymus resembles a retort. Its body, a reddish sheet-like structure which fits into the posterior concavity of the air-chamber, has an anterior concave surface and a posterior convex one. The posterior surface of the body of the thymus lies just in front of the capsule of the air-bladder. The limb of the retort extends forwards and lateralwards along the outer side of the air-chamber, up to the gill-chamber (Fig. 1).

II. Histological structure

The thymus appears to be mainly formed of deeply-staining cells (Fig. 2), which are referred to as thymocytes. Both collagenous and fine reticular connective tissue fibres are scattered all over its substance. The whole thymus is enveloped by a thin connective tissue capsule, in which thymocytes are occasionally seen embedded. Most probably, such cells emigrate to the capsule from the main mass of the thymus substance.

The thymus in 9 mm long specimens is in close connection with the richly vascular epithelial wall enclosing the accessory air-breathing arborescent organs. From this epithelial wall numerous blood capillaries penetrate the connective tissue capsule of the thymus, invading its inner substance. The thymus in such a small specimen appears as a lymphoid organ without any regional differentiation (Fig. 2). However, in specimens of 12—15 mm length an evidence of differentiation can be observed, as the thymus shows a ventral deeply-staining zone (cortex) and a dorsal lightly-staining zone (medulla) (Figs 3 and 4). In a 17 mm long specimen an additional third zone (ventral zone) can hardly be differentiated (Fig. 5), but in larger specimens all three zones can be easily detected (Fig. 6). The zonal differentiation of the thymus persists till the fish reaches about 150 mm in length, then disappears, due to the involutionary processes in the thymus.

In a well-differentiated thymus, there is a ventral zone lying close to the epithelial wall enclosing the accessory air-breathing arborescent organs. This zone is a relatively narrow strand of cells, mostly composed of lightly-staining thymoblasts which presumably will give rise to the thymocytes. The middle zone has a deeply-staining appearance as it is mostly composed of closely

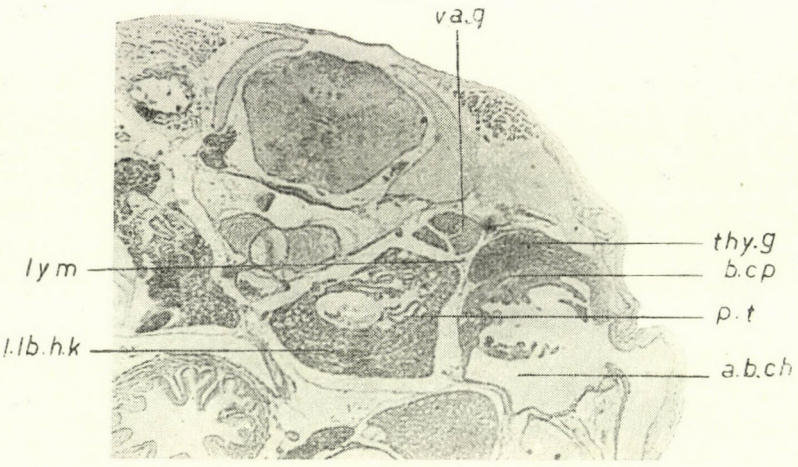

Fig. 2. Transversal section of *Clarias lazera* 9 mm long, showing the thymus and its connection with the head kidney through a trial of lymphocytes. ×80. The symbols see on page 332

Fig. 3. Transversal section of *Clarias lazera* 12 mm long, showing the cortical and medullary zones of the thymus. ×75. The symbols see on page 332

packed thymocytes, thus obscuring the reticulum in which they are held. This middle zone is similar to the cortical region of the thymus of higher vertebrates. Finally, the third, most dorsal zone, appears light as in this region the deeply-staining thymocytes are less in number, while the reticular cells forming the reticulum are more conspicuous. This third zone is analogous to the medullary region of the thymus of higher vertebrates. It is worthy of mention that there is no sharp demarcation between the three thymic zones (Figs 5 and 6).

During the vigorous growth of the thymus in small specimens, emigration of thymocytes was noticed. The emigrating cells are present either in a small number or in considerable numbers in the connective tissue lying dorsal to the thymus (Figs 2—4). This early emigration of cells from the thymus substance could be considered either as a normal continuous process or as a fraying out of the organ in the course of its histogenesis.

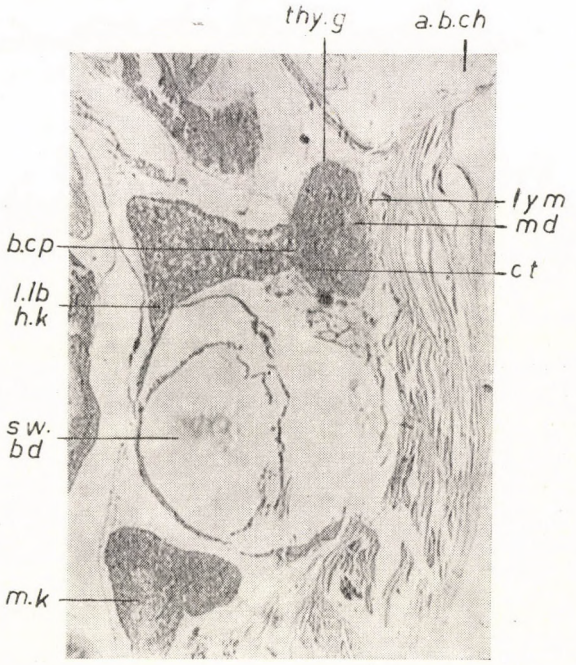

Fig. 4. Longitudinal section of *Clarias lazera* 15 mm long, showing the fusion of the thymus with the homo-lateral lobe of head kidney. ×70. The symbols see on page 332

The thymocytes are small, rounded, deeply-staining cells, quite similar to the lymphocytes. Their nucleus is large and surrounded by a thin sheet of non-granular cytoplasm. The chromatin granules are abundant and scattered all over the nucleoplasm, thus giving a dark tint to the whole nucleus (Fig. 7).

Thymoblasts are present throughout the thymus substance, most abundantly in the ventral zone. These cells are larger and lightly-staining as compared with the thymocates (Fig. 5). The vigorous growth of the thymus in the early stages is presumably interpreted by the mitotic activity of these thymoblasts, giving rise to the smaller thymocytes characteristic of the thymus.

Among the thymocytes there are lightly-staining reticular cells and large cells of irregular forms. The outline of these cells is somewhat indistinct and

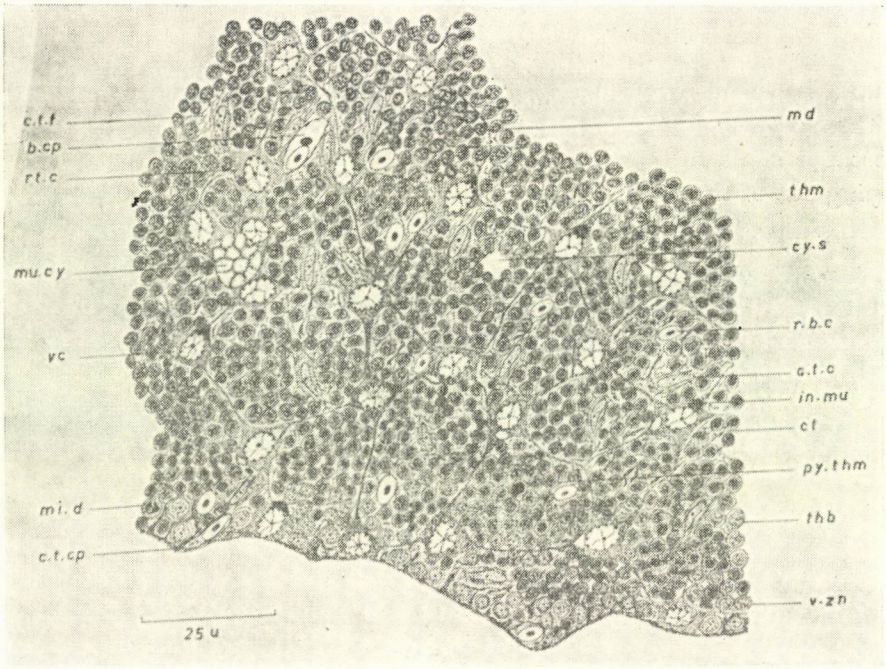

Fig. 5. Drawing of a section of the thymus of *Clarias lazera* 17 mm long, showing its zone differentiation and its structure. The symbols see on page 332

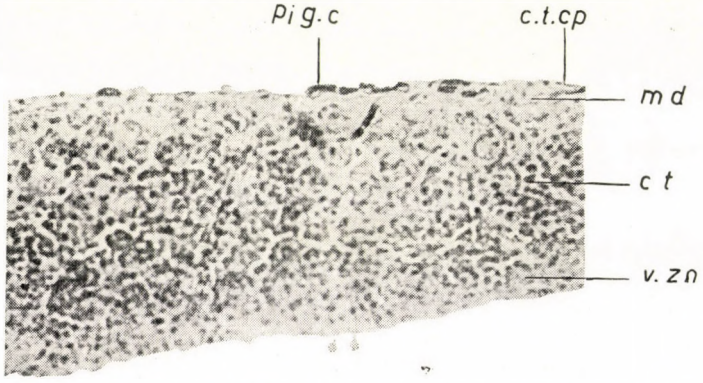

Fig. 6. Section of the thymus of *Clarias lazera* 42 mm long, showing the three zones of the thymus. ×700. The symbols see on page 332

its cytoplasm is drawn out into irregular prolongations which anastomose with similar processes of neighbouring cells. Accordingly, a reticular syncytial system is formed with thymocytes in the meshes (Figs 5 and 7).

The reticular cells are usually larger and more abundant in the medullary zone than elsewhere in the thymus. On the contrary, in the cortical zone it is

somewhat difficult to follow the reticular cells and their protoplasmic processes as the surrounding thymocytes are closely packed around them. The finely granular cytoplasm of the reticular cell stains lightly with eosin, and occasionally contains vacuoles. The nucleus is large, about three times as large as the thymocyte nucleus. The chromatin granules are few and are mostly confined to the nuclear membrane (Figs 5 and 7).

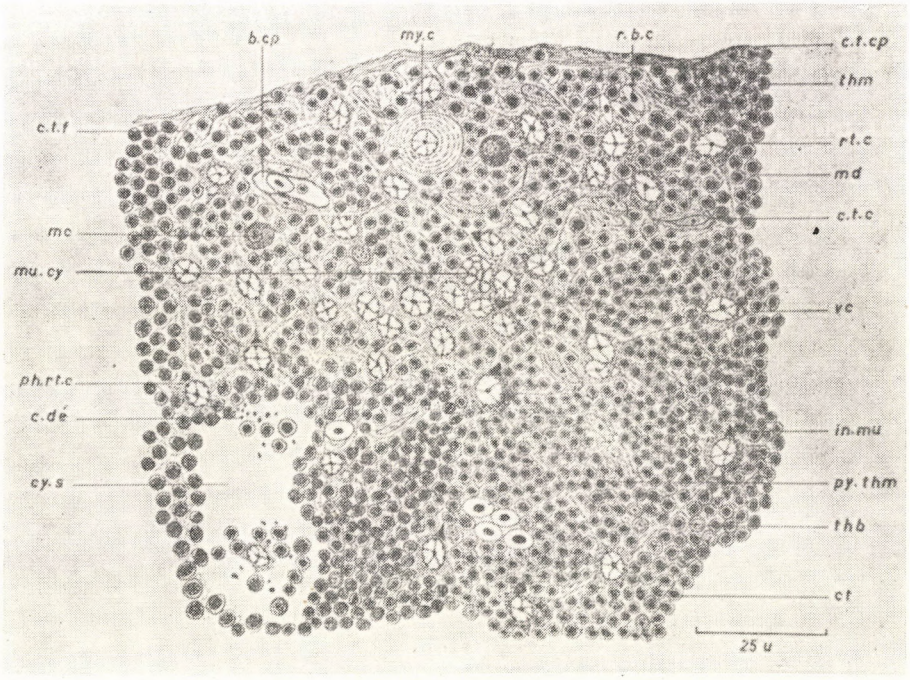

Fig. 7. Drawing of a section of the thymus of *Clarias lazera* 100 mm long, showing the structure of the thymus. The symbols see on page 332

Some of the reticular cells seem to be phagocytic, with cytoplasmic processes tending to retract. Most probably, the phagocytic reticular cells feed on thymocytes as their cytoplasm contains foreign nuclei similar in staining properties to those of the thymocytes (Fig. 7).

Some of the reticular cells are further differentiated as they grow in size and become oval or rounded in shape. The cytoplasm displays concentrically arranged fibrillae which remind of those of the muscle fibres, thus these cells are referred to as myoid cells. Such cells, few in number, are found all over the thymus substance, the superficial layer of the medullary zone being most abundant (Fig. 7).

Macrophages are of frequent occurrence in the medullary and ventral zones of the thymus, just underneath the connective tissue capsule. These

cells are rounded and their cytoplasm shows numerous, very small vacuoles. The nucleus is mostly eccentric in position, and the great number of chromatin granules giving it a darkly-staining appearance (Fig. 7).

Cystic spaces of various shapes are scattered throughout the whole thymus substance, regardless the age of the fish (Figs 5, 7 and 8). Parallel with the age of the fish these spaces increase in number and size and become more irregular in outline. In the thymus of small specimens the cystic spaces are spherical, very small, and their lumina are empty (Fig. 5). On the other hand, in the thymus of large specimens the lumina of the cystic spaces contain degenerated nuclei of both thymocytes and reticular cells, *i.e.*, cell débris or granular detritus (Fig. 7).

Mucous cysts, mostly lined with reticular cells are found in the thymus, irrespective of the age of the fish. The contained mucus stains bright red and appears frothy with Mayer's mucicarmine. In addition, large or small amounts of mucus occur in the cytoplasm of some reticular cells (Figs 5, 7 and 8).

III. Age involution

Actual involution appears in the thymus of specimens of about 150 mm long, and these involutionary changes become more pronounced with the increase of age. When actual involution takes place the zonal differentiation of the thymus becomes indistinct and eventually disappears. Moreover, the surrounding collagenous connective tissue capsule shows a remarkable increase in thickness and becomes hyaline in appearance (Figs 8—10). Also, the connective tissue fibres in the thymus substance increase in amount, particularly in the subcapsular region.

The thymocytes shrink and appear as very deeply-staining cells, and such pyknotic thymocytes may be considered to have reached the adult condition or the last stage of their life span. Later on, the thymus substance becomes loose, as the pyknotic areas of thymocytes show disintegration and degeneration. Accordingly, there is an increase in the number of the irregularly-shaped cystic spaces. Some cystic spaces contain cellular débris, while others appear clear, indicating that the granular detritus found in their lumina has been completely absorbed (Fig. 8).

Beside the process of necrobiosis in the thymus substance, the mitotic activity of the thymus cells seems to cease completely. Moreover, emigrated thymocytes are seen in the neighbouring connective tissue (Fig. 9); most probably the emigrating cells have a negligible effect on the involution of the thymus as they occur in moderate numbers.

As the involution proceeds, the overall reduction in the size of the thymus becomes clear. Moreover, a vigorous infiltration of connective tissue separates the thymus from the adjacent muscles (Figs 9 and 10). In specimens about

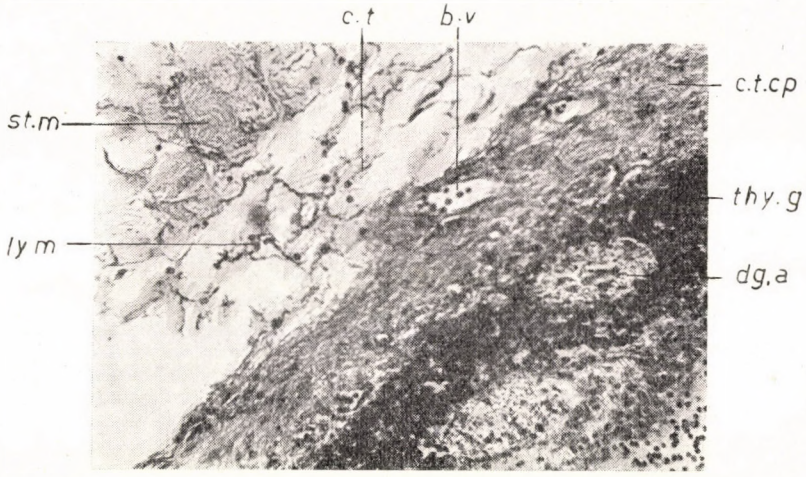

Fig. 8. Section of the thymus of *Clarias lazera* 200 mm long, showing degenerated areas of thymocytes, cystic spaces and mucous cysts. ×150. The symbols see on page 332

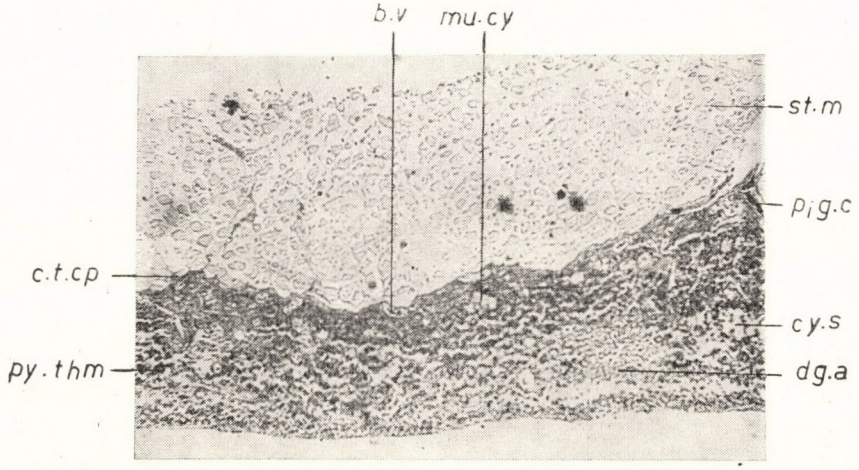

Fig. 9. Section of the thymus of *Clarias lazera* 400 mm long. Reduction in size of the thymus, degenerated areas and a very thick connective tissue capsule. ×335. The symbols see on page 332

650 mm in length, the thymus is very reduced, it consists of a narrow strand of cells, and the connective tissue capsule becomes separated from the substance of the thymus (Fig. 10). Judging from the activity of the involutionary process reducing the thymus to a microscopical vestige, one must expect the complete disappearance of the thymus in larger specimens. This is true as in specimens of about 1050 mm long the thymus is totally absent.

IV. The relation between the thymus and head kidney

During the vigorous growth of the thymus, in small specimens of *Clarias lazera*, due to the multiplication of its cells and consequently its zonal differentiation, another growth of a similar nature takes place in its neighbourhood. It is the growth of the lymphoid head kidney or the pronephros.

In small specimens of *Clarias* a relation exists between the thymus and the lobe of the head kidney of the corresponding side. This is manifested by a direct continuity between these organs through an infiltration of cellular ele-

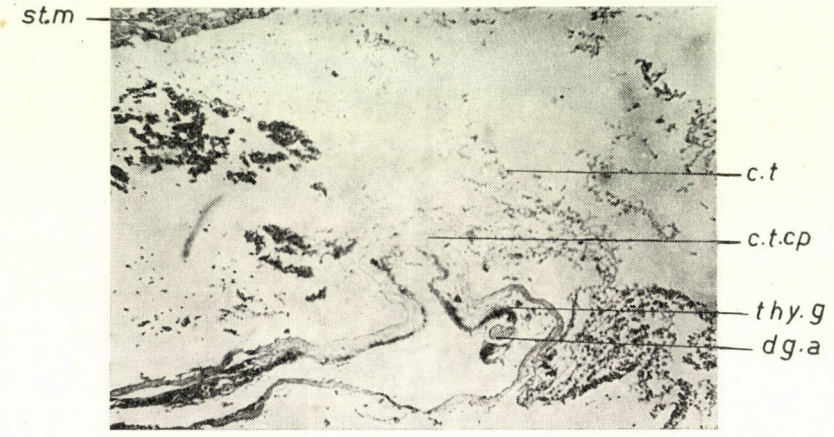

Fig. 10. Section of the thymus of *Clarias lazera* 650 mm long, showing its reduction to a vestige, degenerated areas and a very thick connective tissue capsule. ×80. The symbols see on page 332

ments as well as connective and vascular tissues which appear to be withdrawn from one organ to the other. Embedded in this connective tissue numerous lymphocytes are disposed in tracts or strands uniting the two organs (Fig. 2). In some instances, however, the head kidney and the thymus are completely fused so that at the point of fusion one cannot distinguish the cells belonging to either organ. This is because no morphological difference could be noticed between the thymocytes of the thymus and the lymphocytes of the head kidney. Numerous blood capillaries extend between the two organs, making the fusion more elaborate (Fig. 4). The head kidney, however, is characterized by its degenerating pronephric tubules and its rich vascularization comparable to that of the thymus (Figs 2—4), while the thymus is characterized by its cystic spaces and mucous cysts.

Discussion

The thymus of Clarias is shifted far posteriorly from the position the thymus usually occupies in teleosts, namely just posterior to the gills, inasmuch as that the removal of the operculum reveals the organ clearly (SALKIND, 1915). However, in some teleosts the thymus is partly concealed by the dorsal musculature in the branchial region (DEANESLY, 1927; HAFTER, 1952 and AL-HUSSAINI and RIZKALLA, 1957). The removal of the operculum in Clarias does not expose the thymus; in order to find it the pectoral arch as well as the accessory air-breathing arborescent organs should be removed. Accordingly, SALKIND's generalization (1915) is not justifiable as the thymus of teleosts shows different conditions, varying from completely external to entirely hidden; intermediate cases have also been reported.

In a well-differentiated thymus of Clarias, three distinct zones can be illustrated: a ventral zone, a middle cortex and a dorsal medulla. This is similar to that reported by HAMMAR (1909) and HAFTER (1952) in the thymus of some teleosts. In most teleost fishes, however, two zones (cortex and medulla) can only be distinguished in the thymus (cf. LEIE, 1933; von HAGEN, 1936; ROMIEU and JULLIEN, 1941 and AL-HUSSAINI and RIZKALLA, 1957).

It is worthy of mention that the mammalian thymus is characterized by concentrically hyalinized Hassall's corpuscles, arranged about cores of nuclear débris. HAMMAR (1909) stated that in the thymus of teleosts structures comparable to true Hassall's corpuscles occur but rarely. Moreover, Hassall's corpuscles are frequently seen in the thymus of mammals, less often in those of birds and still more rarely in the thymus of fishes (BLIN, 1951). Generally, hypertrophied reticular cells, concentric complexes of reticular and myoid or mucous cells and multicellular mucous glands have been described in fishes to bear similarity to Hassall's corpuscles (LEIE, 1933; LAGABRIELLE, 1936; von HAGEN, 1936 and HAFTER, 1952). It seems, however, that in the thymus of Clarias the large myoid cells with their concentrically arranged fibrillae are the nearest structures to Hassall's corpuscles.

There is a wide variation, existing in the different species of teleosts, concerning the time at which actual involution of the thymus begins. It is obvious that the reduction of the thymus of Clarias does not coincide with sexual maturity, as actual involution starts in the thymus of specimens about 150 mm long, which are still sexually immature. SALKIND (1915), DEANESLY (1927) and von HAGEN (1936) came to the same results for some teleost fishes. On the contrary, according to HAMMAR (1909), LEIE (1933 and 1934), HAFTER (1952) and AL-HUSSAINI and RIZKALLA (1957) the thymus involution is correlated with sexual maturity.

The involutionary process in the thymus of Clarias is achieved mainly by an overall reduction in its size. This is accomplished by an increase in the

number of pyknotic thymocytes and in turn of degenerating areas due the disintegration of such pyknotic cells. LEIE (1933), HAFTER (1952) and AL-HUSSAINI and RIZKALLA (1957) arrived at findings similar to those recorded in this investigation. On the other hand, MAURER (1886), HAMMAR (1909), DEANESLY (1927) and HILL (1935) concluded that the thymus involution is largely effected by the emigration of thymocytes rather than the degeneration of such cells within the thymus substance.

A complete absence of the thymus in aged fishes, as has been recorded for Clarias, has already been reported for some teleosts by MAURER (1886), DEANESLY (1927) and LEIE (1934). Accordingly, HAMMAR's conclusion (1909) that the thymus never disappears in aged fishes has no justification to be generalized. On the other hand, either microscopic vestiges or reduced thymus, which could be seen with the naked eye, are observed throughout the whole life of some teleost fishes (DEANESLY, 1927; LEIE, 1933; HAFTER, 1952 and AL-HUSSAINI and RIZKALLA, 1957).

SUMMARY

1. The thymus of Clarias has been shifted backwards for some distance from its common position, which is immediately behind the gills, and comes to lie behind the accessory air-breathing arborescent organs.

2. In small specimens, 9 mm long, the thymus does not show any regional differentiation. An evidence of its differentiation into cortical and medullary zones is first observed in specimens 12—15 mm long. An additional third zone (ventral zone) is differentiated in specimens 17 up to 150 mm long. The thymus in larger specimens undergoes involutionary changes, which proceed until the thymus entirely disappears.

3. The bulk of the thymus substance consists mainly of deeply-staining thymocytes, among which there are reticular cells forming a reticulum. Some reticular cells seem to be phagocytic, while others are further differentiated into myoid cells.

4. The thymus is characterized by the presence of cystic spaces which increase in number and size as the fish increases in age. These spaces indicate degenerating areas of the thymus substance. Moreover, cysts of different size, filled with mucus, are found in the thymus, regardless the age of the fish.

5. During the histogenesis of the thymus, in early stages, there is a connection between the thymus and the lymphoid head kidney through trails of lymphocytes. Moreover, a complete fusion of the two organs is also encountered.

Acknowledgement. The author wishes to express his deep gratitude to late Prof. A. H. AL-HUSSAINI, Head of Zoology Department, Faculty of Science, Ain Shams University, Cairo, for suggesting the subject and under whose supervision the work was carried out. Also, the author is greatly indebted to Prof. A. KEMÉNY, Head of Physiology Department, University of Veterinary Science, Budapest and to Dr. G. PETHES, Head of Radioisotope Laboratory, of the same Department for their valuable advice, continuous encouragement and criticism.

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STUDIES ON THE ADRENAL GLANDS AND CORPUSCLES OF STANNIUS OF THE TELEOST NILE FISH, CLARIAS LAZERA C. AND V.

By

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Historical

Small encapsulated grandular bodies, partly embedded in the posterior portions of the kidneys, known as corpuscles of Stannius, were described in some fishes by STANNIUS (1839), who suggested on a morphological basis, that these bodies may represent the adrenal gland of fishes. COLLINGE (1897) and VINCENT (1897, 1898a and 1898b) came to the conclusion that the corpuscles of Stannius represent the adrenal cortex. These authors claimed that the medul lary portion of the adrenal gland is absent in teleosts.

GIACOMINI (1902, 1908a, 1908b and 1910) was the first to discover the chromaffin tissue or adrenal medulla in teleosts, which is represented by groups of cells associated with the adrenal cortical tissue. Both tissues are found in the lymphoid head kidney. GIACOMINI referred to the adrenal cortex as the "anterior interrenal tissue", while he referred to the corpuscles of Stannius as the "posterior interrenal organs" due to the homology of the latter organs to the adrenal cortical tissue.

Later on GIACOMINI (1911a, 1911b and 1911c), RAMALKO (1921 and 1923), VINCENT and CURTIS (1927a and 1927b), ABOIM (1946) and RASQUIN (1951) came to the conclusion that the corpuscles of Stannius bear no relationship to the adrenal cortical tissue, but the true homologue of this tissue is the "anterior interrenal tissue". On the contrary, DIAMARE (1933) and CALLAMAND (1943) claimed that the corpuscles of Stannius have a cortical function and the adrenal cortex of teleosts is represented exclusively by these bodies.

Material and methods

Adult specimens of *Clarias lazera* were killed, and the right lobes of the head kidneys with parts of the posterior cardinal veins were removed quickly and carefully. Also, the corpuscles of Stannius with parts of the surrounding renal tissue were dissected. The materials were fixed in Zenker's formol fluid, dehydrated, cleared and embedded as usual. Serial sections of 6 μ thickness were prepared and stained with Delafield's haematoxylin-eosin.

For demonstrating mitochondria and fat droplets the material was fixed in Regaud's fluid and postchromated with 4% potassium dichromate for a week at 37° C. Serial paraffin sections of 4—5 μ thickness were prepared, and stained with either Heidenhain's iron haematoxylin (mitochondria) or Sudan Black B (fat).

For demonstrating ascorbic acid granules, the material was fixed in acid silver nitrate solution. Serial paraffin sections of 5 μ thickness were prepared and stained with Delafield's haematoxylin-eosin.

Observations

I. Adrenal gland

In specimens preserved in 10% formalin the adrenal tissue could be observed by naked eye. It forms a ring and completely surrounds the anterior part of the posterior cardinal vein in the region where it passes through the right lobe of the head kidney. The adrenal tissue, being formed of a relatively compact tissue faint grey in colour, can be differentiated from the surrounding tissue of the head kidney which is dark brownish in colour. Microscopically, the adrenal gland is composed of the interrenal tissue,* forming the major part of the gland, to which scattered groups of chromaffin tissue (adrenal medulla) are associated (Figs 1 and 2).

A. Interrenal tissue

This glandular tissue is formed of epithelial cords, each being one-cell thick or more, separated by very small blood capillaries (Fig. 2). A network of these capillaries opens in the posterior cardinal vein. The interrenal tissue lies in close contact with the head kidney (Fig. 1). The latter is readily recognized as it is made up of a delicate reticulum, enclosing closely packed lymphocytes in its meshes. Neither the kidney tubules nor the Malpighian corpuscles are represented in the head kidney, which is characterized by its abundant vascularization (Fig. 1).

The cells of the interrenal tissue possess distinct cell outlines, mostly polygonal or rounded, less frequently elongated with their long axes perpendicular to the blood capillaries. The cytoplasm is uniformly finely granular, and the nuclei are central in position. The chromatin granules are abundant and scattered evenly throughout the nucleoplasm (Fig. 2). The mitochondria appear as very minute granules scattered uniformly all over the cytoplasm.

Among the cells of the interrenal tissue some are free of fat droplets, others contain little fat, and still others, mainly those in the close proximity of the posterior cardinal vein show a homogeneous black colour, indicating that great amounts of fat droplets are uniformly distributed in their cytoplasm (Fig. 3).

* The cortical tissue of the adrenal gland is referred to as the interrenal tissue instead of the "anterior interrenal tissue" of GIACOMINI. The first designation dismisses any idea about the existence of a posterior organ.

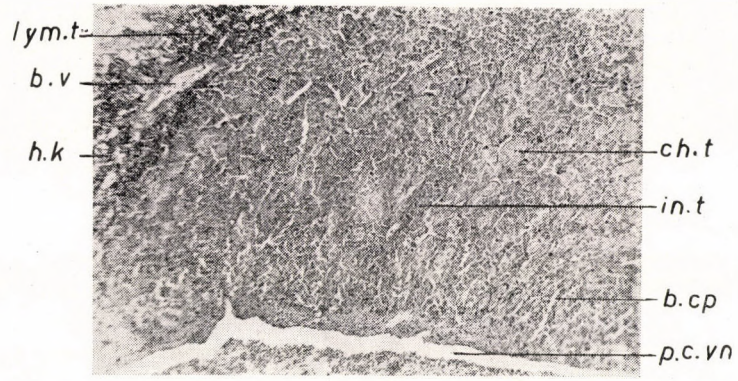

Fig. 1. A section of the adrenal gland of *Clarias lazera*. Interrenal and chromaffin tissues, the posterior cardinal vein and the lymphoid tissue of the head kidney. ×110. Symbols: b.cp, blood capillary; b.v, blood vessel; c.t, connective tissue; c.t.cp, connective tissue capsule; ch.t, chromaffin tissue; f. dr, fat droplets; h.k, head kidney; in. t, interrenal tissue; lb lobule; lym. t, lymphoid tissue; p.c.vn, posterior cardinal vein

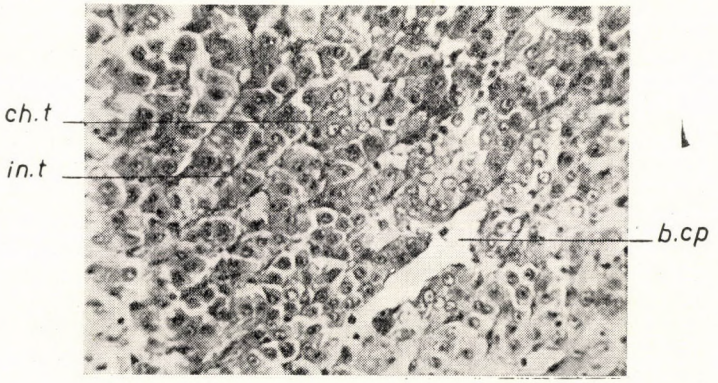

Fig. 2. A section of the adrenal gland of *Clarias lazera*. Interrenal and chromaffin tissues. ×550. For the symbols see Fig. 1

Fig. 3. A section of the adrenal gland of *Clarias lazera*. Interrenal tissue cells with variable fat content. ×320. For the symbols see Fig. 1

The ascorbic acid reaction was positive in groups of cells scattered all over the interrenal tissue. In addition, many black granules indicating ascorbic acid were seen inside the lumina of the blood vessels as well as in their connective tissue adventitia.

B. Chromaffin tissue

This glandular tissue is represented by groups of cells which are scattered all over the interrenal tissue. The chromaffin cells stain faintly as their cytoplasms have a weak affinity for acid dyes (Figs 1 and 2). The cell outlines are mostly indistinct and so the chromaffin cell groups appear like syncytial areas. The cytoplasm is finely granular and are mostly confined to the nuclear membrane. The nuclei of the chromaffin cells are slightly larger than those of the interrenal tissue cells (Fig. 2).

II. Corpuscles of Stannius

The corpuscles of Stannius occur fairly constantly in the posterior third of the median kidney. They are partially embedded in the substance of the ventral surface of the kidney and do not project appreciably from this surface. There are 3 to 7 corpuscles of Stannius in an adult specimen. They are mostly rounded, whitish, fatty-looking, but of hard texture, ranging from 0.5 to 1.5 mm in diameter. The surface of some corpuscles is marbled with very fine blood capillaries.

Each corpuscle of Stannius is formed of epithelial lobules which are separated from one another by richly vascular connective tissue septa (Figs 4 and 5). The latter appear as trabeculae of variable thickness issuing from the connective tissue capsule that surrounds the corpuscle. A considerable number of pigment cells are occasionally found in both the connective tissue capsule and the interlobular connective tissue septa. In the majority of instances there is a local increase in the amount of the renal lymphoid tissue in close contact with the connective tissue capsule of the corpuscle (Fig. 5).

The lobules of the corpuscle of Stannius are formed of closely-packed cells which surround numerous blood capillaries (Fig. 5). The cells, mostly indistinct in outline, appear to be more or less rounded or polygonal in shape. All the cells show cytoplasmic granulation variable from one cell to another, most probably depending on the secretory phase of the cell. The nuclei are prominent and intensely-stained, showing abundant chromatin granules scattered all over the nucleoplasm. Some nuclei appear as deeply-staining spheres which may indicate pyknotic changes.

Some corpuscles of Stannius have the appearance of closely-packed acinar-like lobules which are separated by very thin connective tissue septa (Fig.

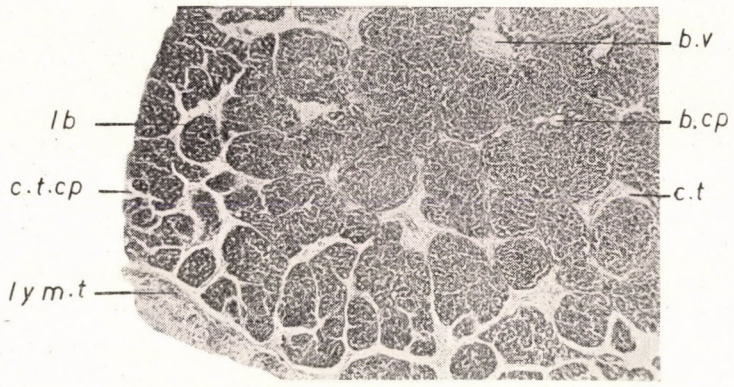

Fig. 4. A section of a corpuscle of Stannius of *Clarias lazera*. Numerous lobules bounded by a richly vascular connective tissue. ×100. The symbols see on page 345

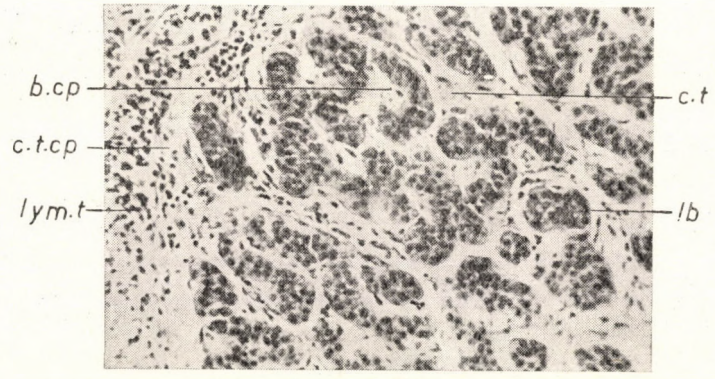

Fig. 5. A section of a corpuscle of Stannius of *Clarias lazera*. Lobules of compact epithelial cells and numerous blood capillaries. ×500. The symbols see on page 345

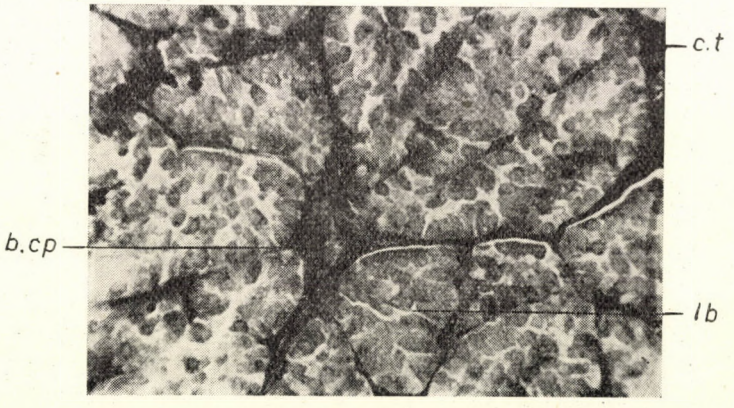

Fig. 6. A section of a corpuscle of Stannius of *Clarias lazera*. Acinar-like arranged cells. ×580. The symbols see on page 345

6). The acini are mostly filled up with cells and strikingly suggestive of secretory glands. The peripheral cells of the acini are elongated and their nuclei are mostly apical in position. On the other hand, the central cells are rounded or polygonal with centrally located nuclei. Occasionally, in the centres of such acini there are many degenerating cells, fragmented nuclei and shreds of protoplasm. Most probably, the central cells undergo rapid postmortem degenerative changes.

Mitochondria appear as numerous cytoplasmic granules, mostly accumulating around the nucleus. The mitochondrial granules in the cells of the corpuscles of Stannius are more conspicuous than those of the interrenal tissue cells. Fat could not be detected in the cells of the corpuscles of Stannius.

Discussion

I. Adrenal gland

The adrenal gland of higher vertebrates is formed of two closely associated components, an outer adrenal cortex and an inner adrenal medulla. In fishes, however, the two components are usually separated, the adrenal cortex being represented by the interrenal tissue, while the adrenal medulla by the suprarenal bodies or chromaffin tissue.

Several authors have come to the conclusion that the "anterior interrenal tissue" in teleost fishes is composed of epithelial cells which, however, do not form a large compact organ or an individualized structure. Thus, the "anterior interrenal tissue" may be arranged in a single or double layer of cells, closely associated with the cardinal veins or their branches inside the head kidney (RAMALKO, 1923 and RASQUIN, 1951). Sometimes, the "anterior interrenal tissue" is represented by encapsulated solid or hollow masses of cells widely scattered throughout the substance of the head kidney (GIACOMINI, 1910; COMOLLI, 1913; VINCENT and CURTIS, 1927a; BAECKER, 1928; ABOIM, 1946 and BOBIN, 1949), whereas the chromaffin tissue either lies between the "anterior interrenal tissue" and the walls of the cardinal veins, or is represented by scattered cells within the individual groups forming the "anterior interrenal tissue". In contrast to all other teleost fishes examined, in *Clarias* the two components of the adrenal gland are closely associated with each other, representing a large and more individualized organ, resembling the adrenal gland of higher vertebrates.

II. Corpuscles of Stannius

GARRETT (1942) suggested that in teleost fishes, in general, the corpuscles of Stannius occur as a single pair. However, *Clarias* has of the corpuscles more than two, ranging from 3 to 7 in number in the specimens dissected.

Histologically, the corpuscles of Stannius in *Clarias* appear to be variable as to their interlobular connective tissue, vascular elements and the arrangement of the epithelial cells in them. Thus, the corpuscles are formed of either richly vascular epithelial masses or of closely-packed small acinar-like lobules. PETTIT (1910), RAMALKO (1921), VINCENT and CURTIS (1927a), GARRETT (1942) and BOBIN (1949) recorded only the acinar arrangement of the cells in the corpuscles of Stannius of some teleost fishes, while ABOIM (1946) stated that the corpuscles in teleosts are formed of epithelial strands, separated by richly vascular connective tissue septa. RASQUIN (1951) described both epithelial cords and acini in the corpuscles.

In fact, the corpuscles of Stannius are the least understood organs of teleost fishes. However, there is a widespread tendency to homologize these organs, in structure and function, with the adrenal cortex of vertebrates. DIAMARE (1933) and BOBIN (1949) demonstrated the presence of lipid droplets in the corpuscles of Stannius of some teleost fishes, similar to what have been recorded for the cortical tissue of higher vertebrates. Moreover, CALLAMAND (1943) recorded that the extracts of the corpuscles of Stannius indicate the presence of a cortical hormone.

The microscopic structure (arrangement of cells and their cytoplasmic inclusions) of the corpuscles of Stannius in *Clarias* is not in harmony with what is almost universally recognized as typical for a cortical or interrenal tissue. Accordingly, one can conclude that the corpuscles of Stannius represent highly organized epithelial organs which are sharply marked off from the renal tissue in which they are embedded. As these corpuscles are richly vascularized, they may be regarded as an endocrine organ the function of which is still obscure. On the other hand, the interrenal tissue of *Clarias* proved to be analogous to the adrenal cortex of other vertebrates.

SUMMARY

1. In *Clarias lazera* the adrenal gland is represented by a highly organized compact tissue, surrounding the anterior part of the posterior cardinal vein, where it penetrates the right lobe of the head kidney.

2. The adrenal gland is composed of interrenal tissue, forming the major part of the gland, to which scattered groups of chromaffin tissue are associated.

3. The interrenal tissue is formed of cellular bands of glandular cells, separated by blood capillaries. The cells contain ascorbic acid and variable amounts of fat.

4. The scattered groups of chromaffin tissue show weak affinity for acid dyes. The chromaffin cells are mostly rounded, and larger than the interrenal cells; their contours are ill-defined.

5. The corpuscles of Stannius are represented by 3 to 7 small, rounded bodies, scattered on, and partially embedded in, the ventral surfaces of the posterior third of the median kidney.

6. The corpuscles, each being enveloped with a connective tissue capsule, are composed of epithelial lobules, which latter are separated by richly vascular connective tissue septa.

7. The corpuscles of Stannius are highly organized epithelial organs which are sharply marked off from the renal tissue in which they are embedded. They may represent an endocrine organ whose role is still obscure.

Acknowledgement. The author wishes to express his deep gratitude to late Prof. A. H. AL-HUSSAINI, Head of Zoology Department, Faculty of Science, Ain Shams University, Cairo, for suggesting the subject and under whose supervision the work was carried out. The author is also greatly indebted to Prof. A. KEMÉNY, Head of Physiology Department, University of Veterinary Science, Budapest, and to Dr. G. PETHES, Head of Radioisotope Laboratory of the same Department, for their continuous encouragement, and criticism.

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SOME OBSERVATIONS ON THE HISTOCHEMICAL FEATURES OF DEVELOPING FOLLICLE AND CORPUS LUTEUM IN THE CAT AND DOG OVARY

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(Received April 2, 1968)

Introduction

After ovulation, the cells of membrana granulosa show hypertrophy, chiefly due to increase in cytoplasm, and become luteal cells which secrete progesterone.

This is the usual event in all mammals (BRAMBELL, 1956; ECKSTEIN, 1962; AMOROSO and FINN, 1962). Our knowledge is meagre about the nature of complex cytoplasmic processes which occur during the transformation (or luteinization) of granulosa cells into luteal cells. However, recent electron-microscopic studies have revealed the development of abundant smooth-surfaced (or agranular) endoplasmic reticulum coincident with the luteinization (YAMADA and ISHIKAWA, 1960; ENDERS, 1962; GREEN and MAQUEO, 1965; BLANCHETTE, 1966a, b). The detailed histochemical nature of lipid inclusion droplets which are formed in the luteal cells (YAMADA and ISHIKAWA, 1960; ENDERS, 1962; GREEN and MAQUEO, 1965; BLANCHETTE, 1966a, b) is not known. In this paper the histochemical features of the developing follicle and corpus luteum in the ovaries of cat and dog will be described. The present study forms a part of histochemical studies on the granulosa and theca interna during follicular development, ovulation, and corpus luteum formation and regression in the ovaries of the American opossum, rabbit and man (GURAYA, 1968).

Materials and methods

Ovaries of sexually mature domestic cats and dogs were used. Follicles were studied in estrous and pregnant cats and pseudopregnant dogs. Corpora lutea were studied in 10 pseudopregnant dogs and 7 pregnant cats. The uterus in dogs showed a progestational endometrium. The cats were either in early stages (three), or in later stages (four), of pregnancy as determined approximately by the development and size of foetuses. The fixatives and histochemical techniques used have been reported previously (GURAYA, 1965, 1966; GURAYA and GREENWALD, 1964a, b; GURAYA, 1965).

Results

Histochemical features of developing follicles. The granulosa cells of normal growing follicles are small (Figs 1—5). Sudanophilic lipid bodies are sparsely distributed among the granulosa cells (Figs 1—5). They consist of phospholipids (GURAYA and GREENWALD, 1964b; GURAYA, 1967b). The cytoplasm of

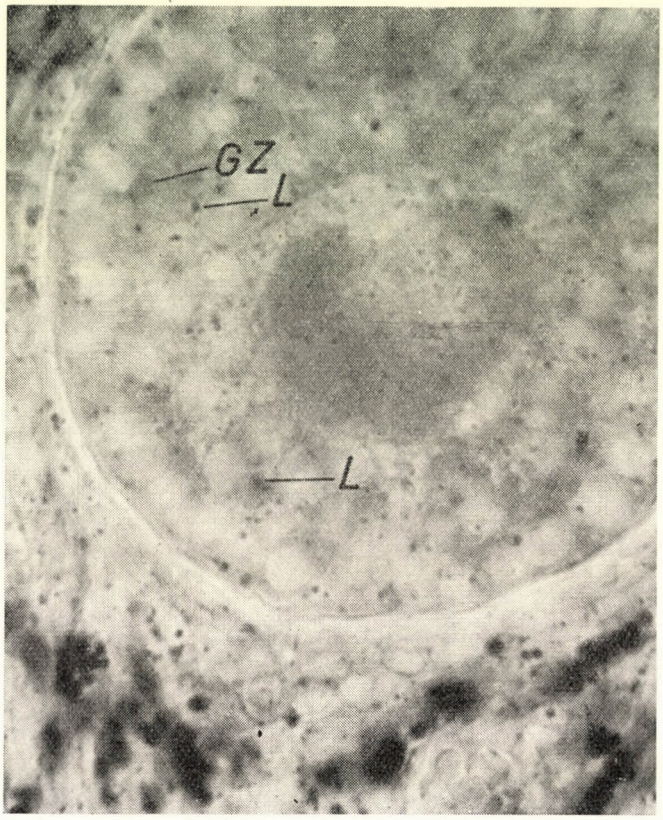

Fig. 1. Part of large preantral follicle of dog ovary, showing sparsely scattered lipid bodies (L) among the granulosa cells. The Golgi zone (GZ) of granulosa cells colours very feebly. Formaldehyde calcium, followed by dichromate calcium, Sudan black B. (×990)

granulosa cells is rich in RNA as determined by the strongly positive reaction in the methyl green-pyronine technique together with a negative reaction in the control sections treated with ribonuclease. In the final stages of follicular growth, the granulosa cells form a relatively thin layer, called membrana granulosa (Figs 4, 5). The theca interna of the growing follicle consists of stromal elements which do not show much cytoplasmic differentiation. Some sparsely scattered lipid bodies are seen which in their histochemical reactions

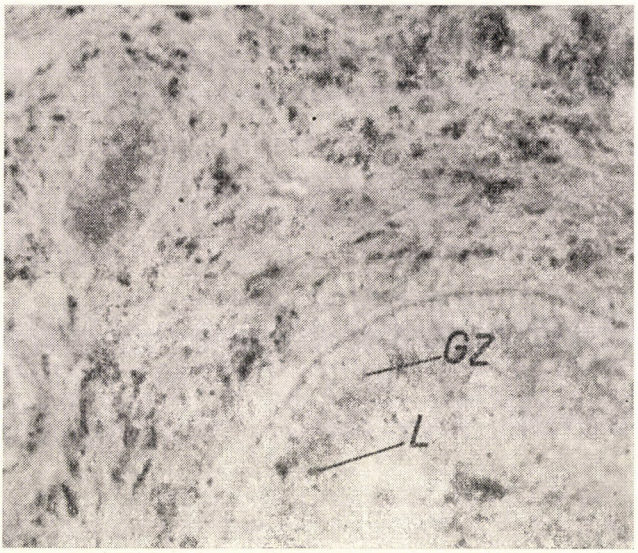

Fig. 2. Part of a small antral follicle of dog ovary, showing sparsely scattered lipid bodies (L) among the granulosa cells, which also show feebly sudanophilic Golgi zone (GZ). Stromal elements of theca interna contain some sparsely scattered lipid granules. Technique as in Fig. 1 (×450)

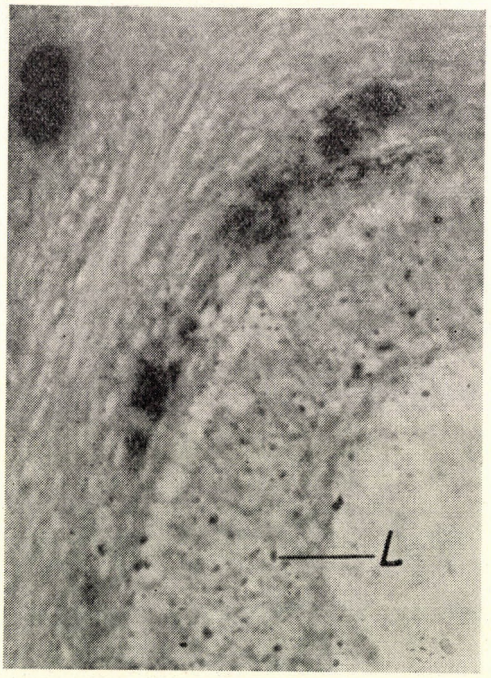

Fig. 3. Part of a small antral follicle of estrous cat ovary, showing sparsely scattered lipid bodies (L) among the granulosa cells. Theca interna shows some cells filled with coarse, sudanophilic lipid bodies. Technique as in Fig. 1 (×440)

resemble those of the membrana granulosa. They also consists of phospholipids. However, the theca interna in the growing follicle of cat ovary contains hypertrophied cells filled with coarse, sudanophilic lipid bodies (Figs 3—5) which consist of phospholipids, triglycerides and cholesterol or cholesterol esters (GURAYA, 1967a).

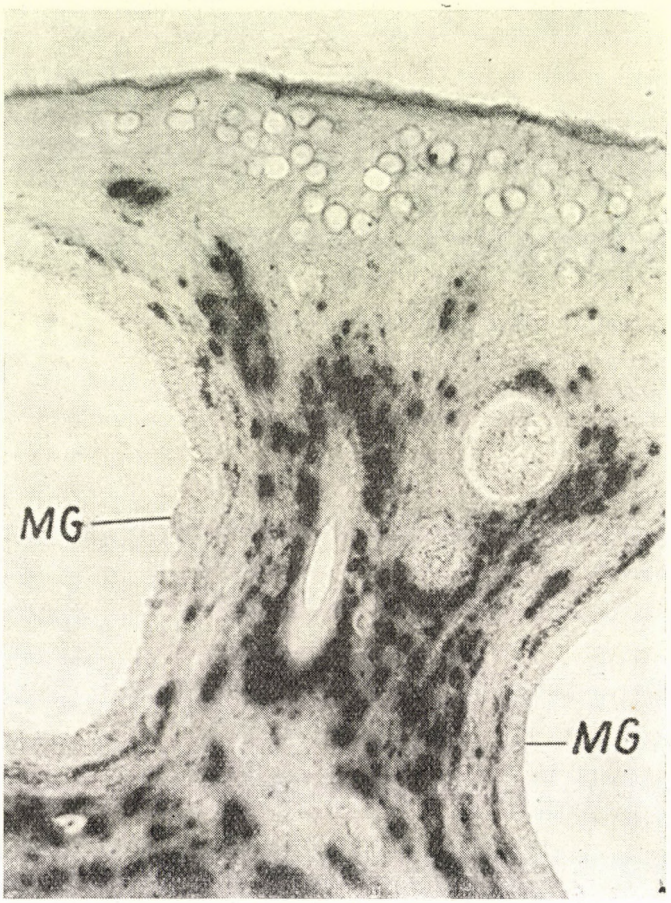

Fig. 4. Part of an estrous cat ovary, showing the membrana granulosa (MG) of large antral follicles. Theca interna of these follicles contains cells filled with sudanophilic lipid bodies. The cells filled with sudanophilic lipids are also seen in the ovarian stroma. Technique as in Fig. 1 (×84)

Histochemical features of corpus luteum: As compared with the original granulosa cells (Figs 1—5), the luteal cells are hypertrophied glandular tissue (Figs 6, 7), which is well vascularized. Each cell has a large vesicular nucleus. The most striking and significant change during the transformation of granulosa cells into luteal cells is the formation of a diffusely distributed sudano-

philic substance throughout the cytoplasm of the latter (Figs 6, 7). The Sudan black-positive reaction of the cytoplasm is negative in material fixed in weak Bouin solution and extractable with hot pyridine, indicating that the cytoplasm of luteal cells has Sudan black B-positive lipids which are not seen in the cy-

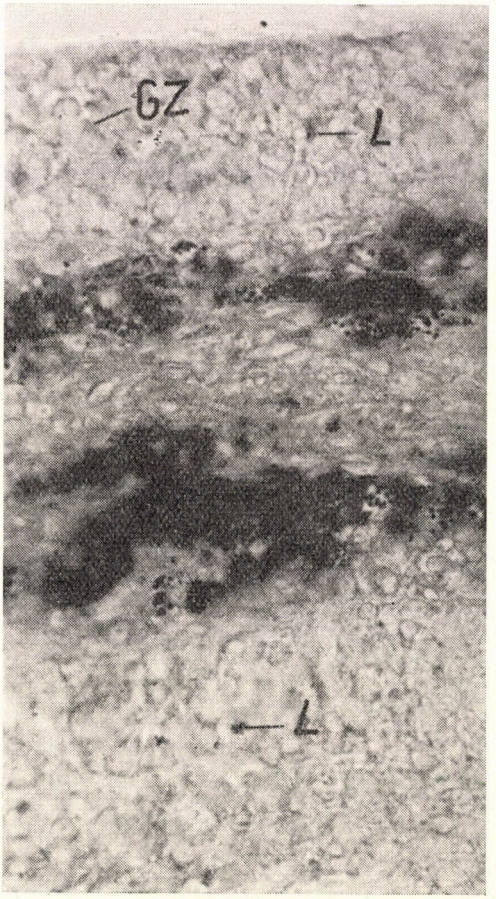

Fig. 5. Parts of two large antral follicles of estrous cat ovary, showing sparsely scattered lipid bodies (L) among the granulosa cells which show feebly sudanophilic Golgi zone (GZ). Theca interna contains cells filled with sudanophilic lipid droplets. Technique as in Fig. 1 (×560)

toplasm of granulosa cells except for a small zone (probably the Golgi zone) lying adjacent to the nuclear envelope (Figs 1—5). The diffuse lipids of luteal cells seem to be lipoproteins as they gave negative reactions with other histochemical techniques for the demonstration of triglycerides, phospholipids, and cholesterol or cholesterol esters (GURAYA, 1968, 1966, 1967a,b). However, the entire cytoplasm gave a positive reaction in the periodic acid Schiff (PAS) test; the distribution of the PAS-positive substance was the same

as that of the Sudan black-positive substance. The PAS-positive reaction continued to persist in the control sections treated for acetylation, showing that the PAS-positive reaction of the cytoplasm in the luteal cells was mainly due to the diffuse lipid component which disappeared after pyridine extractions. Besides the diffuse lipoproteins some lipid inclusions of variable size developed

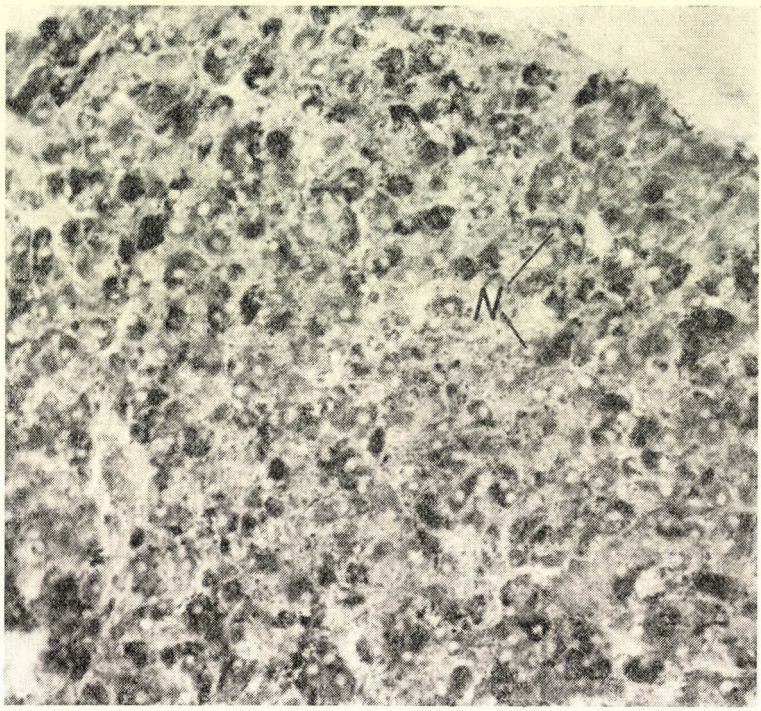

Fig. 6. Part of corpus luteum of pseudopregnant dog ovary, showing the development of diffuse, sudanophilic lipids and lipid bodies in the cytoplasm of luteal cells. The vesicular nucleus (N) does not show lipids. Technique as in Fig. 1 (×168)

in the cytoplasm of luteal cells (Figs 6, 7). Their number also varied in different luteal cells. Compared with the lipid bodies of membrana granulosa they stained less intensely with acid haematin, indicating a different nature of their phospholipids. Some lipid inclusions of luteal cells stained pink in Nile blue, and orange-red in red Sudan dyes, indicating triglycerides in addition to phospholipids. No cholesterol or cholesterol esters demonstrable with histochemical techniques were present in the lipid inclusions of luteal cells.

The stromal elements, which are apparently derived from the theca interna and theca externa, are of small size (Figs 7—9). They did not develop the diffuse lipoproteins formed in the luteal cells of granulosa cell origin. The

hypertrophied thecal cells filled with lipid droplets could not be distinguished in the corpora lutea of the cat ovary.

Regression of the corpus luteum was gradually characterized by the storage of coarse, sudanophilic droplets in the cytoplasm of luteal cells (Figs 8, 9). During regression the luteal cell mass also developed sudanophobic

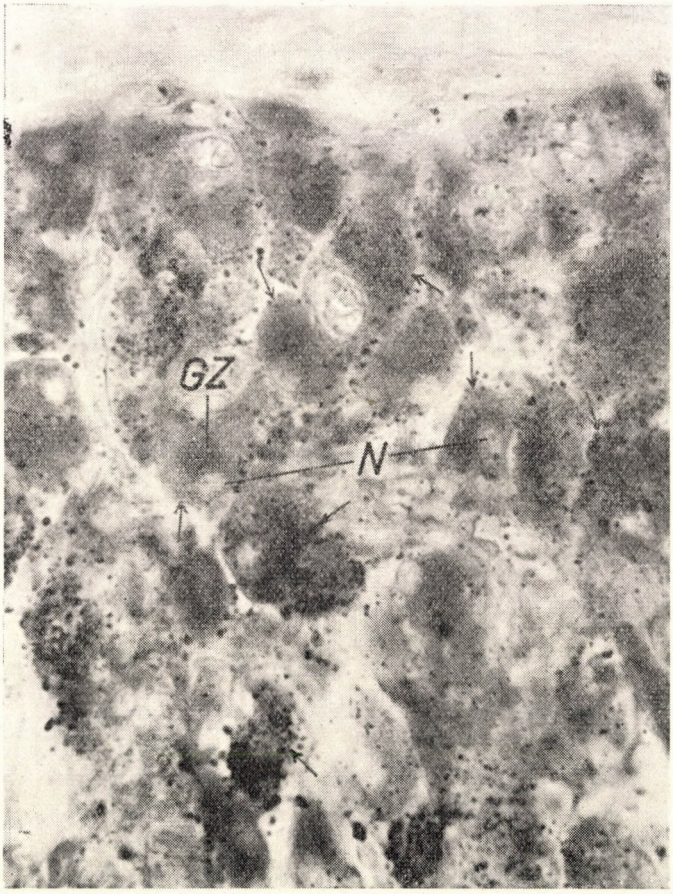

Fig. 7. Details of Fig. 6 showing diffuse sudanophilic lipids and lipid bodies in the cytoplasm of luteal cells (arrows). The Golgi zone (GZ) is also seen. The vesicular nucleus (N) does not show lipids. Technique as in Fig. 1 (×560)

vacuoles of variable size. The lipid inclusions stained pink in Nile blue, and orange-red in red Sudan dyes, indicating triglycerides. In later stages of regression, the lipid inclusions, too, were Schultz-positive and developed cholesterol or cholesterol esters. They stained moderately in acid haematin together with a negative reaction in the control material fixed in weak Bouin solution and were extracted with hot pyridine, thus demonstrating some phospholi-

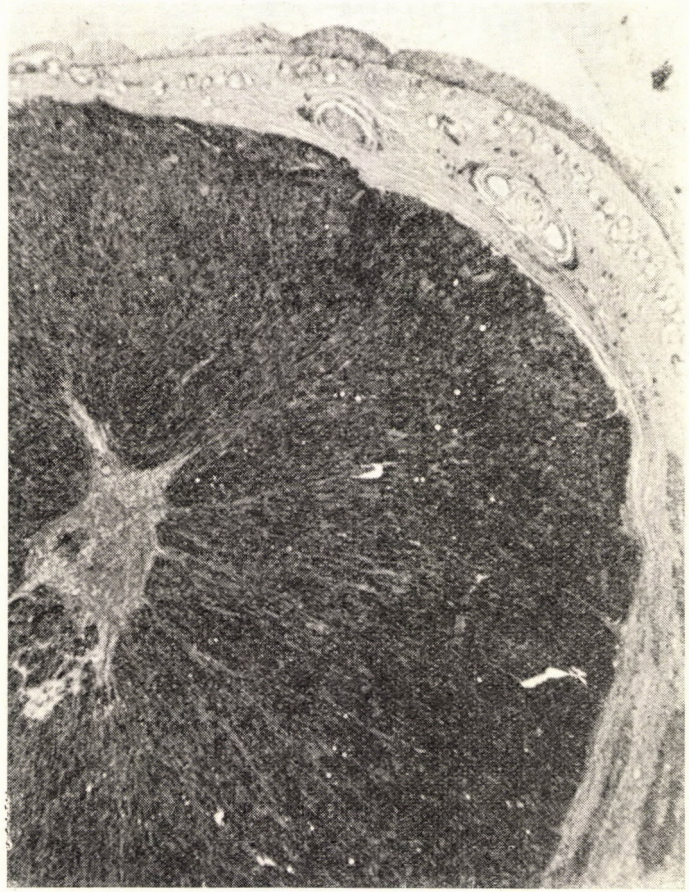

Fig. 8. Part of cat corpus luteum in later stages of pregnancy, showing sudanophilic lipids in the luteal cell mass which contains stromal elements. Technique as in Fig. 1 (×42)

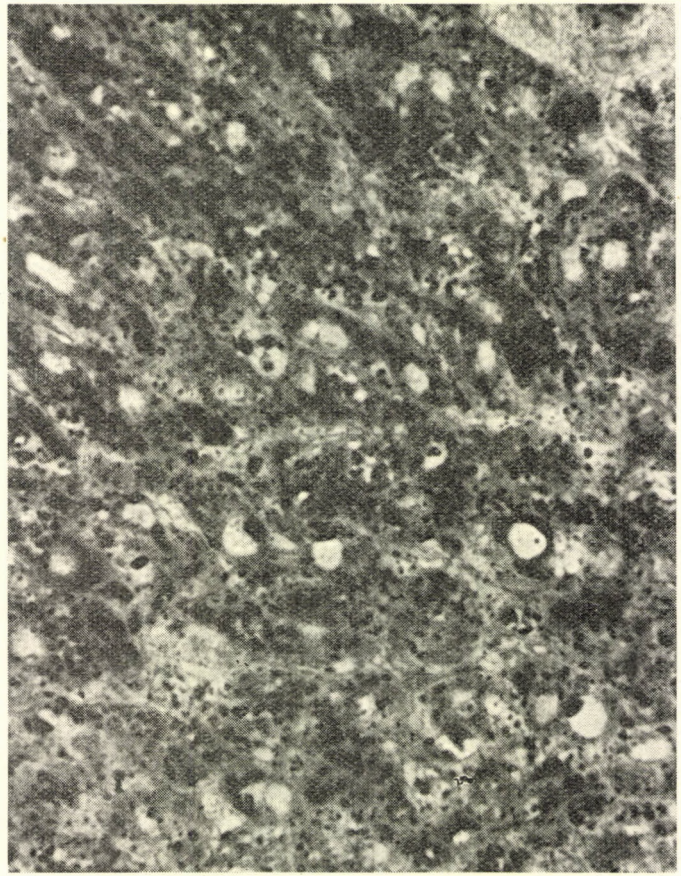

Fig. 9. Details of Fig. 8 showing the accumulation of coarse, sudanophilic lipid droplets of variable size in the luteal cells. Some sudanophilic vacuoles are also seen. Technique as in Fig. 1. (×560)

pids in regressing luteal cells. The increase in the amount of coarse lipid inclusion droplets corresponded to the decrease in amount of diffuse lipoproteins which were present in abundance in earlier stages. In the final stages of regression (Fig. 10) the lipids consisted mainly of triglycerides and cholesterol or

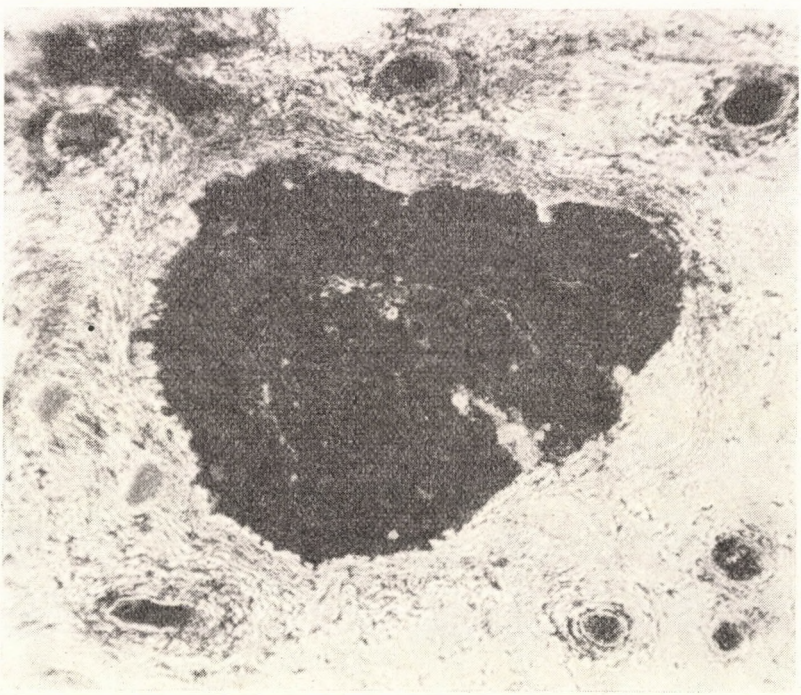

Fig. 10. Corpus albicans from the dog ovary showing the accumulation of sudanophilic lipids. Technique as in Fig. 1. (×66)

cholesterol esters; some pigment, perhaps lipofuscin, developed simultaneously in the lipid droplets as shown by its resistance to fat solvents. The cytoplasmic basophilic substance, due to RNA, was not removed in the control sections treated with ribonuclease. The residual luteal cells filled with coarse lipid droplets persist in the ovarian stroma for several months (Fig. 10).

Discussion

The abundant cytoplasmic RNA in the granulosa cells of developing follicles may be due to the presence of ribosomes or RNP particles as suggested by electron microscopic studies (BLANCHETTE, 1966); they have been associated with the protein synthesis during follicular growth. The lipid bodies consisting of phospholipids invariably occur in the granulosa and oocyte of developing follicles in the ovaries of different mammals

(GURAYA, 1968) The diffuse lipoproteins were not present in the cytoplasm of the granulosa cells during follicular development, preovulatory swelling phase and ovulation in opossum, rabbit and man (GURAYA, 1968a, b,c). In the rabbit ovary, the development of diffuse lipoproteins was first seen in the membrana granulosa cells of newly-ruptured follicles, which were being vascularized simultaneously (see Fig. 2 in GURAYA, 1968). However, they were not observed in the membrana granulosa cells of newly-ruptured follicles in the human ovary, which was still devoid of blood vascularity (see Figs 3, 4 in GURAYA, 1968). The diffuse lipoproteins actually started their development along with the blood vascularity of membrana granulosa. From these observations, it is quite clear that vascularization is the key to this cytoplasmic change (development of diffuse lipoproteins) which occurs during the transformation of granulosa cells into luteal cells; the latter were also hypertrophied simultaneously. The hypertrophy or luteinization of the granulosa cells during the corpus luteum formation was accompanied by the development of diffuse lipoproteins throughout the cytoplasm, a feature which, so far, has been overlooked. The development of diffuse lipoproteins throughout the cytoplasm of hypertrophied steroid cells may serve as a useful histochemical feature of these cells.

What is the functional significance of diffuse lipoproteins which are formed invariably in the cytoplasm of luteal cells in the ovaries of mammals? The cytoplasm of ovarian and testicular interstitial gland cells is also rich in diffuse lipoproteins (GURAYA, 1966; 1967a,b,c, 1968). The appearance of diffuse lipoproteins in the cytoplasm of steroid cells may hold a clue to control mechanisms in the biosynthesis of steroid hormones. It is well established that the luteal cells as well as the other steroid cells, including those in the adrenal cortex, show abundant smooth-surfaced (or agranular) endoplasmic reticulum, which is dispersed throughout the cytoplasm (YAMADA and ISHIKAWA, 1960; ENDERS, 1962; GREEN and MAQUEO, 1965; BLANCHETTE, 1966a, b; MUTA, 1958; CHRISTENSEN, 1965; ZELANDER, 1959). The diffuse lipoproteins demonstrated histochemically apparently derive from the extensively developed agranular endoplasmic reticulum which acts as a site of enzymes involved in the production of steroid hormones (CHRISTENSEN, 1965; CHRISTENSEN and FAWCETT, 1966). The scarcity of diffuse lipoproteins or smooth-surfaced endoplasmic reticulum (BJORKMAN, 1962; BLANCHETTE, 1966) in the granulosa cells of developing follicles as well as preovulatory follicles suggests that they are probably not adapted to the synthesis of steroid hormones. But the *in vitro* experimental studies have implicated the granulosa cells of developing follicles in steroidogenesis (RYAN and SHORT, 1965; RYAN and PETRO, 1966).

The cytoplasm of luteal cells, which are well known to synthesize steroid hormones, contained lipid inclusion bodies consisting of either phospholipids,

or phospholipids and triglycerides (GURAYA, 1968). BLANCHETTE (1966) has stated that the structural relationships between the lipid inclusion bodies and smooth membranous components of the cytoplasm, which vary during life span of the lutein cell, favour the possibility that an interaction between these two cell components results in steroid biosyntheses; the lipid droplets are precursor material stored within the cell. It can now be stated safely that both the lipid inclusions and the diffuse lipoproteins demonstrated in the luteal cells of this study are associated with the synthesis of steroid hormones. Both components are lacking in the cytoplasm of unvascularized granulosa cells during the follicular growth, and preovulatory swelling period.

With the regression of the corpus luteum, the luteal cells retain the coarse lipid inclusions which consist of either phospholipids and triglycerides or triglycerides, cholesterol or cholesterol esters and some phospholipids. Similar lipids are also stored abundantly in the regressing corpora lutea of opossum, rodents and man (BLANCHETTE, 1966; GURAYA, 1968; GURAYA and GREENWALD, 1965; CLAESSON and HILLÄRP, 1948). It is generally believed that when steroidal lipids are accumulated abundantly in steroid-producing cells, hormone storage is taking place, and when the amount decreases, hormone is released (GURAYA, 1966). It can now be added that the luteal cells during the later stages of pregnancy in cat begin to store hormone precursor (steroidal lipids) (Figs 8, 9) rather than secrete hormone and the hormone secretion occurs in those luteal cells which show no accumulation or coarse lipid inclusions. The present histochemical observations are in close agreement with the previous physiological observations (GROSS, 1962) which have shown that removal of both ovaries in later stages of pregnancy in cats was followed by normal parturition at term (64 days), although spaying at 46 days or earlier regularly interrupted pregnancy. Storage phases for Schultz-positive material during the lutein cell cycle of the rabbit have also been demonstrated (CLAESSON and HILLÄRP, 1948) to occur during the second third of pregnancy and post partum. The changes in the metabolism of steroidal lipids reflect certain alterations in the gonadotropins. This leads to the storage of hormone precursor (lipid inclusions). The gonadotropins of pituitary and placenta origin are now well known to influence steroidogenesis or lipid metabolism in the different compartments of mammalian ovary (GURAYA, 1966; GURAYA and GREENWALD, 1964a,b; GURAYA, 1967a,b; GURAYA and GREENWALD, 1965; CLAESSON and HILLÄRP, 1948; SAVARD *et al.*, 1965; CLAESSON *et al.*, 1948; GURAYA, 1967c,d).

In the late corpus luteum function, the cytoplasm of luteal cells apparently undergoes some drastic physicochemical changes as suggested by the decrease in the amount of diffuse lipoproteins and the accumulation of lipid inclusion bodies of variable size and cytochemical nature (Figs 8—10). It appears that some of the lipid inclusions are actually derived from the diffuse

lipoproteins by some pathological change, since the decrease in amount of diffuse lipoproteins is concomitant with the increase in the amount of some coarse lipid bodies consisting of triglycerides, cholesterol or its esters, pigment and some phospholipids. In electron-microscopical studies BLANCHETTE (1966) has also described some inclusion bodies in the regressing luteal cells of rabbit corpus luteum, which are derived from the agranular endoplasmic reticulum; the latter, which is decreased in amount correspondingly, is constituted by the diffuse lipoproteins of this study.

SUMMARY

The histochemical features of developing follicle and corpus luteum in cat and dog ovaries have been studied. The granulosa cells of developing follicles show abundant cytoplasmic RNA. Some lipid bodies consisting of phospholipids are sparsely distributed among the granulosa cells. The most conspicuous histochemical change involved in the differentiation (or luteinization) of the granulosa follicle cell into a luteal cell is the development of abundant diffuse lipoproteins throughout the cytoplasm. Lipid bodies consisting of either phospholipids or phospholipids and triglycerides are also formed in the cytoplasm of luteal cells. Both the lipid inclusions and the diffuse lipoproteins are apparently associated with the synthesis of steroid hormones.

The stromal elements of theca interna contain some sparsely scattered phospholipid bodies. However, the hypertrophied cells seen in the theca interna of cat ovary are filled with coarse lipid bodies consisting of phospholipids, cholesterol or cholesterol esters and triglycerides. They could not be distinguished in the corpus luteum.

With the regression of luteal cells, coarse lipid inclusions consisting of either phospholipids and triglycerides, or phospholipids, triglycerides and cholesterol or cholesterol esters accumulate abundantly in the cytoplasm. Some pigment, perhaps lipofuscin, is also formed in the later stages of corpus luteum regression. The increase in amount of coarse lipid inclusions is concomitant with the decrease in amount of diffuse lipoproteins, suggesting that the former may be formed from the latter by some pathological change. The regressing luteal cells filled with coarse lipid bodies persist in the ovarian stroma for months.

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DIAGNOSTIC STUDIES ON THE EXTRAGONADAL (EPIDIDYMAL) RESERVES OF SPERMATOZOA IN WATER BUFFALO

By

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The general use of artificial insemination (A.I.) directed the attention of many investigators to study the spermatozoa-producing capacity of bulls. The quantitative determinations of spermatozoa produced, is of great importance as regards of the intensive utilization of bulls at A.I. Centres. In relation to the European cattle, such investigations have already been carried out. There is no information however in connection with buffalo, which occupies a particular position in the dairy industry of Egypt.

Accordingly, attempts were made to establish correlations between the total number of spermatozoa present in the different regions of the epididymis, the weight of the epididymis and the weight of the testicle.

With the exception of EL AZAB and OSMAN's work (in press) on the morphological characters of epididymal spermatozoa of buffalo, the available literature lacks information on this subject. In connection of cattle however, a number of reports are at our disposal (BRANTON, 1946; BIALLY and SMITH, 1956, 1958; ORTAVANT, 1956, 1959; ALMQUIST, AMANN and O'DELL, 1958; SALISBURY and VAN DEMARK, 1961).

Material and methods

Male buffaloes, including 14 1.5 years old and 9 2.5 years old animals, were studied. After slaughtering, the testicles and epididymides of each animal were removed together. After the disjunction of epididymis, the head, body and tail of each one were separated and weighed to the nearest 10 mg. The volumes were also determined by water displacement to the nearest 1/10 of ml. The number of spermatozoa was determined in cytometer. The material was previously macerated in saline, using an electromixer, and stained with a 3% solution of water-soluble eosin. To 5 ml of this fluid 3 drops of sodium hydroxide (2%) and 2 drops of the eosin solution were added during gently shaking. The statistical analysis of the obtained data was performed by methods described by SNEDECAR (1956).

Results

The results obtained are presented in Tables 1—8.

Table 1

Weights and volumes of different regions of the epididymis

Regions of the epididymis	1.5 years age group				2.5 years age group			
	Mean	St. dev. ±	Min.	Max.	Mean	St. dev. ±	Min.	Max.
Head (caput)								
weight (g)	4.650	1.320	2.350	7.750	6.080	1.080	4.800	8.450
volume (ml)	4.5	1.3	2.3	7.7	5.9	1.2	4.7	8.2
Body (corpus)								
weight (g)	2.430	0.690	1.300	3.600	2.630	0.610	1.500	3.500
volume (ml)	2.3	0.7	1.1	3.5	2.5	0.6	1.3	3.3
Tail (cauda)								
weight (g)	3.160	0.970	1.200	4.500	3.450	0.410	2.980	4.300
volume (ml)	3.0	1.0	1.1	4.4	3.2	0.4	2.8	4.1

Table 2

Total weight of the epididymis and testicle

Organ	1.5 years age group				2.5 years age group			
	Mean	St. dev. ±	Min.	Max.	Mean	St. dev. ±	Min.	Max.
Epididymis	10.240	2.190	6.400	14.120	12.160	2.570	9.700	14.950
Testis	94.280	22.1	62.000	140.000	109.110	12.510	90.000	129.000

Table 3

Percentage participation of the three regions in the total weight of the epididymis

Region of the epididymis	1.5 years age group %	2.5 years age group %
Head	45.4	50.1
Body	23.7	21.6
Tail	30.9	28.3
Total	100.0	100.0

Table 4

Numbers of spermatozoa ($\times 10^6$) present in the different regions of the epididymis

Region of the epididymis	1.5 years age group				2.5 years age group			
	Mean	St. dev. ±	Minimum	Maximum	Mean	St. dev. ±	Minimum	Maximum
Head:								
sperm/ml	308	142	129	662	315	135	149	510
sperm/g	293	131	125	574	307	133	144	507
Body:								
sperm/ml	393	145	135	746	436	244	163	793
sperm/g	366	125	124	631	409	229	143	762
Tail:								
sperm/ml	835	625	216	2,606	810	255	316	1,084
sperm/g	777	580	211	2,389	763	238	295	1,004

Table 5

Total numbers of spermatozoa ($\times 10^6$) present in different regions of the epididymis

Region of the epididymis	1.5 years age group				2.5 years age group			
	Mean	St. dev.	Minimum	Maximum	Mean	St. dev.	Minimum	Maximum
Head	1,298	531	664	2,310	1,827	712	744	2,754
Body	873	415	311	1,849	1,149	803	214	2,577
Tail	2,139	981	630	4,406	2,457	1,140	885	3,794
Total	4,310	1,420	2,035	7,327	5,433	2,334	1,849	8,953

Table 6

Spermatozoa present in different regions of the epididymis, per cent of the total sperm count

Region of the epididymis	Percentages in	
	1.5 years age group	2.5 years age group
Head	30.116	33.628
Body	20.255	21.148
Tail	49.629	45.224
Total	100.000	100.000

Table 7

Simple correlations

	1.5 years age group+			2.5 years age group*		
	r =	P	Results	r =	P	Results
Total sperm count × Total weight of epididymis	+0.973	<0.1%	Highly significant	+0.884	<0.27%	Highly significant
Total sperm count × Total weight of testis	+0.654	<5%	significant	+0.65	>5%	not significant
Total weight of epididymis × Total weight of testicle	+0.651	<5%	significant	+0.670	>5%	not significant

+n = 14; *n = 9

Total number o spermatozoa ($\times 10^9$) present in each region of the epididymis of cattle and buffalo

Region of the epididymis	Almquist (1956*)	Bially and Smith (1956*)	Ortavant (1959)		Present work	
			2 years	2 years	1.5 years	2.5 years
Head	20.3	—	14.3	12.0	1.298	1.827
Body	4.8	—	5.2	4.5	0.873	1.149
Tail	37.3	18.0	13.8	24.1	2.139	2.457

* for the combined right and left regions of the epididymis.

Discussion

It is well known that the epididymis plays an important role in the maturation and preservation of spermatozoa.

On the basis of the above-mentioned data, it can be pointed out that in mature buffalo the total weight of both of the epididymis and testicle is markedly smaller than the corresponding data recorded for the bull by Asdell (1955) and Abdel-Raouf (1960).

It has been found that the head makes the largest part, while the body the smallest part of the total epididymal weight, the tail being between them in size. The value of these results lies in the fact that the total number of spermatozoa counted in each region of the epididymis is variable (Table 6), especially with regard to the head and tail regions, a large percentage of spermatozoa being present in the last one. Moreover, the number of spermatozoa

counted per gram of tissue in the head region is much less than that counted in the tail. Accordingly it can be concluded that the size of the epididymal tube and consequently the sperm reserve markedly increases from the head to the tail region of the epididymis. This can be supported by the findings of OSMAN and FAHMY (1969) in buffalo and ORTAVANT (1959) in the bull.

Regarding the total number of spermatozoa present in each region of the epididymis in per cent of the total sperm count, our results are closely similar to those recorded for the bull by BIALLY and SMITH (1958). With the advance of age, the ever increasing weight of the testicle, the ever growing of the epididymis and the continually increasing number of spermatozoon production allow us to conclude that both of these glands reach their full function by the age of 2.5 years.

The total number of spermatozoa in the epididymis is, in buffalo, much lower than those recorded for the bull by ORTAVANT (1956), VAN DEMARK (1956) and SALISBURY and VAN DEMARK (1961). From Table 8 it can be concluded that the reproductive capacity of buffalo bull is much less than that of the bull. Therefore it is of importance to avoid extensive use of buffalo bull for breeding and never use this animal in routine A.I. technique as the bull is used. Naturally, from the results of this study, the experiment of BOYD and VAN DEMARK (1956) cannot be made for the buffalo bull.

Our findings are in accord with the results of some investigations concerning the semen characteristic of buffalo. PRABHU and SHARMA (1953) indicated that it is undesirable to take more than 2 ejaculates at a time from these animals. Moreover, SAYED and OLOUFA (1957) found that there is a highly significant difference in the concentration of the semen among buffalo bulls from which semen was collected once every week, and those from which collection was done once every other day (three times a week), for 13 weeks. The present results seem to be also in accord with the finding of HAFEZ and DARWISH (1956).

In the present work a great individual variation within each age group was met with, especially with regard to the total number of spermatozoa. Similar findings were recorded previously by MAHMOUD (1949) on buffalo semen.

On the basis of the statistical analysis of the present data, the results have revealed that the relation between the total sperm count in the epididymis and the total weight of this organ is highly significant within both age groups (Table 7). This indicates that in normal animal one may expect a large number of spermatozoa, if the clinical examination shows large epididymal size. It may be noted that OSMAN (1965) found no marked difference between the results of clinical and anatomical measurements of the buffalo's epididymis. For the bull ALMQUIST, AMANN and O'DELL (1958) and BIALLY and SMITH (1958) reported that no significant correlation was found between the extragonadal reserves and epididymal weight.

Regarding the relation between the testicle and epididymis and between the total sperm count and the weight of the testicle, the present results lead us to conclude that, at early ages, the factors controlling the growth and function of the testicle and epididymis may be limited and nearly regular. These factors seemed to be the growth-accelerating factors mentioned previously by BROOKS and VINCETT (1950) for the live weight generally. With the advance of age other variable factors may greatly affect this relation. It is generally accepted that if ejaculation does not occur, these spermatozoa undergo liquefaction and absorption in the epididymis (SIMEONE and YOUNG, 1931; COLLERY, 1944). This may play a role in the absence of significant correlation between the sperm reserves in the epididymis and the testicular weight, especially in adult subjects. The absence of such relation in the second age group is in accord with the findings of ALMQUIST et al. (1958) and BIALLY and SMITH (1958) in the bull. On the other hand, ORTAVANT (1952) demonstrated that there is a significant correlation ($r = 0.80$) between testicular size and number of spermatozoa in the excised reproductive tracts of the rams.

Regarding the relation between the testicle and epididymis our results are in accord with the findings of ABDEL-RAOUF (1960) for young growing bulls and with BRIGATTI (1951) for adult bulls.

As regards the extragonadal reserves of spermatozoa in buffalo, we can draw the conclusion that the male must be chosen for breeding at the age of 1.5 years and attention should be paid to evaluate the size of the testicle and epididymis besides the characteristic genetic and hygienic features of genetics and health. Later on and just before using such animal for A. I., the general characteristic features of its semen must be carefully evaluated.

SUMMARY

The extragonadal reserve of spermatozoa was studied on two groups of buffaloes aged 1.5 and 2.5 years in order to establish a possible correlation between the total number of spermatozoa present and the weight and volume of the epididymis and of the testicle. For counting spermatozoa a new method involving maceration and measurement with cytometer was used. The results obtained and the correlations are presented in Tables. In both of the age groups significant correlation was found between the total number of spermatozoa and the weight of epididymis (Group 1: $r = +0.973/P < 0.1\%$; Group 2: $r = +0.884/P < 0.27\%$). Significant correlation between the total number of spermatozoa and the total weight of testicle however was found but in the age group 1 ($r = +0.654$, $P < 5\%$).

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DETERMINATION OF MANGANESE IN THE LIVER OF WL CHICKENS IN THE EARLY POST-INCUBATION PERIOD

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Introduction

The preceding two reports of this series (SOVA *et al.*, 1969; and MAREK *et al.*, 1969) dealt with the determination of Fe and Cu in the livers of chicken in the course of development.

This report presents the Mn^{++} values. The importance of Mn for the live organism and the Mn ratio in the organs of mammals and birds were first studied by BERTRAND and MEDICRECEANU (1912—1913), the role of Mn in the activity of certain enzymes was mentioned by VELIKÝ (1964) and BERNSTEIN (1958) among others, and in the metabolism of lipids by VELIKÝ (1964). Mn^{++} was determined in certain organs and tissues by VELIKÝ (1964), in livers of chickens by ANKE *et al.* (1967), in bones by HILL (1965), and in eggs and egg-shells by OPICHAL (1965) and LYONS (1939) respectively. The relation between the quantity of Mn^{++} in the feed ration and hatchability and laying was mentioned in the papers of OPICHAL (1965), ERMENKOV (1964), and of KRIEG (1965). Several authors (PATRICK, 1957; LYONS, 1939; HUNT, 1960; CASKEY *et al.*, 1944; RYS, 1959; ERMENKOV, 1964; WILGUS, 1936; and MANDELLI and RIZZI, 1966) have dealt with the symptoms of Mn^{++} deficiency in poultry. The required concentration and quantity of Mn^{++} in feeds were determined by PATRICK (1957) and by GALLUP and NORRIS (1939). We have failed to find any data on the Mn^{++} content in the livers of chickens in the post-incubation period.

Material and method

Mn^{++} was determined in the livers of 58 chickens from 1 to 31 days of age. The chickens were kept under the usual zootechnical conditions and were fed a complete feed mixture used for rearing of chickens. The mixture contained a 1% mineral admixture containing 0.55% $MnCO_3$. Mn^{++} was determined photometrically after oxidation in permanganic acid and from the sulphate ashes obtained after the burning of the dry substance of the livers.

Results and discussion

Results are presented in Table I and illustrated in Fig. 1.

After hatching the chicks had, on the average, only 1.33 μg of Mn^{++} in the whole livers, *i.e.*, 0.124 mg per 100 g, the total quantity in the liver being 1/7th to 1/15th of that found in the whole egg by OPICHAL (1965). The average Mn concentration makes up only 41% of the value (0.303 mg per

100 g) given for livers of poultry by VELIKÝ (1964). Table 1 also shows that the concentration of Mn^{++} in the liver increased in the post-incubation period and at the age of 1 month it approximated the value reported by VELIKÝ (1964). Thus, newly hatched chicks had only 1.33 μg of Mn^{++} in the livers, *i.e.*, some-

Table 1

The quantity of manganese in the liver of chickens in the first month of age

Age of chickens, day	Weight of liver (g)	Dry weight of liver per cent $\bar{x}$	Dry weight of liver per cent s	n	Mn in the liver mg/100 g	Mn in the liver mg/100 g of dry weight $\bar{x}$	Mn in the liver mg/100 g of dry weight s	Mn in the liver mg/100 g of dry weight V	Total manganese in liver (μg)
1	1.078	36.4	0.57	18	0.124	0.34	0.086	0.25—0.42	1.33
3	0.998	31.9	0.91	6	0.161	0.57	—	—	1.61
4	1.286	32.5	1.5	6	0.130	0.38	—	—	1.67
7	2.004	27.4	1.4	6	0.096	0.35	—	—	1.92
11	1.931	26.0	0.52	12	0.143	0.55	0.031	0.54—0.57	2.76
18	3.761	25.5	0.23	7	0.140	0.55	0.26	0.35—0.58	5.26
25	5.797	24.8	1.2	1	0.234	0.94	—	—	13.57
31	7.456	27.55	0.95	2	0.291	1.06	—	—	22.0

what less than was found by MANDELLI and RIZZI (1966) in the livers of older chicken suffering from porosis [1.4—1.9 μg /1 g]. The Mn^{++} value of 0.291 mg per 100 g for our 1-month-old chickens is very close to the values found in the livers of healthy chickens by the mentioned authors [3.2 μg Mn/1 g]. However,

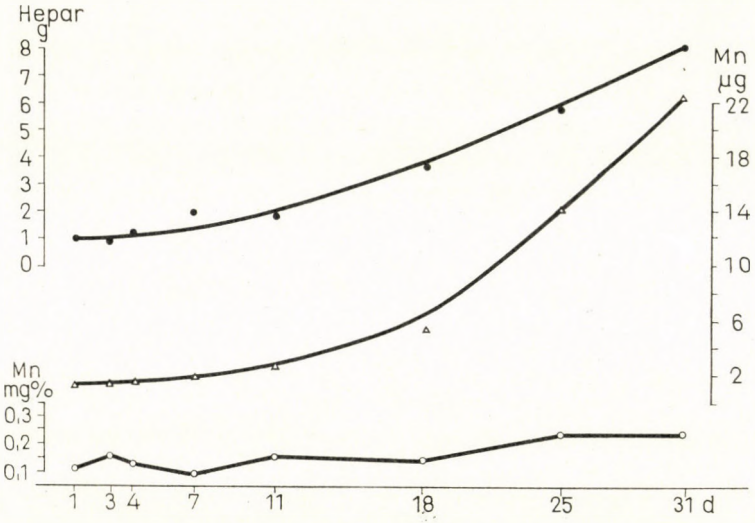

Fig. 1. Manganese in the liver in the first month of age

our values are considerably lower (roughly 9 times) than those given for chickens by ANKE *et al.* (1967). It cannot be excluded that these considerable differences are caused by a different quantity of absorbable Mn^{++} in the feed ration. According to the state standard ČSN 55, our feed ration should contain 55 p.p.m. of $MnCO_3$, *i.e.*, approximately 26 p.p.m. of Mn^{++}. One week after hatching the concentration of Mn^{++} in the dry substance of livers equalled to the values found at hatching, while the concentration calculated for the wet substance decreased, the difference being due to the declining percentage of the dry matter in the livers. In the first week after hatching the total weight of the livers was almost redoubled. Fig. 1 shows the dynamics of changes in the Mn^{++} content in livers in relation to the weight of the livers.

Up to the age of 18 days the total quantity of Mn^{++} increased more or less regularly in correlation to the growth of the livers; beginning from the 18th day of age, however, the curve illustrating the absolute quantity of Mn^{++} in the livers is steeper. By the age of 31 days the weight of the livers increased seven times compared with the value after hatching, while the quantity of Mn^{++} in the livers increased almost 17 times. It may be assumed that the feed ration had been sufficiently enriched with Mn^{++}, so that the chickens were able to store this supply. Only further experiments will show whether the concentration of Mn^{++} found in chicken of an age of 31 days corresponds to the values found in older poultry.

SUMMARY

In chicken of the WL breed, from 1 to 31 days of age the quantity of Mn^{++} in the livers was examined. Immediately after hatching the whole livers of the chicken contained only 1.33 μg of Mn^{++}, *i.e.*, 0.34 ± 0.086 mg/100 g Mn^{++} in the dry substance and 0.124 mg/100 g whole liver. In the first week of life the weight of the livers increased 2 times while the total quantity of Mn^{++} in the livers rose only insignificantly to 1.92 μg. Beginning from the 18th day of life the concentration of Mn^{++} in the livers rose, so that while the weight of the livers increased 7 times in the course of 31 days, the Mn^{++} content in the livers increased almost 17 times.

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THE QUANTITY OF COBALT IN THE LIVER OF WHITE LEGHORN CHICKENS IN THE EARLY POST-INCUBATION PERIOD

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Introduction

The previous three reports of this series (MAREK *et al.*, 1969; SOVA *et al.*, 1969a, b) dealt with the Fe, Cu, and Mn contents in chicken livers in the course of development. This report presents Co^{++} values. Cobalt, a component of vitamin B_{12}, influences a number of important processes in the organism (VELIKÝ, 1964; EDWARDS, and CARTER, 1953, ERICKSON *et al.*, 1955; DRYDEN, 1952; KOVALSKIY, 1958). The Co content in the liver and other organs was dealt with by STARE and ELVEHJM (1933), and by MELEHSKO (1959). COMAR and DAVIS (1947) studied the absorption of the radioactive Co. The daily requirement of Co in feed was stated by MCNAUGHT (1948) and HARLEY and BECK (1958), the influence of the Co concentration in the feed on its concentration in the organs was determined by ASKEW and MAUNSELL (1937), KONSTANTINOV *et al.* (1963) and by DAVIS *et al.* (1953). The relation between a Co supplement and hatchability, laying, and size of eggs was studied by BERZIN (1950), and by VELIKÝ (1965). The influence of Co on the composition of blood was proved by OBENKO and ANTAKOV (1965) and by POPESCU *et al.* (1966). The synthesis of B_{12} was studied with labelled Co by MENKE *et al.* (1965), the synthesis of vitamin B_{12} in relation to certain antibiotics or therapeutic preparations was investigated by MENKE *et. al.* (1965). Finally, Co deficiency in poultry was studied by GRIGOR (1959).

Material and method

Co was determined in livers from 74 chickens aged from 1 to 31 days, divided into groups as shown in Table 1. The chickens were kept under the usual zootechnical conditions and were fed the mark "K" feed mixture for chicken. They received feed and water *ad libitum*. The prescription of the mixture does not state the Co content in the feed. The quantity of Co was determined photometrically in the sulphate ashes obtained after burning of the dry substance. As reagent nitroso-R-salt was used.

Results and discussion

The results obtained are given in Table 1 and Fig. 1. The Co values obtained in the livers are mentioned together with total weight and dry substance of livers. The smallest quantity of Co was found in chicken livers immediately after hatching (18.2 μg per 100 g liver, *i.e.*, 50 $\pm$ 14 μg per 100 g dry substance).

Table 1

The quantity of cobalt in the liver of chickens in the first month of age

Age in days	Weight of liver	Dry weight of liver, per cent		n	Co in the liver				
					mg/w 100 g of liver	mg per 100 g dry weight			Total cobalt of liver, μg
		$\bar{x}$	s			$\bar{x}$	s	V	
1	1.078	36.4	0.57	12	18.2	50	14	40— 60	196
3	0.998	31.9	0.91	12	46.3	145	21	130—160	462
4	1.286	32.5	1.5	18	29.2	90	36	60—130	376
7	2.004	27.4	1.4	6	30.1	110	—	—	604
11	1.931	26.0	0.52	12	15.6	60	56	20—100	302
18	3.761	25.5	0.23	7	38.9	150	38	140—240	1460
23	5.096	26.8	0.7	3	46.0	180	40	130—210	2340
25	5.797	24.8	1.2	1	29.4	120	—	—	1710
31	7.456	27.55	0.95	3	35.7	130	37	90—160	2660

In whole livers of newly-hatched chickens our experiments revealed, on the average, 196 μg Co. These values are higher than those found by MELESHKO (1959) per 100 g liver of cattle (13.4 μg), sheep (14.83 μg) and pigs (11.45 μg). In the following days of life these values double in the chicken liver. Compared with the concentration of Co in yolk (2.5 μg per 100, MELESHKO, 1959), a 7 times higher concentration of Co was found in the livers of our hatched chicks.

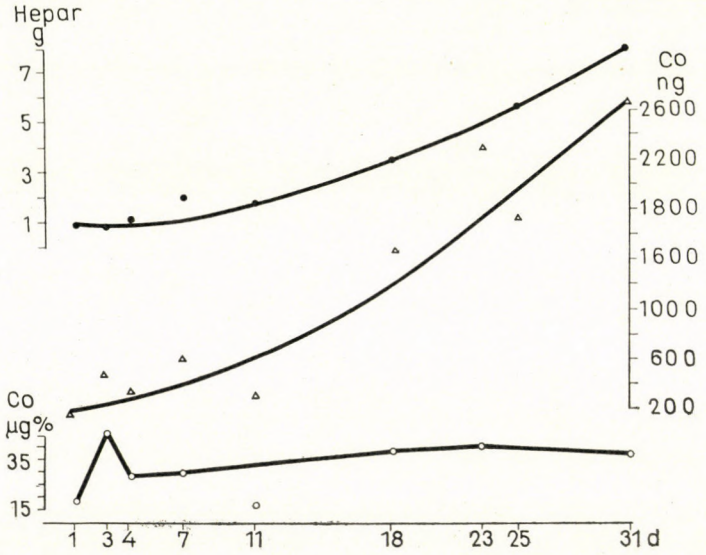

Fig. 1. Cobalt in the liver of chicken in the first month of age

In chicks of an age of 3 days a concentration of 46.3 μg per 100 g liver, *i.e.*, 145 ± 21 μg per 100 g dry substance was found. The absolute value was 462 μg/liver.

Fig. 1 shows that in the course of development the Co values in the livers fluctuate rather strongly, a minimum value being 15.6 μg per 100 g on the 11th day and two maxima, 46.3 and 46.0 μg per 100 g, on days 3 and 23, respectively. We assume that this considerable dispersion of Co values in livers is caused by a not fully perfect grinding of microelements in the feed mixture and so by a fluctuating Co intake on different days. On the other hand, after confrontation with the data published by HARLEY and BECK (1959), our observations have shown that the "K" feed mixture for chicken used in Czechoslovakia contains a fully sufficient quantity of Co even if its concentration is not stated. The fact that the mixture ensures a sufficient supply of Co has been proved by the 13.6-fold rise of the absolute quantity of Co in the livers within 31 days as compared with the 7-fold increase in the liver's weight.

SUMMARY

In chickens of an age of from 1 to 31 days the quantity of Co in the livers was examined. Immediately after hatching the livers contained 18.2 μg 100 g liver, *i.e.*, 196 ng of Co in whole livers. Later the Co content in the livers rose, so that in the first month of life the Co values in whole livers ranged between 15.6 and 46.3 μg/100 g liver, *i.e.* between 60 ± 56 μg% and 180 ± 40 μg/100 g dry weight. Within 31 days of age the weight of livers increased 7 times and the Co content in the livers increased 13.6 times, so that the absolute Co quantity in the whole livers of 1 month old chicken amounted to 2660 μg.

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EINIGE HÄMATOLOGISCHE PARAMETER BEI WIEDERKÄUERN BEI NORMALER UND HALBSYNTHETISCHER DIÄT

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Das Blut als eines der spezialisiertesten Gewebe der Lebewesen widerspiegelt — wie dies HOŘEJŠI und Mitarb. (1964) betonen — teils die metabolischen Vorgänge der Blutelemente, teils auch die des im ganzen Organismus verlaufenden Stoffwechsels. Aus diesen Gründen bietet die biochemische Blutanalyse eine Reihe wichtiger Informationen für die Diagnostik der normalen und abnormalen Funktionen des Organismus.

In Anbetracht der erwähnten Bedeutung der Blutuntersuchungen wurde die Frage gestellt, ob bei Verwendung halbsynthetischer Diät die unerwünschte Beeinflussung einiger wichtiger Funktionen des Organismus bei Wiederkäuern berechnet werden kann. Jene Parameter wurden gewählt, die die Verteilung des Stickstoffes und der Proteine, sowie die Aktivität einiger Enzyme im Blutserum der Versuchstiere (Ziegenböcke) charakterisieren.

HACKETT und Mitarb. (Zit.: LONG und Mitarb., 1961) fanden bei Schafen im Blutserum einen durchschnittlichen Reststickstoffwert von 34 mg%, während BARLETT und Mitarb. (Zit.: LONG und Mitarb., 1961) bei Kühen bei der Winterfütterung 21,5 $\pm$ 1,0 mg% und beim Weiden 28,2 $\pm$ 3,3 mg%.

Der durchschnittliche Gesamteiweißgehalt ist nach AFONSKIJ (1960) bei Ziegen 7,7 g% und bei Ziegenböcken 7,6 g%.

Elektrophoretische Untersuchungen der Serumeiweiße bei Ziegen führten GORCZYCA und Mitarb. (1959, 1960) durch. Bei einem durchschnittlichen Bluteiweißgehalt von 6,25 g% wurden folgende Durchschnittswerte der Eiweißfraktionen festgestellt: 63,3% Albumin, 6,7% β_2-Globuline und 15,5% γ-Globuline. Die Autoren wiesen ferner die verhältnismäßig bedeutenden ontogenetisch bedingten Veränderungen der Serumeiweiße bei Ziegen und Ziegenböcken im Alter von 7—9 Monaten und von 24—30 Monaten nach.

Verteilung der Glykoproteine im Blutserum von Rindern nach NILSSON und ALBERG (Zit.: LONG und Mitarb., 1961), 26,9% Verbindung mit Albumin — 20,6% α-Globulin —, 25,9% β-Globulin und 25,2% γ-Globulin.

Alkalische Phosphatasewerte im Blut nach NEGI und Mitarb. (1960): bei Rindern 4,35 $\pm$ 0,57 Einheiten und bei alten Ziegen 8,65 $\pm$ 1,70. In einer vorangehenden Mitteilung haben wir uns (PAVEL und TYLEČEK, 1968) mit der Aktivität der alkalischen Phosphatase, Lipase und Transaminase befaßt.

Über die oxydoreduzierenden Enzyme haben wir in der Literatur keine Angaben gefunden.

Material und Methodik

Zu den Versuchen wurden 5 erwachsene Ziegenböcke verwendet. Drei Tieren wurde eine Kontrollfutterdosis verabreicht, die 1 kg Wiesenheu, 0,5 kg Gerste, 5 g mineralisches Gemisch, 5 g Salz enthielt. Die beiden anderen Tiere erhielten eine Versuchsdiät (1 kg Weizenstroh, 0,46 kg Stärke, 30 g Harnstoff, 0,5 g Sacharin, 5 g Salz, 5 g mineralisches Gemisch). Nach dreiwöchiger Adaptation der Tiere zu den erwähnten Diäten wurde Blut durch Punktion der Jugularvene zur Zeit kurz vor der Morgenfütterung entnommen. In wöchentlichen Intervallen wurde das Blut noch zweimal untersucht. Folgende Bestimmungen wurden ausgeführt.

Gesamt-N, Eiweiß-N- und Reststickstoffgehalt wurde durch Nesslerisation nach der von HOŘEJŠÍ u. Mitarb. (1964) angeführten Methodik bestimmt.

Die papierelektrophoretische Fraktionierung der Serumeiweißstoffe, der Serum-Lipoproteine und Glykoproteine wurde nach den üblichen Methoden (DITTMER, 1961; BÜCHER, 1961) durchgeführt.

Bei der Fraktionierung der Eiweißstoffe des Blutserums durch Gelfiltration auf der Sephadex G-200 wurde nach der Methode FLODINS und KILLANDERS (1962) vorgegangen.

Die Enzymeaktivität wurde folgendermaßen bestimmt: Malat- und Laktat-Dehydrogenase nach ŠEVELA und TOVÁREK (1959, 1960), Sorbit-Dehydrogenase nach POJER u. Mitarb. (1963), Transaminase des Glutamatpyruvats (GOT) nach REITMAN und FRANKEL (1957), Amylase nach STREET (Zit.: BERGMAYER, 1965) und alkalische Phosphatase nach BESSEY und Mitarb. (1946).

Die Ergebnisse wurden statistisch bewertet.

Ergebnisse und Besprechung

Die Ergebnisse sind in den Tabellen I—III zusammengefaßt.

Tabelle I enthält die Stickstoffwerte und die Ergebnisse der Gelfiltration. Es sei erwähnt, daß die Fraktion »S« Eiweiße mit einem Molekulargewicht über 200 000 enthält, die Fraktion »M« um 150 000, und die Fraktion »L« wird von leichten Molekülen (M. G. um 70 000) gebildet. Zur besseren Veranschaulichung dient die Abbildung 1, die die einzelnen Eiweißfraktionen auf Grund der Gelfiltration bei einem Tier der Kontrollgruppe darstellt.

Aus den Angaben der Tabelle I geht weiterhin hervor, daß die Wirkung der halbsynthetischen Diät nur in bezug auf den Reststickstoff signifikant war, während die Veränderungen der übrigen Werte die Grenze der Signifikanz nicht erreichten. Dabei wurden die besonders geringen Unterschiede bei der Gelfiltration beider Gruppen verzeichnet.

Tabelle II enthält übersichtlich die relative Verteilung der einzelnen Fraktionen der Serumproteine und deren komplexen Verbindungen — der Lipo- und Glykoproteine. Zur besseren Veranschaulichung dient die Abbildung 2, wo die Kurven der Elektrophoregramme von demselben Tier dargestellt sind. Verwendet wurde das Densitometer ERI-10 (Zeiss).

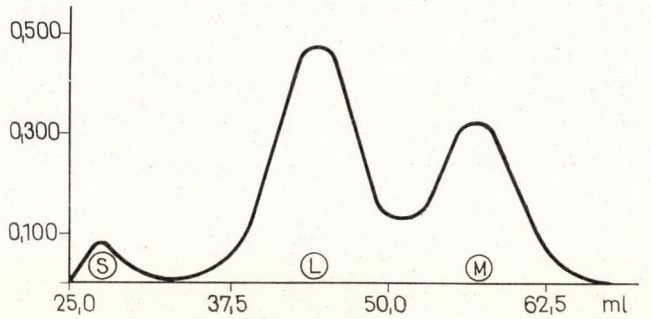

Abb. 1. Die einzelnen Eiweißfraktionen auf Grund der Gelfiltration bei einem Tier der Kontrollgruppe. Fraktion mit Molekulargewicht über 200,000 (S), um 150,000 (M), um 70,000 (L)

Vergleicht man die elektrophoretisch bestimmte Verteilung der Serumproteine beider Tiergruppen, so läßt sich feststellen, daß die Unterschiede recht gering und nicht signifikant sind.

Die dritte Gruppe bilden die Enzymuntersuchungen, deren Ergebnisse in der Tabelle III zusammengefaßt wurden. Unter den Transferasen, Hydrolasen und Oxydoreduktasen weisen nur zwei Enzyme auf Grund der Varianzanalyse eine signifikante Differenz auf. Das erste von diesen, die Sorbitdehydro-

Tabelle I

Serum-Stickstoffwerte und Serumeiweißgelfiltrationswerte (Sephadex G-200)

	Diät		Signifikanz der Unterschiede
	Kontroll- $\bar{x}\pm s_{\bar{x}}$	Halbsynthet. — $\bar{x}\pm s_{\bar{x}}$	
Gesamt-N — mg%	927,6±32,1	1012,2±41,8	—
Eiweiß-N — mg%	907,6±26,4	975,7±34,2	—
Rest-N — mg%	20,0± 3,9	36,5± 3,9	+
Gelfiltration der Eiweiße			
Fraktion S — %	4,2±0,59	5,4±0,92	—
Fraktion M — %	46,3±2,18	46,2±1,94	—
Fraktion L — %	49,5±2,98	48,4±3,04	—

+ Signifikant; — Nicht signifikant

Tabelle II

Eiweißfraktions-, Lipoprotein- und Glykoproteinwerte im Blut

	Diät		Signifikanz der Unterschiede
	Kontroll-	Halbsynthet.	
Eiweißfraktionen			
Albumin — %	44,7	41,2	—
α-Globuline — %	11,1	9,5	—
β_1-Globuline — %	10,3	10,5	—
β_2-Globuline — %	6,1	8,5	—
γ-Globuline — %	27,8	30,3	—
Lipoproteine			
Fraktion Nr. 1 — %	55,1	58,6	—
Fraktion Nr. 2 — %	25,6	27,7	—
Fraktion Nr. 3 — %	19,3	13,7	—
Glykoproteine			
Fraktion Nr. I — %	16,4	14,5	—
Fraktion Nr. II u. III — %	58,8	62,6	—
Fraktion Nr. IV — %	24,8	22,9	—

\+ Signifikant; — Nicht signifikant

Tabelle III

Enzymaktivitätswerte

	Diät		Signifikanz der Unterschiede
	Kontroll-	Halbsynthetische	
Laktatdehydrogenase U/l g Eiweiß	98,3	79,8	—
Malatdehydrogenase U/l g Eiweiß	157,6	158,5	—
Sorbitdehydrogenase U/l g Eiweiß	1,23	3,09	+
Transaminase des Glutamat-Oxalazetats U/l g Eiweiß	21,0	20,3	—
Transaminase des Glutamat-Pyruvats U/l g Eiweiß	10,3	12,0	—
Alkalische Phosphatase U/l g Eiweiß	92,9	48,7	+
Amylase U/l g Eiweiß	3,22	3,52	—

\+ Signifikant; — Nicht signifikant

genase im Blutserum der Tiere mit halbsynthetischer Diät zeigte eine sehr erhöhte Aktivität, während sich die Aktivität des zweiten Enzyms — der alkalischen Phosphatase — bei diesen Tieren im Gegenteil fast auf die Hälfte der Normalwerte verminderte. Die Unterschiede der Aktivität bei den übrigen untersuchten Enzymen waren in der Mehrzahl der Fälle sehr gering und auch statistisch nicht signifikant.

Die Ergebnisse der eigenen Untersuchungen weichen von den der anderen Autoren ab (GORCZYCA und Mitarb. 1959), teils stimmen sie im Gegenteil verhältnismäßig gut überein (HACKETT, zit.: LONG und Mitarb., 1961).

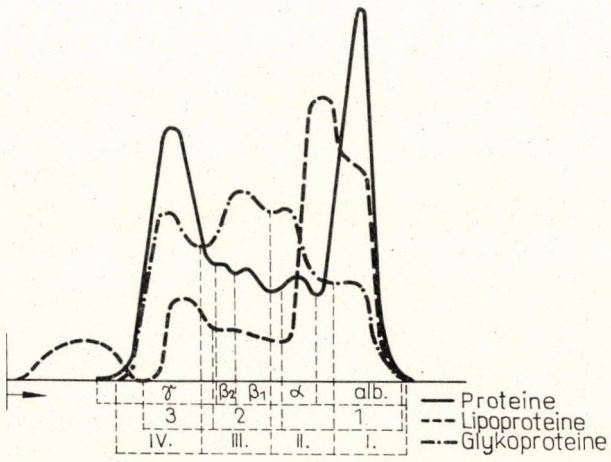

Abb. 2. Die relative Verteilung der einzelnen Fraktionen der Serumproteine und deren komplexen Verbindungen

Die Ursachen können möglicherweise eher in der Uneinheitlichkeit der experimentellen Bedingungen erblickt werden, dennoch lassen sich die erhaltenen Abweichungen nicht als unverhältnismäßig groß bewerten. Es sei auch der Umstand erwähnt, daß Serum-Normalwerte noch nicht festgestellt sind, so daß man sich bei der Beurteilung der gefundenen Werte auf keinen anerkannten Maßstab stützen kann.

Wir nehmen deshalb an, daß die gestellte Frage, sowohl vom laboratorischen Gesichtspunkt aus, als auch vom gesundheitlichen Gesichtspunkt gegenwärtig nicht genügend objektiv beurteilt werden kann. Aus unseren Ergebnissen ergibt sich lediglich die Schlußfolgerung, daß bei der Verfütterung halbsynthetischer Diät bei Wiederkäuern die Abweichungen von den normalen Werten im Blute sehr gering sind, was dafür zeugt, daß es im Gesamtmetabolismus der Tiere zu keinen wesentlichen Abweichungen von dem normalen Zustand des Organismus kam.

ZUSAMMENFASSUNG

Es wurden Blutanalysen bei drei Ziegenböcken ausgeführt, die eine Normaldiät (Wiesenheu und Gerste) und bei zwei Ziegenböcken, die eine halbsynthetische Diät (Weizenstroh, Stärke, Harnstoff mit der üblichen Mineralstoffergänzung) erhielten. Folgende Blutanalysen wurden durchgeführt: Gesamteiweiße, Reststickstoff, Gelfiltration der Serumeiweiße auf Sephadex G-200; Papierelektrophoretische Fraktionierung der Serumproteine, Lipoproteine und Glykoproteine, Serum-Laktat-, Malat-, Sorbit-Dehydrogenase, alkalische Phosphatase und Amylaseaktivität.

Die Ergebnisse wurden durch Varianzanalyse ausgewertet. Es wurde festgestellt, daß bei der halbsynthetischen Diät im Blutserum nur die Zunahme des Reststickstoffs und der Aktivität der Sorbitdehydrogenase, ferner die Abnahme der Aktivität der alkalische Phosphatase signifikant war.

*

Für die Ermöglichung der Blutentnahme von den Versuchstieren danke ich dem Lehrstuhl für Ernährung und Fütterung der Wirtschaftstiere der Landwirtschaftlichen Hochschule in Brno.

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RAPID DEMONSTRATION OF BULL TRICHOMONADOSIS IN UNSTAINED SMEAR PREPARATIONS FROM PREPUTIAL SCRAPINGS

By

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The introduction of artificial insemination has greatly reduced the spread of infection with *Trichomonas foetus*. In Hungary, the trichomonadosis of bulls has recently occurred exclusively in cattle farms where service is still by natural way or, infrequently, in artificial insemination centres among newly procured sires. Since the *Trichomonas* remains viable both in diluted semen stored at 2—4° C and deep frozen semen stored at —79 or —196° C, in the latter case even for 5 years, the semen from contaminated bulls may become a source of infection in several farms. Thus, a rigorous checking of newly procured sires in the artificial insemination centres is imperative.

Practical experience has shown that in the acute stage when the parasites are present only under the prepuce, trichomonadosis is well controllable by the common contact drugs. But in the chronic stage, when parasites have gained entrance to the secondary reproductive organs, all therapeutic measures fail and temporary improvement is followed by recurrence. Therefore, the early demonstration of trichomonadosis is extremely important.

The common methods of the examination of preputial contents do not appropriately serve this purpose. In the practice, examination has often to be repeated several times until infection can be demonstrated. This is due partly to the anatomical structure of the preputial mucosa, which has many folds, partly to the hiding of few protozoa at the bottom of the folds. In addition, the applied procedure (centrifugation, cultivation, microscopic examination) takes 10—15 days after sampling. Thus, information on the contamination or "cleanness" of the sire reaches the practitioner too late.

With these facts in mind, attempts to develop new methods for the demonstration of trichomonadosis have been made in many countries. In the U.S.A., WITHERSPOON and WALKER (1966) have carried out sampling by sterile gauze tampons instead of rinsing fluid, taking samples from the exposed penis itself and from the folds of the preputial vault. With this procedure, many of those sires which had been found "clean" by the rinsing method proved to be infected.

In the Soviet Union, chiefly in the Ukraine, instead of rinsing fluid, preputial scrapings are used as samples. They are obtained by a special instrument constructed by KORTCHAK

(see AVANESOV and PYASIK, 1968). The ensheathed instrument is passed under the prepuce, then the sampling head is thrusted forward and the sample thus obtained is inoculated into nutrient medium.

In a comparative study on 3000 preputial scrapings and semen samples, OSETROV and ZHADOVEC (1968) have found that the most reliable results can be obtained with the scrapings.

In 1967, we examined many preputial scrapings parallel with rinsing fluids.

Experimental

On a total 451 pedigree sires from 11 artificial insemination stations which had been found "clean" by the rinsing method, and 71 breeder bulls from 33 farms, were tested for trichomonadosis. Preputial scrapings were obtained with modified KORTCHAK's instrument (Figs 1 and 2). Sampling was undertaken either immediately before collection of the ejaculate or 48 hours after the last service. Prior to sampling, the surroundings of the prepuce were thoroughly washed with soap and water of body temperature, and dried. Since in most cases several bulls were examined in sequence, the instrument was disinfected in boiling water for 5—10 minutes prior to each use, and rinsed with neutral saline immediately before application. In most cases the sample was taken in the stable or on the pasture and only infrequently were the bulls restrained. The animals were steadied by the nose, and at the hock on the side where the sampling was made. In this position the instrument hold in one hand was inserted with a single down-to-upward movement behind the prepuce, which was hold open with 3 fingers of the other hand to prevent contamination. The head of the instrument was thrusted forward to the reflection fold, then drawn back and thrusted upward again. In this way 21—30 uniform scraping movements were made. To prevent erection, care was taken to operate lightly and without strain. If erection had yet occurred, it ceased after slowly moving the instrument and scraping thus could be continued. Removal of the instrument was made cautiously, holding the prepuce open again with three fingers to prevent contamination.

One drop of each scraping was placed on a slide, covered with a coverslip and examined in dark field at a 100—120fold magnification. The bulk of the scrapings consisted of mucus from the preputial vault and a mass of epithelial cells. If required, the material was diluted with saline of body temperature. In positive cases the protozoa, performing typical vigorous movements, were regularly detected in the 1st—5th field. The rest of the scrapings was rinsed from the instrument with maximum 2 ml of neutral saline or Manninger's rinsing solution (HUTYRA *et al.*, 1959) and collected in a test tube for further examination. After sedimentation for 40 minutes, the sediment was re-examined. To check the observations made on the unstained slides, 0.2—0.3 ml amounts of the sediment were inoculated into appropriate media.

In the case of positive results, the examination was repeated several times. In the infected herds control examinations were carried out every month. The samples taken from sires at the farms were collected in test tubes in Manninger's rinsing fluid and were immersed in ice for shipping to the laboratory, where they were inoculated into growth media. In cases of therapeutical treatment, the examinations were repeated after 10, 30, 60 and 90 days.

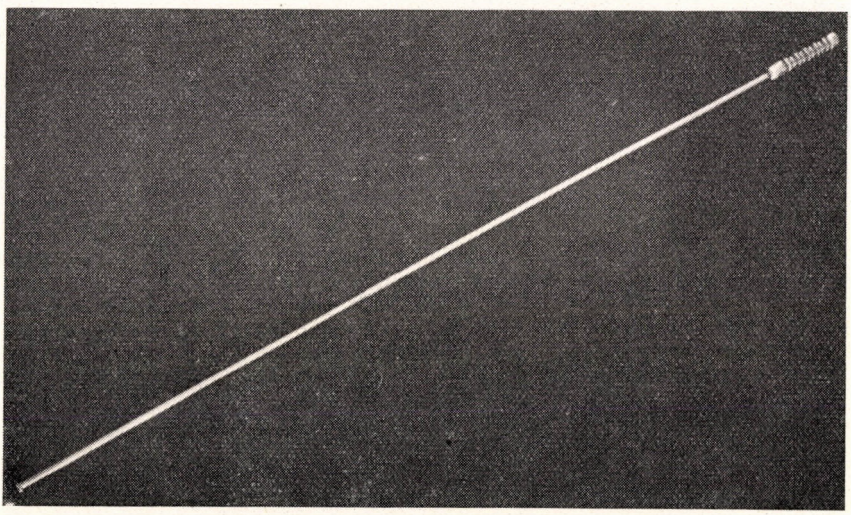

Fig. 1

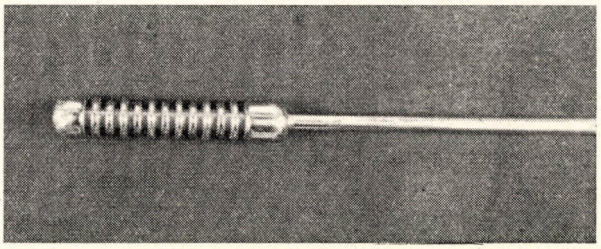

Fig. 2

Results

In none of the 522 animals examined did the intervention cause traumatic injury or complication. In the artificial insemination centres, sampling immediately preceding the collection of the ejaculate had no influence on semen production. The results obtained by rinsing and scraping in one and the same farm have been compared (Table I).

It is shown that the scraping method immediately enabled the detection of the sires infected with *Trichomonas foetus*. Spread of the parasitosis could be checked by the usual measures (isolation, *etc.*). The later checkings agreed well with the initial findings. The results obtained by the microscopic examination of preputial scrapings were confirmed by the culturing experiments. This implies that by microscopic examination of unstained preputial scrapings the infection of sires with *Trichomonas foetus* can be demonstrated in 10—15 minutes.

Table I

Comparative data on demonstration of *Trichomonas foetus* organisms in preputial scrapings and rinsing fluid from sires in a cattle farm

Number of bulls examined	Date	Number of bulls with trichomonadosis diagnosed by traditional rinsing fluid test	Date	Number of bulls with trichomonadosis as assessed from preputial scrapings in			Date	Number of bulls with trichomonadosis as assessed by comparative rinsing test
				unstained slides	40-minute sediment of suspended scrapings	culture		
20	1966		1967				1967	
	28.12	3	28.1	9	9	9	30.1	3
			30.3	9	9	9		
			13.4	2*	2*	2*		
			26.5	2*	2*	2*		

* 7 pedigree bulls could be cured

To obtain reliable results, it is essential that scrapings should be collected 48 hours after the last service as before that time the number of protozoa may by too low. Drug treatment of the prepuce must not be made for 10 days prior to sampling. Disregarding of theses circumstances may lead to false results: it is therefore recommended that in unknown herds the examination should be repeated after 3 days. If no microscopic examination can be performed on the spot, the sediment of rinsed scrapings should be used either for microscopic examination or for culturing. For culturing rinsed scrapings should be stored at +5° C.

SUMMARY

Though the extensive practice of artificial insemination has greatly reduced the *Trichomonas foetus* infection of bulls, it still occurs among newly procured sires in artificial insemination centres or among pedigree bulls in cattle herds where service is still by the natural way. Thus, early detection of the parasitosis is essential to prevent its extensive dissemination. The common method of the diagnosis, based on the isolation of the parasite from preputial rinsing fluid, has failed to serve this purpose. Initiated by similar attempts reported in the literature

the authors describe a rapid reliable method of demonstration of *Trichomonas foetus* by microscopic examination of unstained preputial scrapings taken with an appropriate instrument. The results obtained by screening of a total of 522 bulls have been confirmed by culturing experiments.

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ERYTHROZYTENSEDIMENTIERUNG UND HÄMATOKRITWERTE BEI GESUNDEN ENTEN

Von

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(Eingegangen am 4. Dezember 1968)

Die Aufgabe dieser Arbeit ist die Bestimmung der Erythrozytensedimentation und der Hämatokritwerte bei gesunden Enten mit Rücksicht auf Alter und Geschlecht.

Literatur

Die bisherigen Erfahrungen weisen auf die Abhängigkeit der Sedimentierungsgeschwindigkeit der Erythrozyten auch bei den Hühnern von der Erythrozytenzahl (STURKIE und TEXTOR, 1960), der Temperatur der Umgebung (FRIED und JANTOŠOVIČ, 1962), der Lagen der Sedimentierungspipetten (STURKIE und TEXTOR, 1958; ŠTERNOVSKÝ, 1962) sowie von dem Alter (JANTOŠOVIČ, 1967) hin.

Nach DIESEM und Mitarb. (1958) ist es möglich, die Hämatokritmethode zur Feststellung des Volumens der Blutkörperchen als eine diagnostische Hilfsmethode auch beim Vogelblut anzuwenden. Diese Autoren bewiesen einen direkten Zusammenhang zwischen der Erythrozytenzahl und des Hämetokritwertes bei Hühnern. Die Mikrohämatokritmethode soll konstant einen höheren Prozentsatz des gesamten Volumens der Blutkörperchen als die WINTROBsche Methode bieten.

Die zugänglichen Literaturangaben über die Hämatokritwerte und Sedmentierungsgeschwindigkeit der Erythrozyten bei gesunden Enten scheinen mangelhaft zu sein, obwohl diesbezügliche Untersuchungen bei den Hühnern mehrere Autoren durchgeführt haben. Über die Hämatokritwerte bei gesunden Enten finden sich derzeit nur 2 uns zugängliche Angaben, die aber voneinander weitgehend abweichen. BOND und GILBERT (1948) haben folgende Werte ermittelt: Tauchenten (redhead x canvasback) 37 $\pm$ 3 und Fleckenten (mallard $\times$ black) 43 $\pm$ 2. SOLIMAN und Mitarb. (1966) geben bei 6—10 Monate alten Enten einen durchschnittlichen Wert von 33,0%. Über die Sedimentation der Erythrozyten bei gesunden Enten haben wir bisher nur eine einzige Literaturquelle gefunden (GONGAZA und CADUNGOG-ZABALLERO, 1964). Es wurde in dieser Hinsicht kein bedeutender Unterschied zwischen den Enten und Enterichen festgestellt. Die gefundenen Werte nach einer Stunde sind, wie folgt: bei Enten 8,0—16,5 mm (Ø 10.86 $\pm$ 0,17), bei Enterichen 8,5—16,5 mm (W 11,05 $\pm$ 0,17); gemeinsame Werte: 8,0—16,5 mm (Ø 10,94 $\pm$ 0,14).

Methodik

Das Blut wurde aus der V. cutanea ulnaris, für die Sedimentierung durch Herz-Punktion gewonnen. Die Sedimentierung wurde in den Westergreenschen Pipetten, beim Neigungswinkel 60° und bei Zimmertemperatur 18—22° C durchgeführt. Das Blut wurde mit 3,8%iger Natriumzitratlösung im Verhältnis 4 : 1 versetzt.

Die Sedimentierung wurde insgesamt bei 46 Enten, 21 Enterichen und bei 10 zweimonatigen Enten durchgeführt. Bei 7 Enten wurde zuerst orientierungshalber die senkrechte Sedimentierung vorgenommen.

Die Hämatokritwerte wurden mittels des WINTROBschen Hämatokrits festgestellt, wobei die Blutmuster 30 Minuten unter 3000 U/M zentrifugiert wurden. Anzahl der untersuchten Tiere: 47 Enten, 20 Enteriche und 10 zweimonatige Enten.

Ergebnisse

I. Die Sedimentierung der Erythrozyten verläuft bei der senkrechten Stellung der Pipetten sehr langsam (Tab. I), besonders in der ersten Stunde.

Mit Rücksicht auf die langsame Sedimentierung wurde im weiteren die Sedimentierung unter der schiefwinkligen Pipettenstellung (60°) untersucht. Die Ergebnisse sind in Tabelle II zusammengefaßt.

Tabelle I

Erythrozytensedimentation bei gesunden Enten
(durchschnittliche Werte)

Pipettenstellung	n	15 Min.	30 Min.	1 St	6 St	24 St	48 St
90°	7	0,0	0,14	1,71	23,42	74,0	98,0
60°	46	4,97	12,5	25,15	73,54	106,60	110,43

Tabelle II

Schiefwinklige Sedimentation bei gesunden Enten
(Neigungswinkel: 60°)

Kennziffern	15 Min	30 Min	1 St	6 St	24 St	48 St
n	46	46	46	46	46	46
$\bar{x}$	4,97	12,5	25,15	73,54	101,60	110,43
s	2,08	4,24	5,74	8,06	6,14	3,03
Min-Max	1—9	6—22	16—37	47—92	90—116	95—125

Bei den Enterichen, im Vergleich zu den Enten, wurden nur kleine Unterschiede in den Sedimentierungswerten festgestellt. In der ersten Phase des Sedimentierungsvorganges (die erste Stunde) sedimentieren die roten Blutkörperchen etwas langsamer als bei den Enten, von der 6. Stunde ab nimmt die Sedimentierungsgeschwindigkeit mäßig zu. Werden die Sedimentierungs-

werte in der Periode zwischen 24 und 48 Stunden verglichen, dann ist festzustellen, daß die Durchschnittsunterschiede fast 10 mm betragen. Daraus geht hervor, daß die Sedimentierung bei Enten wie bei Enterichen binnen 24 Stunden nicht beendet ist.

Bei jungen Individuen (im Alter von 2 Monaten) verläuft die Sedimentierung schneller als bei erwachsenen Tieren.

Tabelle III

Schiefwinklige Sedimentation bei gesunden Enterichen
(Neigungswinkel: 60°)

Kennziffern	15 Min	30 Min	1 St	6 St	24 St	48 St
n	21	21	21	21	21	21
$\bar{x}$	4,33	11,04	24,09	74,85	103,14	112,19
s	1,6	5,9	5,2	7,5	6,0	5,9
Min-Max	2—7	6—16	15—33	59—86	92,111	97—121

Tabelle IV

Erythrozytensedimentation bei jungen Enten
(Alter: 2 Monate)

Kennziffern	15 Min	30 Min	1 St	6 St	24 St	48 St
n	10	10	10	10	10	10
$\bar{x}$	4,8	12,0	25,7	81,0	107,0	117,0
s	1,2	2,3	5,0	6,9	4,0	3,6
Min-Max	3—6	9—17	20—36	71—94	101—114	112—123

Tabelle V

Hämatokritwerte und Erythrozytenzahl

	Hämatokritwerte				Erythrozyten	
	n	$\bar{x}$	s	Min-Max	$\bar{x}$	s
erwachsene Enten	47	42,78	2,20	39—47	2 733 599	321 170
Enteriche	20	43,00	2,20	39—46	2 716 666	277 810
junge Enten	10	39,80	1,95	37—43	2 674 000	266 350

II. Hämatokritwerte. Der Durchschnittsunterschied zwischen den Hämatokritwerten bei Enten und Enterichen ist verhältnismäßig gering (0,22%). Bei dieser Gattung des Wassergeflügels ist also praktisch kein Geschlechtsunterschied der Hämatokritwerte zu beobachten, der bei Hennen und Hähnen so stark ausgeprägt ist; bei den jungen Enten wurden niedrigere Werte registriert. Die Ergebnisse sind in Tabelle V dargestellt.

Mathematisch-statistische Analyse

Die ermittelten numerischen Daten wurden auch der mathematisch-statistischen Analyse unterworfen, um die biologische Variabilität des Versuchsmaterials festzusetzen und die Signifikanz der beobachteten Unterschiede zu prüfen. Die Berechnung der statistischen Angaben, d. h. $\bar{x}$ und t, geschah nach den üblichen Formeln. Die Signifikanz der Differenz zwischen den betreffenden arithmetischen Mittelwerten wurde nach STUDENT vorgenommen.

Folgende Ergebnisse sind zu verzeichnen:

1. Die Unterschiede zwischen den Mittelwerten bei der senkrechten und schiefwinkligen Sedimentation (Tab. I) sind so offensichtlich, daß sie die statistische Prüfung überflüssig machen.

2. Unterschiede zwischen der Sedimentationsgeschwindigkeit bei den Enten und Enterichen (Tab. II und III): die Differenzen zwischen den Mittelwerten in allen Zeitpunkten lassen sich statistisch nicht beweisen (bei Irrtumswahrscheinlichkeit $\alpha = 5\%$).

3. Unterschiede zwischen der Sedimentationsgeschwindigkeit bei den erwachsenen und jungen Enten (Tab. III und IV): die Differenzen zwischen den Mittelwerten sind in der Anfangsperiode (innerhalb der ersten Stunde) nicht signifikant, von der 6. Stunde ab dagegen hochsignifikant ($\alpha = 1\%$).

4. Die Differenzen der Hämatokritwerte zwischen den erwachsenen Enten und Enterichen (Tab. V) sind nicht signifikant, zwischen den erwachsenen und jungen Enten dagegen schon mit der statistischen Sicherheit von 99% nachweisbar.

Besprechung

Der Sedimentierungsverlauf der Ertyhrozyten bei Enten, ähnlich wie bei Hennen, ist langsam. Deshalb ist es zweckmäßig, die schiefwinklige Sedimentation durchzuführen. In unseren Versuchen hat sich die 60°ige Pipettenstellung bewährt. Wie aus unseren Untersuchungen folgt, sind die Unterschiede bei Enten und Enterichen sehr gering (1—2 mm), was auch GONZAGA und CADUNGOG-ZABALLERO (1964) bemerkten. In den ersten Stunden sedimentieren die Erythrozyten der Enteriche langsamer als die der Enten, von der 6. Stunde ab ist es umgekehrt.

Obwohl der Geschlechtsdimorphismus bei Hennen und Hähnen sich auch in der Zahl der Erythrozyten äußert (STURKIE und TEXTOR, 1960; LUCAS und JAMROZ, 1961; JANTOŠOVIČ, 1967) und auch die Unterschiede im Verlauf der Sedimentierung auffallend sind (STURKIE und TEXTOR, 1960), ist die Zahl der Erythrozyten bei Enten und Enterichen beinahe gleich (Tab. V), was auch den ungefähr gleichen Werten der Sedimentierung entspricht.

Bei der Bewertung der Sedimentierungsgeschwindigkeit soll auch auf das Alter der Tiere Rücksicht genommen werden. ŠTERNOVSKY (1962) und JANTOŠOVIČ (1967) stellten fest, daß die Sedimentierung bei jüngeren Hühnern schneller verläuft, was von uns auch bei Enten beobachtet wurde. Die Beschleunigung der Sedimentierung wird wohl durch die niedrigere Zahl der Erythrozyten bei jüngeren Tieren bedingt.

Analog der Sedimentierung stehen auch die Hämatokritwerte und die Erythrozytenzahl in einer und geraden Korrelation: bei der Verminderung der Erythrozytenzahl nehmen auch die Werte des Hämatokrits ab und umgekehrt. Bei Hennen und Hähnen wurden auffallende Unterschiede der Hämatokritwerte festgestellt (LUCAS und JAMROZ, 1961; JANTOŠOVIČ, 1967). Die nachgeweisenen Unterschiede der Hämatokritwerte und der Erythrozytenzahl haben wir auch bei den Truthennen und Truthähnen festgestellt (JANTOŠOVIČ und FRIED, 1968). Die gleiche Gesetzmäßigkeit gilt auch für die Enten.

Im Vergleich mit den Werten bei Hennen sind die Hämatokritwerte bei Enten bedeutend höher, wenn auch offensichtliche Unterschiede der Erythrozytenzahl nicht vorhanden sind, was hauptsächlich durch die Größe der Erythrozyten bedingt ist. Größenverhältnisse der Erythrozyten bei Enten: $8{,}1 \times 13{,}4\ \mu$ (eigene Beobachtung), beim Huhn: $7{,}5 \times 12{,}0\ \mu$ (WIRTH, 1950; SCHERMER, 1954). Die Werte, die KOLB (1952) für die Enten angibt, sind niedriger, als wir festgestellt haben. Fast die gleichen Werte wurden aber bei zweimonatigen Enten registriert. Wesentlich kleinere Durchschnittwerte, im Vergleich mit unseren Ergebnissen, stellten SOLIMAN und Mitarb. (1966) fest.

ZUSAMMENFASSUNG

Die Geschwindigkeit der Erythrozytensedimentation und Hämatokritwerte werden bei gesunden, erwachsenen und jungen (zweimonatigen) Enten studiert.

Die Geschwindigkeit der Sedimentation wurde bei 46 Enten, 21 Enterichen und 10 Entchen in den Westergreenschen Pipetten von Lichte 2,5 mm und bei Neigung von 60° verfolgt. Bei beiden Geschlechtern sind die Unterschiede der durchschnittlichen Werte gering (1—2 mm) und nicht signifikant. Die Erythrozytensedimentation wird von dem Alter in dem Sinne beeinflußt, daß sie bei jungen Tieren schneller verläuft.

Die Hämatokritwerte wurden bei 47 Enten, 20 Enterichen und 10 Entchen üblicherweise festgestellt. Geringe nicht signifikante, vom Geschlecht abhängige Unterschiede wurden beobachtet. Die niedrigeren Werte wurden bei jungen Enten vorgefunden.

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ERFAHRUNGEN BEI DER FLEISCHBESCHAU VON RINDERN, DIE AN RETICULOPERITONITIS PERFORATIVA LITTEN, MIT BESONDERER BERÜCKSICHTIGUNG AUF DAS VORKOMMEN DES CORYNEBACTERIUM PYOGENES TYP. ANIMALIS

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Die Probleme, die sich bei der Diagnostik, Prophylaxe, der konservativen und der chirurgischen Therapie der Reticuloperitonitis perforativa (RPP) und ihrer Komplikationen melden, sind allgemein bekannt (Balázs, 1967; B. Kovács und Szokolóczy, 1965; Horváth und Mitarb., 1966). Leider ist der tierärztliche Eingriff oft erfolglos. In diesem Falle ist die Rettung des Fleischwertes das Ziel. Die Beurteilung der RPP-Fälle wirft aber oft auch vom Gesichtspunkt des Tierarztes, der die Beschau vornimmt, zahlreiche Probleme auf.

Ebendeshalb dürfte die Veröffentlichung unseres mehr als anderthalbjährigen Materials von Nutzen sein, wobei wir auch die pathologisch-anatomischen, mikrobiologischen und nahrungsmittelhygienischen Zusammenhänge zu besprechen wünschen.

Material und Methodik

In unserer Veröffentlichung verarbeiteten wir die auf dem Schlachthof von Pécs (zwischen 31. 1. 1966 und 31. 7. 1967) bei den üblichen und Notschlachtungen beobachteten Fälle sowie die uns zur Laboruntersuchung von der Umgebung eingesandten RPP-Fälle.

Im Laufe unserer Untersuchungen führten wir die Summierung und die statistische Verarbeitung der Anamneseangaben, der pathologisch-anatomischen und der mikrobiologischen Befunde sowie die der endgültigen Fleischbeschauungsangaben durch.

Zu den ergänzenden bakteriologischen Untersuchungen wendeten wir folgende Methode an: Von den Organen der Tiere impften wir aus der Milz, der Leber, der Niere, aus zwei Muskelproben, aus den präskapularen und den gegenseitigen poplitealen Lymphknoten sowie aus sonstigen pathologisch veränderten Teilen (z. B. aus abgekapselten interabdominalen Abszessen, Lungensegmenten) auf Nährboden über. Als Nährboden verwendeten wir Bouillon (pH: 7,4), 10% Rinderblutagarplatten, 5% Rinderserum enthaltende Agarplatten sowie Drigalski-Medium mit Bromthimolblau als Indikator. Zum

Nachweis der Salmonellen benutzten wir die Bouillon mit Kaliumtetrathionat nach PREUSS. Die Muskelproben und die Lymphknoten wurden auch in Leberinfusionsbouillon mit Vitamin B_1-Gehalt und Traubenzucker überimpft und wegen Vernichtung der vegetativen Formen bei 80° C Wärme behandelt. Bei der Züchtung der anaeroben und mikroärophilen Keime wurde zur Sicherung der anaeroben Verhältnisse die Methode von KOCH (1934) angewandt. Auf die Innenfläche der Petri-Schale mit dem Blutagar brachten wir Papierbeutel mit Pyrogallussäure, Kaliumkarbonat und Bolus alba an, wonach die Petri-Schale mit Paraffin luftdicht verschlossen wurde. Die Bouillon-Röhrchen wurden nach 24stündiger Inkubation wiederholt auf Blutagarplatte überimpft und einer anaeroben Inkubation unterworfen. Die endgültige Beurteilung der Untersuchungen erfolgte erst nach 48 Stunden.

Vorkommenshäufigkeit

Das prozentuelle Verhältnis der RPP-Fälle zu der Zahl der gesundgeschlachteten und notgeschlachteten Tiere zeigt die Tabelle 1.

Tabelle 1

Prozentuelles Verhältnis der RPP-Fälle

	Zahl der geschlachteten Tiere	Zahl der RPP-Fälle	% der RPP-Fälle
übliche Schlachtung	20 257	65	0,32%
Notschlachtung	645	60	9,34%
Provinz*	—	14	—
insgesamt	20 902		9,66%

* Nur für bakteriologische Untersuchungen bestimmtes Material ist eingelangt.

Erwähnt sei, daß 46,7% aller RPP-Fälle aus Tieren stammten, die im Laufe der normalen Schlachtungen geschlachtet wurden und als gesund erschienen. Sicherlich ist dies damit erklärbar, daß sich die RPP-Symptome nicht in allen Fällen klar manifestieren, fernerhin, daß die Eigentümer ihre Tiere nach der Beobachtung der ersten Symptome nicht immer behandeln lassen, sondern versuchen, sie als Fleisch zu verwerten.

Pathologisch-anatomische Gruppierung

Die pathologisch-anatomische Gruppierung des Materials ist wegen Überdeckungen nicht einfach. Bei der Einordnung der Fälle stellten wir stets die bedeutendste Veränderung in den Vordergrund (Tabelle II). In 83 (59,7%) von 139 Fällen ging die Perforation des Netzmagens mit akuter bzw. chroni-

scher Bauchfellentzündung von wechselnder Ausbreitung einher. In 25 von 83 Fällen war die Perforation frisch, die Bauchfellentzündung lokalisierte sich im allgemeinen nur auf einige, etwa männerhandgroße Gebiete, wobei es in der Regel gelang, auch den Fremdkörper zu finden. In 10 Fällen beobachteten wir, daß sich zwischen dem Netzmagen, der Bauchwand, dem Zwerchfell und der Milz — die sich infolge der akuten Perforation verklebten — ein unangenehm riechendes, dünnes, fibrinöseitriges Exsudat in ansehnlicher Menge ansammelte (»Diafragmaabszeß«).

In 56 Fällen drang der Fremdkörper nach Durchbohrung des Netzmagens weiter und beschädigte auch die Lunge (14,4%), das Herz (10,0%), die Milz (7,9%) und die Leber (7,1%). Endokarditis wurde in einem Fall beobachtet. Unseren Angaben und früheren Beobachtungen gemäß ist die Endocarditis ulcerosa eine überaus selten auftretende Komplikation der RPP. Mit Hilfe bakteriologischer Untersuchungen gelang es uns auch in diesem Fall nicht, die Krankheitserreger nachzuweisen, sondern nur die Saprophyten. Dies ist auch schon deshalb interessant, weil bei Rindern im Falle einiger Krankheiten, z. B. fibrinöse Gelenkentzündung bei den vergesellschafteten Endokarditiden oft eine *Corynebacterium-pyogenes*-Sepsis festzustellen ist. Es gilt als eine schwere Komplikation, wenn der Fremdkörper in die Milz perforiert. In diesen Fällen sind in der Leber häufig viele, erbsengroße Abszesse zu beobachten, die infolge des Milztraumas durch die portale Streuung zustande kamen.

Bei den Komplikationen konnte selbstverständlich stets auch eine in der Regel akute Bauchfellentzündung mit verschiedener Ausbreitung beobachtet werden.

Ergebnisse der bakteriologischen Untersuchung

In unseren 139 RPP-Fällen wurden bei 125 ergänzende bakteriologische Untersuchungen durchgeführt. Bakteriologische Untersuchungen wurden nur bei jenen Tieren nicht unternommen, bei denen mit Hilfe der Koch-Probe ein ekelerregender fremder Geruch wahrnehmbar war.

Bei der Beurteilung der Fälle wurden folgende Gesichtspunkte vor Augen gehalten. Sepsis wurde diagnostiziert, wenn der Krankheitserreger mindestens aus einem Eingeweide und einem Muskel oder Lymphknoten, oder aus einem der Organe und dem pathologischen Teil (meist aus einem Abszeß) herausgezüchtet werden konnte. Es sei bemerkt, daß in der Mehrzahl der Fälle die Krankheitserreger nur aus zwei Organen nachgewiesen wurden. Konnte der Krankheitserreger nur aus zwei Eingeweiden nachgewiesen werden, so wurde der Fall (diskutierbar) nicht als Sepsis bewertet.

Das Ergebnis der 125 ergänzenden bakteriologischen Untersuchungen gestaltete sich wie folgt: steril in 43 Fällen, positiv in 82 Fällen. Die Verteilung

der 82 positiven Fälle war folgende: *Corynebacterium pyogenes*-Sepsis wurde bei 27 Tieren, durch *Corynebacterium pyogenes* und beta-hämolysierende Streptokokken gemeinsam verursachte Sepsis bei 2 Tieren, durch *C. pyogenes* und (koagulase-positiven) *Staphylococcus albus* gemeinsam verursachte Sepsis in einem Fall wahrgenommen. Nach CSISZÁR (1964) können sich bei RPP auch verschiedene anaerobe Keime im Organismus vermehren. In unserem Material trat die durch anaerobe Keime verursachte Sepsis in zwei Fällen auf. Einfachheitshalber sei es hier erwähnt, daß in der Muskulatur Saprophyten in großer Anzahl nur ein einziges Mal beobachtet wurden.

Die veterinären Handbücher (SCHÖNBERG und ZIETZSCHMANN, 1951; CSISZÁR, 1964) weisen darauf hin, daß die RPP auch mit Salmonellen-Sepsis einhergehen kann. In unseren 125 ergänzenden bakteriologischen Untersuchungen war eine solche Salmonellenkomplikation nicht einmal wahrnehmbar.

In den Eingeweiden und in der Muskulatur wurden Saprophyten in kleiner Zahl bei 15 Tieren festgestellt (meist alfahämolysierende Streptokokken, Gram-positive Diplo- und Mikrokokken). Diese Resultate wurden dann festgestellt, wenn diese Keime auf mehreren Nährböden und aus Bouillons nach 24 Stunden bei Blutagar-Überimpfung herausgezüchtet werden konnten.

Die Kolonienzahl war nie höher als 10/Organ. Die Umstände sprechen dafür, daß die herausgezüchteten Bakterien aus dem Organismus des Tieres stammten und nicht durch künstliche Kontamination auf die Nährböden gelangt waren.

Der Nachweis des *C. pyogenes* gelang nur aus je einer Stelle, und zwar aus dem verkapselten Abszeß der Bauchhöhle in 15 Fällen, aus Leberabszessen in 7, aus der Lunge in 2, aus der Niere in 2 Fällen, während aus der Leber und Milz das Ergebnis in je einem Fall positiv war. Gleichzeitig aus zwei Organen (aus der Leber und der Niere zweimal, aus der Milz und der Niere ebenfalls zweimal) wurde *C. pyogenes* in 4 Fällen nachgewiesen. Aus einem Leberabszeß wurden Gram-positive Streptokokken, aus einem anderen Gram-positive Diplokokken gezüchtet.

Auf Grund unserer Erfahrungen sind wir der Meinung, daß bei der ergänzenden bakteriologischen Untersuchung des RPP-Materials in erster Linie mit dem Vorkommen des *C. pyogenes* zu rechnen ist.

Im Zusammenhang mit der Herauszüchtung des *C. pyogenes* möchten wir folgendes bemerken. Frühere Facharbeiten (OSTERTAG, 1932; SCHÖNBERG-ZIETZSCHMANN, 1951; STANDFUSS, 1952) erwähnen dieses Bakterium nicht als einen bei der bakteriologischen Fleischuntersuchung erwartbaren Keim, und auch die neuere Literatur befaßt sich verhältnismäßig selten mit diesem Krankheitserreger. Unseres Wissens erwähnt nur SMITH (1966) das Vorkommen von *C. pyogenes* bei Rindern, die an RPP erkrankt waren. Dieser überraschende Umstand ist wohl darauf zurückzuführen, daß die früheren Untersuchungsmethoden die Herauszüchtung dieses Bakteriums nicht ermög-

lichten, zumal sich die Untersuchungen in erster Linie auf den Nachweis der sog. fleischvergiftenden Keime richteten und zum Nachweis der sonstigen Krankheitserreger höchstens Bouillons und einfache Agar-Platte verwendet wurden. Bekanntlich gehört das *C. pyogenes* zu den anspruchsvolleren Bakterien und benötigt zu seinem Wachstum unbedingt natives Eiweiß. Dies ist für seine Auszüchtung aus dem tierischen Organismus in erhöhtem Maße gültig. Unseren Erfahrungen gemäß kann — bei Verwendung von Blutagar — durch Sicherung von anaeroben Keimen die Möglichkeit seines Nachweises (ZIERDT und Mitarb., 1968) wesentlich erhöht werden, weshalb wir in jedem Fall die hier ausführlich beschriebene anaerobe Auszüchtungsmethode anwendeten. Dabei wurde beobachtet, daß bei anaerober Züchtung das *C. pyogenes* fast doppelt so große Kolonien bildet als auf dem gleichen Nährboden unter aeroben Verhältnissen. Die Zuverlässigkeit der anaeroben Methode wird auch dadurch bewiesen, daß es uns im Verlauf unserer Untersuchungen, öfters gelang, aus Bauchhölenabszessen, aus nekrotisierten Lebergeweben auf mit anaerober Methode inkubierten Blutplatten mit *C. pyogenes* zusammen auch in Kolonien mit einem Durchmesser von 0,1—0,2 mm, wachsende, unangenehm stinkende, gasproduzierende, Gram-negative polymorphe Keime herauszuzüchten. Da die Weiterimpfung selbst unter Sicherung von streng anaeroben Verhältnissen nicht immer erfolgreich war, gelang die genaue Bestimmung ebenfalls nicht. Über ähnliche Erfahrungen berichteten HAUPT (1964) und FIEVEZ (1965), die gleichzeitig mit dem *C. pyogenes* Nekrosebazillen aus den Eutern an Mastitis erkrankter Kühe herauszüchteten.

C. pyogenes-Stämme tierischen Ursprungs verflüssigen das geronnene Löffler'sche Serum und das Gelatin, während sie die Lakmus-Milch gerinnen lassen, ansäuern und das Gerinnsel peptonisieren. Sie produzieren beta-Hämolysin. Sie agglutinieren die roten Blutkörperchen von Haustieren, in erster Linie die der Rinder und der Schafe (FORRAY, 1968).

Die Rolle von *C. pyogenes* in den eitrigen Prozessen der Haustiere ist allgemein bekannt, wobei sie aber auch aus der Schleimhaut, den Rachenmandeln, dem Uterussekret gesunder Tiere und dem Sperma von Zuchtstieren nachgewiesen wurden. SCHÖNE (1965) erklärt in seiner Mitteilung, daß dieses Bakterium ein krankheiterregender Keim sei, bei dessen Anwesenheit das Fleisch des Tieres für den Konsum ungeeignet ist.

Zusammenhang zwischen der endgültigen Fleischbewertung und dem pathologisch-anatomischen Bild

Tabelle III zeigt die endgültige Beurteilung des Fleisches in unseren Fällen. Wie ersichtlich, mußte bei 81 Tieren — von insgesamt 139 RPP-Fällen — das Fleisch in irgendeiner Form beanstandet werden (58,3%). Wie

Tabelle 2

Häufigkeit der pathologisch-anatomisch gruppierten Fälle und deren Einfluß auf die Fleischbewertung

Pathologisch-anatomische Gruppierung der Fälle	Zahl	Endgültige Fleischbewertung				
		a	b	c	d	e
akute RP mit kleiner Ausbreitung (der Fremdkörper wurde meistens gefunden)	25	4	3	2	3	13
akute RP, »Diaphragma-Abszeß«	10	2	2	2	2	2
RP, ausgebreitete fibrinös-eitrige P	8	3	—	3	2	—
RP, ausgebreitete eitrig-putride P	7	1	1	1	2	2
RP, ausgebreitete subchron. fibrin. P	18	3	—	—	3	12
RP, P mit wechselnder Ausbreitung (chronische), zähe Verwachsungen, eingekapselte Abszesse	15	—	1	1	3	10
RP, akute P, Endocarditis	1	—	—	1	—	—
der Fremdkörper drang in die Lunge ein. Putrider Abszeß in der Lunge, ausgebreitete Pleuritis	7	2	3	1	—	1
der Fremdkörper drang in die Lunge ein. Eingekapselter Abszeß in der Lunge, fibrinöse Pleuritis	13	2	—	2	1	8
der Fremdkörper drang in das Herz ein. Fibrinös-putride Pericarditis, P	14	5	3	1	2	3
der Fremdkörper drang in die Leber ein, Abszeß, P	10	2	—	1	1	6
der Fremdkörper drang in die Milz ein. Eitrige Gänge in der Milz. Multiple Abszesse in der Leber, Blutungen in den Nieren, parenchymatöse Degeneration	8	8	—	—	—	—
der Fremdkörper drang in die Milz ein. Eingekapselter Abszeß, halb-akute P	3	1	1	—	—	1
Insgesamt	139	33	14	15	19	58

Erklärung der Abkürzungen (Tabellen 2 u. 3): RP, Retikulo-Peritonitis; P, Peritonitist a, für den Konsum ungeeignet (Sepsis); b, für den Konsum ungeeignet (stinkender Geruch); c, geringerer Nähr- und Genußwert (schwacher Geruch); d, nur bedingt zum Konsum geeigne; (Saprophyten in der Muskulatur in geringer Zahl); e, zum Konsum unbedingt geeignet

Tabelle 3

Die endgültige Fleischbewertung der untersuchten Fälle

	Zahl	% der Fälle
a	33	23,7%
b	14	10,0%
c	19	13,3%
d	15	10,8%
e	58	41,7%
Insgesamt	139	—

aus Tabelle 2 hervorgeht, die den Zusammenhang zwischen dem pathologisch-anatomischen Bild und der endgültigen Fleischbeurteilung zeigt, ist bei der Beurteilung des Fleisches der RPP-Fälle die makroskopische (pathologisch-anatomische) Fleischuntersuchung allein nicht genügend; es ist unerläßlich, gleichzeitig auch bakteriologische Untersuchungen und Kochproben durchzuführen. Wir fanden nur eine pathologisch-anatomische Gruppe, deren Verhalten im Laufe der weiteren Untersuchungen eindeutig war. Bei den 8 Fällen der akuten Milzperforation stellten wir mit der bakteriologischen Untersuchung ausnahmslos *C. pyogenes*-Sepsis fest. In den Fällen, wo die Milzverletzung abgekapselt schien, ergab die bakteriologische Untersuchung im allgemeinen gleichfalls ein ungünstiges Resultat: bloß einer der drei Fälle erwies sich unbedingt für den Konsum geeignet, während wir in einem anderen Fall *C. pyogenes*-Sepsis und im anderen Fall einen stinkenden Geruch wahrnahmen.

Als weitere schwere Komplikation kann die putride Perikarditis, sowie der putride Lungenabszeß und auch die putride Pleuritis betrachtet werden. Nur 4 von den 21 Fällen erwiesen sich als uneingeschränkt für den Konsum geeignet, während bei dem Fleisch von 4 Tieren eine Wertverminderung eintrat. 13 Tiere waren für den Konsum ungeeignet; in sieben Fällen war die Diagnose *C. pyogenes*-Sepsis.

Mit einem verhältnismäßig günstigeren Ergebnis kann man bei akuten Netzmagenperforationen, bei subakuten fibrinösen Bauchfellentzündungen von größerem Umfang sowie in Fällen von abgekapselten Lungen- und Leberabszessen rechnen. In 49 von den 81 Fällen erwies sich das Fleisch uneingeschränkt für den Konsum geeignet; in 11 Fällen wurde *C. pyogenes*-Sepsis durch bakteriologische Untersuchung festgestellt, was gleichzeitig auch dafür zeugt, daß bei RPP selbst bei einem weniger schweren pathologisch-anatomischen Bild die Durchführung der bakteriologischen Untersuchung unerläßlich ist.

ZUSAMMENFASSUNG

Bei 139 an Reticuloperitonitis perforativa erkrankten Rindern wurden pathologisch-anatomische, und bei 125 von denen auch bakteriologische Untersuchungen durchgeführt. Es wurde festgestellt, daß auf dem Schlachthof von Pécs die Reticuloperitonitis perforativa im Laufe von anderthalb Jahren in 0,32% von 20 257 gesund erscheinenden Tieren, und in 9,3% der notgeschlachteten 645 Tiere auftrat. In 83 (59,7%) von 139 Fällen vergesellschaftete sich eine akute bzw. chronische Bauchfellentzündung zur Perforation des Netzmagens, in 56 Fällen drang der Fremdkörper weiter vor und schädigte meistens auch die Lunge (14,4), das Herz (10%), die Milz (7,9%) und die Leber (7,1%).

Auf Grund der Beobachtungen ist die Durchführung der bakteriologischen Fleischuntersuchung auch mit kleinerer Ausbreitung oder bei chronischen Fällen unbedingt begründet. Mit Hilfe der bakteriologischen Untersuchung wurde in 49,6% der Fälle die Anwesenheit des *Corynebacterium pyogenes* festgestellt. *C. pyogenes*-Sepsis wurde in 27 Fällen diagnostiziert. Bei der pathologisch-anatomischen und bakteriologischen Beurteilung der 139 Fälle erwies sich das Fleisch von 58 Rindern uneingeschränkt für den Konsum geeignet, 19 zeigten einen geringeren Nähr- und Genußwert, das Fleisch von 15 Tieren wurde nur bedingt zum Konsum geeignet qualifiziert, während sich das Fleisch von 47 Rindern für den Konsum ungeeignet erwies.

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SUPPRESSION OF THE DEVELOPMENT OF PRIMARY ANTIBACTERIAL IMMUNITY BY HETEROLOGOUS ANTI-LYMPHOCYTE SERUM*

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Introduction

Recently, the effect of anti-lymphocyte serum (ALS) and the mechanism of its immunosuppressive activity have been extensively dealt with in the literature. There is experimental evidence that ALS as a biological immunosuppressive agent is capable of preventing the development of many immunological reactions, *viz.*, of temporarily reversing an established immune state under given conditions (LEVEY and MEDAWAR, 1967).

The available results indicate that ALS suppresses primarily the "delayed-type" allergic and "cell-mediated" immunological reactions. It has been shown to prevent sensitization, *viz.*, to erase immunological memory. It prolongs the survival of allografts in the host organisms and enables the acceptance of xenografts. According to our earlier studies (KASSAI *et al.*, 1968) ALS is capable of preventing the development of anti-helminth immunity. Yet in studies on the mechanism of its immunosuppressive activity little attention has been paid to the extent and mode of influence of ALS on immunological reactions characterized by production of humoral antibodies.

In this context, examinations have been carried out on few antigens. It was found that ALS may suppress heterohaemagglutin formation and may prevent the immune effect of Salmonella H antigen (MONACO *et al.*, 1966; WOODRUFF *et al.*, 1967; JAMES and ANDERSON, 1967). In recent studies we have ourselves dealt with suppression of the development of primary antitoxic immunity by heterologous ALS (RÉTHY and HEGEDÜS, 1969), in mice immunized with graded doses of tetanus toxoid and challenged by tetanus toxin. Heterologous ALS effectively inhibited the development of primary immunity to tetanus. Immunosuppressive treatment was effective exclusively in the early phase of immunization. Once the antitoxic immunity had become established, no immunosuppression could be produced by ALS. A supposed competition of antigens between ALS and tetanus toxoid as a potential cause of immunosuppression was excluded by experimental evidence.

By the experiments reported in this paper we extended our observations concerning the immunosuppression of humoral immunity to the antibacterial immunity.

The following problems were investigated:

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1. To what an extent ALS would influence the development of primary antibacterial immunity if immunosuppression is initiated prior to the administration of antigen, *viz.*, in the phase when no contact has yet occurred between antigen and immunocompetent cells.

2. To what an extent ALS would exhibit immunosuppressive activity in the inductive and/or productive phases of immunity, that is, in the phase when the antigen has already primed the immunocompetent cells.

(a) Immunosuppressive activity of ALS was examined in the inductive phase of immunity when humoral antibodies had not yet appeared, and

(b) when humoral antibodies were likely to be present in the circulation, *viz.*, in the productive phase of immunity.

3. Taking into consideration that ALS *per se* may act as an antigen (LANCE and DRESSER, 1968), the eventual role of competition of antigens in the immunosuppressive action of ALS was also examined (RÉTHY *et al.*, 1967).

In our model experiments aluminiumhydroxide-adsorbed swine erysipelas vaccine was used as antigen. The antigen incorporated in the vaccine contains both bacillary and hapten-protein-complex type components (TRAUB, 1947; 1948; DEDIÉ, 1948; GELENCZEI, 1955). The mouse immunization model was used as, on the analogy of antitetanus immunity, it enables a good assessment of the degree of protection.

Materials and methods

1. Test antigen

Erysipelothrix rhusiopathiae strain was cultivated on a 10% horse serum-containing medium for 48 hours. The culture was adsorbed on 10 mg $Al(OH)_3$ gel/ml and 0.4 w/v p. c. of formalin was added for killing and detoxicating the culture (GELENCZEI, 1955).

2. Active immunization

Mouse groups selected at random were immunized on one occasion with 0.2 ml individual doses of twofold serial dilutions of the vaccine.

3. Challenge

Two weeks after immunization the experimental animals were challenged each with 100 000 live virulent *E. rhusiopathiae* germs. (The challenge dose corresponded to 10^5 MLD per mouse 1 MLD $\sim$ 1 germ.)

4. Preparation of ALS

Thymuses of male mice weighing 8—10 g, were removed after exsanguination by decapitation, and homogenized. The homogenate, which beside

thymocytes contained also tissue debris, was suspended in Hanks' solution, and coarse tissue remnants were removed by sedimentation. Live thymocytes were adsorbed on to aluminium phosphate gel. This adjuvant-containing antigen was used to immunize rabbits on two occasions at a 2-week interval. The single dose contained $3 \cdot 10^8$ thymocytes per 1 kg body weight. On the 7th day after the last immunization the rabbits were bled-out and the pooled sera were kept in deep-freeze until use, without inactivation.

5. *Examination of the effects of ALS*

In vitro methods

1. Determination of the lymphocyte-agglutinating capacity of ALS; 2. Determination of lymphocytotoxic effect of ALS incativated at 56° C. As complement, normal rabbit serum was added to the system (AMOS and PEACOCKE, 1963; GRAY *et al.*, 1966). 3. Determination of heterohaemagglutinin (antimouse) titre of ALS.

In vivo methods

1. *Examination of graft protective effect.* Tail-skin particles from CBA female mice were transplanted to random-bred Swiss female mice weighing 18—20 g, in Nembutal narcosis according to the standard method of MEDAWAR's school (BILLINGHAM and MEDAWAR, 1951). The grafts were fixed for 9 days by gyps-bandages. After removal of the bandages, the condition of the CBA tail grafts was checked daily. The medium survival time (MST) of CBA grafts was calculated and the graft-protecting activity of ALS was expressed by this term.

As a control, grafted mice were treated with normal rabbit serum (NRS) on the same schedule as the experimental mice with ALS. Another control group consisted of grafted mice given no immunosuppressive treatment whatever.

2. *Innocuity-test.* Prior to application, ALS was assayed for toxicity. Mice weighing 16—18 g were injected on 5 occasions at 2-day intervals with 0.5 ml individual doses of the serum. This dose was 2.5fold that used in the immunosuppression experiment. The mice were examined daily and their weights were measured.

Immunosuppression of the effect of swine erysipelas vaccine. The schedule of the experiment is shown in Table I. Treatment with ALS in relation to administration of the test antigen was made at the following times:

1. On the 5th and 2nd days (—5, —2 day) preceding immunization with the vaccine;

2. on the 2nd and 5th days (+2, +5 day) following immunization;

3. on the 10th and 12th days (+10, +12 day) following immunization.

Table I

Scheme of experiments

Experimental groups		Days of treatment before and after immunization							
Number	Designation	−5	−2	0*	+2	+5	+10	+12	+14
1	Control	—	—	imm.	—	—	—	—	Challenge
2	ALS, −5, −2	ALS	ALS	imm.	—	—	—	—	Challenge
3	NRS, −5, −2	NRS	NRS	imm.	—	—	—	—	Challenge
4	ALS, +2, +5	—	—	imm.	ALS	ALS	—	—	Challenge
5	NRS, +2, +5	—	—	imm.	NRS	NRS	—	—	Challenge
6	ALS, +10,+12	—	—	imm.	—	—	ALS	ALS	Challenge

ALS, antilymphocyte serum, single dose 0.5 ml; NRS, normal rabbit serum, single dose 0.5 ml; imm., immunization with swine erysipelas vaccine; Challenge dose = 10^5 virulent *E. rhusiopathiae* organisms; *, day of immunization, regarded as "0-day" of the experiment

Controls

1. One control group was immunized exclusively with swine erysipelas vaccine for determining the protective value of vaccine.

2. Groups of mice were treated with NRS on the analogy of ALS treatment, to check the supposed competition of antigens.

Dosage

The individual dose of all dilutions of swine erysipelas vaccine was uniformly 0.2 ml.

2. The single doses of ALS and NRS were uniformly 0.5 ml.

6. Evaluation of results

The PD_{50} values (50% protective doses) were calculated for each group and compared to PD_{50} values of the groups given different treatments. The relative potency (RP) of the individual conditions of experiment was determined by comparing the PD_{50}-values of individual groups to the PD_{50}-value of untreated control (P. JUHÁSZ, 1968).

For mathematical-statistical analysis the "t" test was applied. In addition, for certain results 95% fiducial limits were determined.

Results

The results of *in vitro* and *in vivo* examinations of the rabbit anti-mouse ALS used to suppress antibacterial immunity and of the control NRS are summarized in Table II.

Table II

In vitro and *in vivo* examinations of the ALS used in the experiments

Tested for	Test results		
	ALS*	NRS*	Control
Lymphoagglutination	1 : 64++	neg.	□
Cytotoxicity	1 : 64++	neg.	□
Agglutination of mouse red blood cells	1 : 4++	neg.	□
⊞ Innocuity	neg.	neg.	□
Graft protection; MST, days	27.5	11.6	11.3**

* 2 × 0.5 ml dose on days +2 and +5; ⊞ dose for innocuity tests 5 × 0.5 ml at 2-day intervals;
** no immunosuppressive treatment was made; □ not examined; MST, medium survival time.

As shown in Table II, both the lymphoagglutinating effect and lymphocytotoxic effect of ALS was demonstrable up to the 1 : 64 dilution.

The rabbit anti-mouse ALS agglutinated mouse red blood cells in 1 : 4 dilution.

With NRS, no lymphoagglutinating, lymphocytotoxic or haemagglutinating antibodies were found.

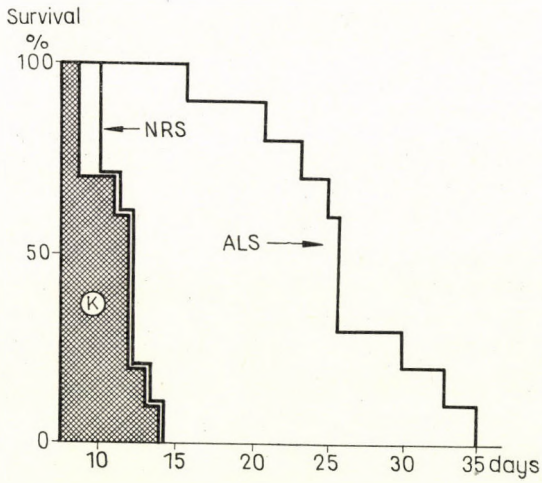

Fig. 1. In vivo graft-protective effect of the heterologous anti-mouse ALS. Survival %, per cent survival of grafts on hosts; days, times of immunological graft rejection; K, untreated controls; NRS, grafted host treated with normal rabbit serum; ALS, grafted host treated with antilymphocyte serum

The results of innocuity tests on mice were also favourable: ALS proved nontoxic for mice. The results of *in vivo* experiments are shown also in Fig. 1 to illustrate the dynamics of graft rejection.

Comparing the data in Table I and Fig. 1, it appears obvious that the survival of CBA grafts on Swiss mice was notably prolonged by ALS-treatment. In ALS-treated hosts, the medium survival time (MST) of grafts was 27.5 days as compared to the 11.3 and 11.6 days in the untreated and NRS-treated controls, respectively.

Table III

Suppression of the immune effect of adsorbed swine erysipelas vaccine by heterologous ALS

Number	Immunosuppressive treatment		Potency of adsorbed swine erysipelas vaccine		
	Agent	Days*	PD_{50} ml	95% fiducial limits of RP	RP
1	—	—	0.010	0.007—0.015	1
2	ALS	— 5, — 2	0.333	0.295—0.365	0.03
3	NRS	— 5, — 2	0.019	0.008—0.044	0.53
4	ALS	+ 2, + 5	0.024	0.011—0.039	0.42
5	NRS	+ 2, + 5	0.008	0.004—0.015	1.25
6	ALS	+10, +12	0.010	0.008—0.013	1

* Related to day of immunization; ALS, antilymphocyte serum; NRS, normal rabbit serum

Fig. 1 clearly shows that the dynamics of graft rejection was the same in the untreated (K) and NRS-treated (NRS) controls. Rejection started on the 9th, respectively, 10th day, and was completed by the 14th day.

Fig. 1 also reveals that all grafts protected with heterologous ALS have survived the maximum survival time of unprotected grafts. In the protected group, graft rejection started by the 17th day and was completed by the 35th day. Also, it appears that the rejection phase of the ALS-protected grafts distributed uniformly before and after the MST (27.5 days).

Using the ALS characterized by the above outlined parameters to suppress primary immunity conferred by swine erysipelas vaccine, the following results were obtained (see Table III).

1. The control group was given exclusively swine erysipelas vaccine. 0.010 ml of this vaccine protected 50% of the immunized mice against challenge with a suspension of virulent *E. rhusiopathiae* organisms equivalent to 10^5 MLD (PD_{50} = 0.010 ml; RP = 1.00).

2. Applying the ALS on days −5 and −2 the vaccine's PD_{50} rose to 0.333 ml. RP = 0.03.

3. Applying ALS on days +2 and +5 the PD_{50} value of the *E. rhusiopathiae* vaccine rose to 0.024, RP = 0.42.

4. Immunosuppressive treatment with ALS on days +10 and +12 was ineffective as related to the efficacy of the untreated control (PD_{50} = 0.010 ml; RP = 1.00).

5. Immunity to *E. rhusiopathiae* of controls treated with NRS on the analogy of ALS, to check a possible antigenic competition, developed in the following way.

(a) Preimmunization treatment with NRS on days −5 and −2 decreased the RP of the vaccine to 0.53 (PD_{50} = 0.019).

(b) NRS applied shortly after immunization (days +2 and +5) "increased" the immunogenic effect of the vaccine by 25% (PD_{50} = 0.008; RP = 1.25). Data obtained by mathematical-statistical analysis of the differences between the PD_{50} values are shown in Table IV.

The results are presented schematically in Fig. 2. Comparing the graphically plotted data to those shown in Tables III and IV, the following conclusions were drawn.

1. Under the given conditions of experiment, ALS reduced the potency of swine erysipelas vaccine exclusively in the cases when it was applied shortly before, or after immunization. In the cases when immunosuppressive treatment was started on the 10th postimmunization day, no immunosuppression took place. (P% $ALS_{\pm 2,5} \rightarrow$ Control < 0.1; $ALS_{+10,+12} \rightarrow$ Control = homogeneous data !)

2. A statistically significant decrease was observed in the immune effect of *E. rhusiopathiae* vaccine when NRS was administered — on the analogy of ALS treatment — prior to immunization, *i.e.*, on days −5 and −2, as control of heterologous serum-antigen in supposed competition of antigens (P% $NRS_{-5,-2} \rightarrow$ Control, < 0.1).

The immunization followed by NRS injections (days +2 and +5) showed slightly "increased" potency as compared to that in the untreated control group (RP = 1.25). Though this increase in the effectiveness was found statistically hardly significant, the phenomenon needs further investigations (P% $NRS_{+2,+5} \rightarrow$ Control, 2.0).

3. Considering that the results do not exclude that also NRS changed the immune effect of swine erysipelas vaccine on both pre- and postimmunization treatments the immunity-affecting effects of ALS and NRS applied at the same phase of immunization were statistically analyzed.

ALS applied prior to immunization decreased the potency of the vaccine 33 times, while NRS applied at the same point of time by only 1.9 times, as related to the untreated controls. This implies that at that point of time, ALS

Table IV

Mathematical-statistical analysis* of differences between experimental results

	Control	ALS −5, −2	NRS −5, −2	ALS +2, +5	NRS +2, +5
Control	—				
ALS, −5, −2	<0.01	—			
NRS, −5, −2	<0.1	<0.01	—		
ALS, +2, +5	<0.1	<0.01	homog.	—	
NRS, +2, +5	2.0	<0.01	<0.1	<0.1	—
ALS, +10, +12	homog.	<0.01	<0.1	<0.1	2.0

* The figures represent P% values

exhibited an approximately 15 times higher degree of immunosuppression than NRS (P% $ALS_{-5,-2} \rightarrow NRS_{-5,-2} < 0.01$).

ALS applied on days +2 and +5 reduced the immunity conferred by the vaccine to about one half, as compared to the immunity-degree of untreated controls. This difference was statistically significant, as outlined above. NRS applied at the same time produced an "increase" of the immunogenic effect.

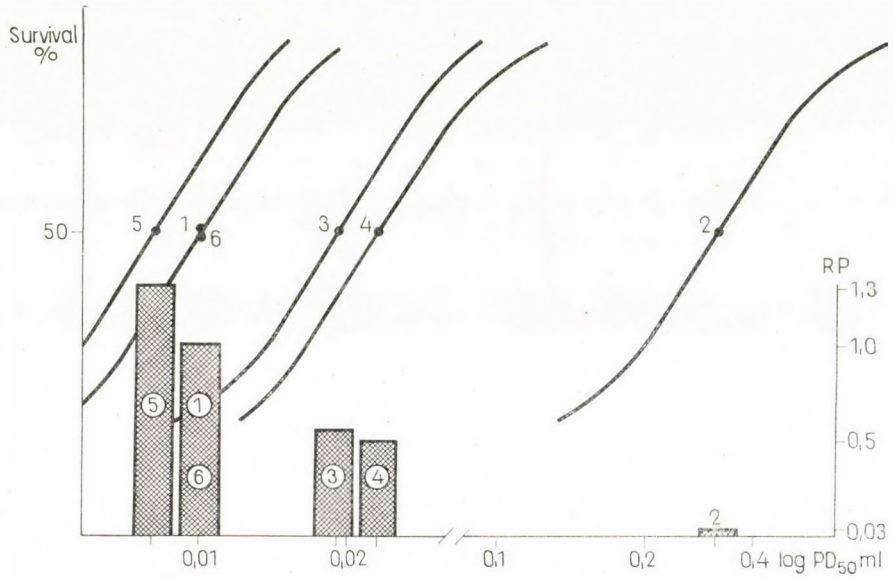

Fig. 2. Suppression of primary immune response to swine erysipelas vaccine by heterologous ALS in the mouse immunization model. 1 control group treated exclusively with swine erysipelas vaccine; 2, ALS given on days −5 and −2; 3, NRS given on days −5 and −2; 4, ALS given on days +2 and +5; 5, NRS given on days +2 and +5; 6, ALS given on days +10 and +12; RP, relative potency related to control (group 1)

Considering that the effect of ALS was immunosuppressive, whereas that of NRS "enhanced" immunity, the difference between the two results is regarded statistically sigificant. This is indicated also by the statistical data, *i.e.*, $ALS_{+2,+5} \rightarrow NRS_{+2,+5}$ $P\% < 0.1$.

Discussion

The results obtained in the present experiments permit the following conclusions.

1. ALS significantly reduced the primary immunity conferred by swine erysipelas vaccine, when applied shortly before or shortly after immunization.

2. ALS was found ineffective when the immunosuppressive treatment was started on the 10th postimmunization day.

3. Although in the present experiments the competitive action of heterologous serum protein came to effect during preimmunization treatment, its primary role in immunosuppression could be excluded with great certainty.

In our model experiments aimed at the suppression of the development of primary antibacterial immunity by ALS, the serum was applied at times when

(a) the bacterial and hapten-protein complex antigen had not yet contacted the immunocompetent cells, that is, on the 5th and 2nd preimmunization days;

(b) part of the adsorbed antigen complex may have entered immunologically competent cells, but demonstrable antibody production had started hardly or not at all, that is, on the 2nd and 5th postimmunization days;

(c) antibody production may have become manifest, that is, on the 10th and 12th postimmunization days.

In a recent paper on the effect of ALS on primary immunity to tetanus we have expressed the view that the immunosuppressive action of ALS given in the preimmunization phase might have been due to temporary reduction in the number, or at least certain "blindfolding" of immunocompetent cells which largely inhibited either the taking of the antigen or the transfer of information (LEVEY and MEDAWAR, 1967; JAMES, 1967). If the ALS is given after the contact between antigen and immunocompetent cells has taken place, the degree of immunosuppression will already depend on the amount of circulating antibodies (RÉTHY and HEGEDÜS, 1969).

Studies on suppression with ALS of primary immune response to swine erysipelas vaccine appear to confirm this view. In immunosuppression experiments with tetanus toxoid, correlation between the timing of ALS treatment and the degree of immunosuppression was less pronounced than in the present experiments. The inhibition of the development of antitoxic immunity was of approximately the same degree whether ALS was given shortly before or shortly

after immunization. In contrast, in the present studies, ALS produced a significantly higher immunosuppression when applied shortly before than shortly after immunization. So far no feasible explanation has been found for this phenomenon. It may have been due to higher immunosuppressive activity of "avidity" of ALS as compared to the ALS-preparation used in the tetanus toxoid experiments. Based on our own experience on the biological "half life" of heterologous ALS, concerning its *in vivo* graft-protecting action, the batch used in the present studies may have had an immunosuppressive activity approximately 50% higher than that used in the previous experiments (RÉTHY *et al.* 1959).

Like in the previous experiments, ALS applied in the productive phase days (+10 and +12) of antibacterial immune response failed to produce immunosuppression. For this phenomenon we propose the same explanation as in the case of tetanus toxoid; the productive phase of the immune process is characterized not so much by cellular function as by the amount of actually circulating antibodies. Thus, the late ALS effect, though probably influencing the immunocompetent cells, can presumably not reduce the level of antibodies already in circulation.

In the experimental system used, also a significant heteroprotein (antigen) effect of ALS has been demonstrated, manifesting itself under the given conditions by competition of antigens. NRS administered prior to immunization with swine erysipelas vaccine significantly reduced the immune effect. Yet this phenomenon must not be considered the exclusive cause of immunosuppression, as NRS reduced the immune effect to only one-half, while ALS did 30fold. Difference was significant also between the actions of ALS and NRS applied immediately after immunization. In this phase, ALS decreased the immune effect to one half, whereas NRS "increased" it. A closer study of this phenomenon remains to be carried out.

In the present studies on suppression of antibacterial immunity by ALS, no complete inhibition was observed in any case. This may be explainable by the prolonged absorption of the adsorbed antigen, which may have still continued in the phase when part of the immunocompetent cells may have been released from suppression and/or new cells may have entered the circulation where they were no longer accessible to the immunosuppressive action of ALS.

SUMMARY

The effect of heterologous anti-lymphocyte serum (ALS) on the development of active primary antibacterial immunity was examined on mice primed with graded doses of aluminium-hydroxide-adsorbed swine erysipelas vaccine and challenged with 10^5 MLD of a virulent *E. rhusiopathiae* strain. The following observations were made.

1. The heterologous ALS effectively suppressed the development of primary immunity to swine erysipelas.

2. Effective immunosuppression was produced exclusively by ALS applied in the early phase of the primary immune process. Significantly higher degree of immunosuppression was obtained if ALS was given in the preimmunization phase than if it was applied after the vaccine.

3. ALS failed to exert immunosuppressive activity when applied in the productive phase of immune response.

4. Examinations of the role of the heterologous protein-antigen nature of ALS in immunosuppression have shown that in the experimental system used the heteroprotein part of ALS interfered with the effect of the test antigen. But this "competition of antigens" did not notably alter the specific immunosuppressive action of ALS.

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EFFECT OF PMS GONADOTROPIC HORMONE ON THE REPRODUCTIVE ORGANS OF LAYERS IN THE PHASE OF REPRODUCTIVE INACTIVITY

UPTAKE OF OXYGEN AND GLUCOSE, AND RELEASE OF LACTIC ACID BY OVARY, MAGNUM AND UTERUS

By

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Little is known on the metabolic processes of the avian oviduct. Examining the metabolism of the albumen-producing magnum and the egg-shell-producing uterus, BROWN and BADMAN (1962) and MISRA and KEMÉNY (1964) have found a greater respiration rate in the latter.

The relevant examinations were carried out on the oviducts of hens in various phases of the egg production cycle. To obtain further information on the relationship between the structural differentiation of the oviduct and the related metabolic processes it seemed worthwhile to extend our studies to tissues of hens in the phase of reproductive inactivity which had been treated with gonadotropic hormone.

The hens were treated throughout with the pregnant mare serum gonadotropin (PMSG) "Gestyl". The experiments were undertaken to clarify

(1) how the atretic ovaries and oviducts of adult hens in the phase of reproductive inactivity would respond to a one-week intensive hormonal treatment and what changes the treatment would effect in them after an interval of one week;

(2) how the uptake of oxygen and glucose, and the production of lactic acid by the follicular wall, magnum and uterus would vary at individual and group level.

Methods

Healthy two-year-old hens of 2.5 kg average weight, with reproductive inactivity, were selected from the layer flock G-56 of the Small Animal Research Institute,* Gödöllő. The birds were observed for 8—10 days after transportation to the laboratory. Both the experimental and control groups were kept under standardized conditions. Illumination: natural light; temperature:

* The help of the Institute's Directorial Board and personally of Dr. J. GUOTH, Chief Veterinary Officer, in obtaining the appropriate birds is truly appreciated.

15—17° C; air humidity: 60—70%; ventilation: fan; floor space density: 5 birds/square meter. The hens received granulated raising feed (Erőtakarmánygyár, Budapest) in feeding troughs, and self-disposed drinking water supply.

Preparation and surgery

In Nembutal narcosis, a 2—3 cm long transversal incision was made on the left side of the abdominal wall, 4—5 cm distant to the midline. The inactivity of the reproductive organs was checked by direct palpation in the exposed abdominal cavity. The ovaries were infiltrated with the hormone preparation, using a lumbar needle under continuous control with the fingertip. The control birds received saline by the same route. Then the abdominal wall and skin were closed in succession by suture.

Treatment

Thirty-five hens were divided into 3 groups. Groups I (12 birds) and II (6 birds) were treated with gonadotropic hormone. Group III (17 birds) served as control.

The first intramuscular dose of hormone was given on the first postoperation day. This treatment was repeated on further 7 occasions at regular daily intervals (between noon and 1 p.m.).

Group I (designated PMSG), was treated intramuscularly on one occasion with 500 I.U. of Gestyl* dissolved in 1 ml of the solvent which was delivered together with the preparation. The birds were killed on the first day following the last treatment.

Group II (designated PMSGp). Treatment and dosage were the same as in Group I, but the birds were sacrificed one week after the closing of treatment.

Group III (control). Control birds were treated each with 1 ml of saline in the same way as the experimental birds; part of them were killed simultaneously with the PMSG, part simultaneously with the PMSGp group.

By the end of the experiment the birds were weighed, exsanguinated, and their reproductive organs were excised for further examination.

Analysis

The ovary and oviduct were weighed rapidly. From the ovary, magnum and uterus collected in a Petri dish immersed in ice, specimens weighing 150 mg were rapidly excised and immediately cut to small pieces weighing 4—5 mg. In every case, the dry material content of the organs was determined by drying

* The "Gestyl" used in the experiments was obtained through the courtesy of the N. V. Organon-OSS-Holland Agency with the kind help of Mr. L. Szántó and Miss E. Neuber.

Fig. 1 Stigmated follicles after PMSG-treatment

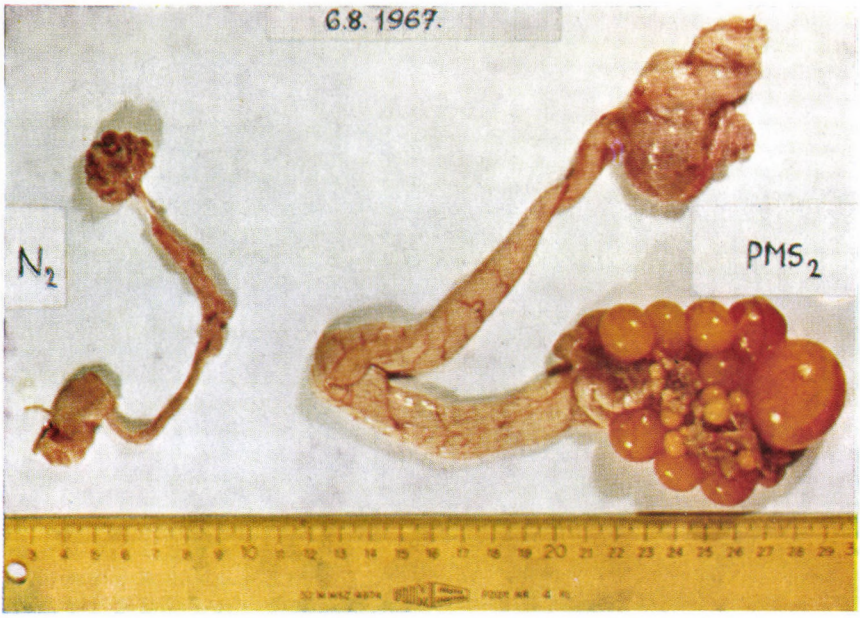

Fig. 2. Reproductive organs of control and PMSG-treated hens

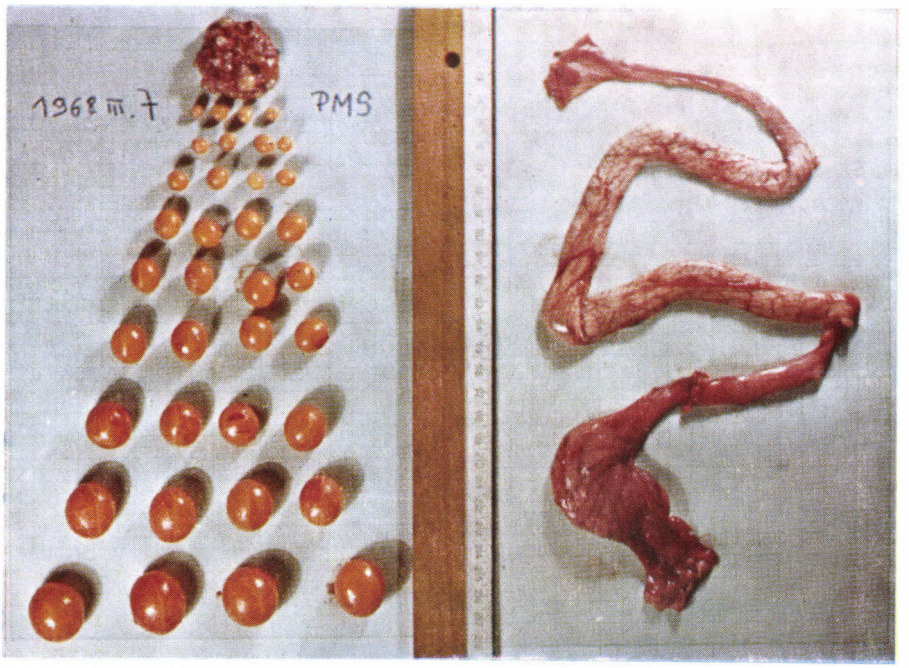

Fig. 3. Hierarchy of follicles and oviduct after PMSG-treatment

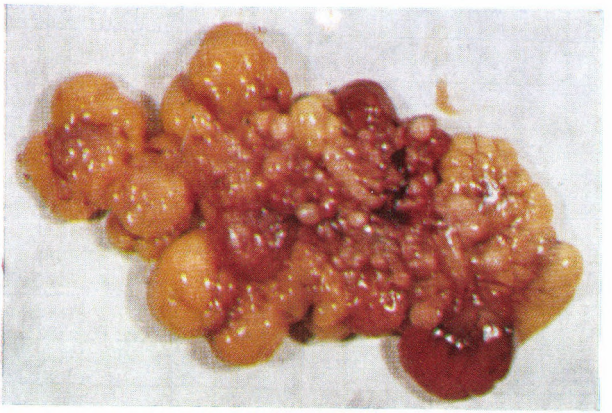

Fig. 4. Follicles in stage of regression after cessation of PMSG-treatment

to constant weight. The minced organ pieces were placed in a Krebs-Ringer phosphate medium containing 120 mg/100 ml glucose, and their oxygen uptake was measured with Warburg's manometric method (UMBREIT, 1957). Gas phase: atm. air. Afterwards the rates of the uptake of glucose and release of lactic acid were determined, with SCHAFFER and SOMOGYI's (1933) and BARKER and SUMMERSON's (1941) methods, respectively. The validity of the findings was checked with the "t" test.

Results

1. Alteration and weight changes of ovaries and oviducts of hormone-treated birds

a) Treatment with PMSG resulted in a hypertrophy of the reproductive organs. The ovary contained 8 to 12 stigmated follicles of nutmeg size (Fig. 1). The average weight of the ovaries rose to 72.6 ± 11.40 g, as compared to an average 4.5 ± 0.57 g in the control group ($p < 0.01$). Average weight of the oviducts of inactive and PMSG-treated birds were 7.0 ± 0.07 g and 61.3 ± 5.00 g, respectively ($p < 0.01$) (Fig. 2). The oviduct showed full development of size, structure and vascularization.

The uniform size of the follicles, representing an abnormal hierarchy (Fig. 3), and the observation that in several cases also the atretic right oviduct showed signs of development have indicated that the PMSG treatment had a greater influence on the reproductive organs than the normal physiological stimulus.

b) One week after the completion of the 8-day treatment with gonadotropic hormone, Group II (PMSGp) was sacrificed. Post mortem examination revealed regression and wilting of the follicles and also the vascularization of the ovary tended to regress (Fig. 4). The weight of the ovary decreased by approximately 45% as compared to that measured in the PMSG group. But the oviducts were still fully developed, like in the PMSG birds killed immediately after the completion of treatment.

2. QO_2 values of ovary, magnum and uterus in groups PMSG and PMSGp

Table I shows that oxygen uptake was highest in the uteri of PMSG birds. An average value of 7.12 ± 0.34 was calculated from 18 measurements on 8 birds. Limit values varied between 5.27 and 9.55. It is of interest that in the uteri of PMSGp birds the QO_2 values of the uteri fell by approximately 60%, though the oviduct itself showed no regression.

Values of oxygen uptake by the magnum indicated that, as a result of treatment with PMSG, the respiration of the albumen-producing oviduct portion varied reciprocally with structural differentiation, whereas the cessation of gonadotropin effect resulted in an increased respiration rate of the magnum.

Table I

Average QO_2* values measured in ovary, magnum and uterus

Group	Ovary			Magnum			Uterus		
	No. of samples	$\bar{x} \pm$ S. E.	p	No. of samples	$\bar{x} \pm$ S. E.	p	No. of samples	$\bar{x} \pm$ S. E.	p
PMSG	28	1.65±0.042	N. S.	16	1.10±0.140	0.01	18	7.12±0.341	0.01
PMSGp	25	0.98±0.120	0.01	5	1.59±0.468	N. S.	7	3.43±0.352	N.S.
Control	19	1.84±0.211	—	22	2.78±0.177	—	30	2.73±0.193	—

* QO_2, μl, O_2 taken up per mg dry weight of tissue per hour; S.E., standard error; N.S., not significant statistically

The QO_2 values measured in the magnum and uterus of controls were nearly the same; this correlates well with our earlier findings (MISRA and KEMÉNY, 1964).

The average QO_2 values measured in the walls of follicles in the control group were conspicuously lower (1.8—2.42) as compared to those measured in the uterus and magnum, that is, even their upper limit did not reach the lowest value found in the latter two. Though under the influence of treatment the diameter of the follicles grew from the original 2 to 30 mm, no change occurred in the respiration rates of their walls. But the regression of the follicles brought about a reduction of the respiration rate. The individual values varied from 0.45 to 1.78.

3. *Glucose uptake and lactic acid production by ovary, magnum and uterus in the PMSG and PMSGp groups*

a) Data of glucose uptake shown in Table II reveal that the gonadotropic hormone effected no change in the glucose uptake by the uterus, but reduced significantly ($p < 0.01$) the glucose uptake by the magnum.

b) As shown in Table III, hormone treatment resulted in an approximately 60% reduction of lactic acid production by the magnum.

Percentual changes in the tissue metabolism of the reproductive organs during the experiment are shown in Fig. 5.

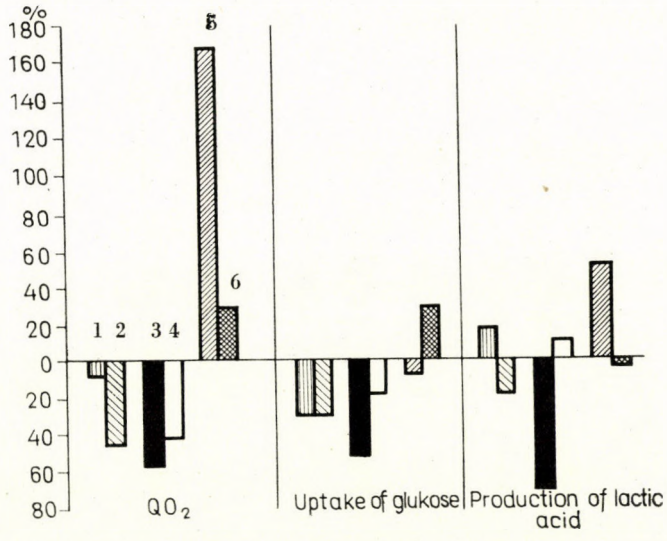

Fig. 5. Uptake of oxygen and glucose, and production of lactic acid by ovary, magnum and uterus, as related to the corresponding values measured in the control group. 1 ovary PMSG; 2 ovary PMSGp; 3 magnum PMSG; 4 magnum PMSGp; 5 uterus PMSG; 6 uterus PMSGp

Table II

Average values of glucose uptake* by ovary, magnum and uterus

Group	Ovary			Magnum			Uterus		
	No. of samples	$\bar{x} \pm$ S. E.	p	No. of samples	$\bar{x} \pm$ S. E.	p	No. of samples	$\bar{x} \pm$ S. E.	p
PMSG	28	33.0±2.64	0.01	14	19.5±2.46	0.01	16	35.4±3.93	N. S.
PMSGp	24	33.0±2.99	0.01	6	33.5±8.57	N. S.	6	47.9±4.54	N. S.
Control	19	47.4±2.97	—	19	41.9±3.44	—	26	37.7±5.82	—

* mg glucose taken up per g dry weight of tissue per hour

Table III

Average values of lactic acid production* by ovary, magnum and uterus

Group	Ovary			Magnum			Uterus		
	No. of samples	$\bar{x} \pm$ S. E.	p	No. of samples	$\bar{x} \pm$ S. E.	p	No. of samples	$\bar{x} \pm$ S. E.	p
PMSG	20	9.4±0.890	N. S.	16	3.3±0.848	0.01	18	21.7±1.34	N. S.
PMSGp	25	6.5±0.530	N. S.	4	12.1±2.44	N. S.	7	15.6±0.89	N. S.
Control	17	7.9±0.856	—	21	11.0±1.12	—	27	16.7±1.35	—

*mg lactic acid produced per g dry weight of tissue per hour

Discussion

The highly developed ovaries found after hormonal treatment suggest that PMSG produced an "overstimulation" of the gonads. The appearance of the ovaries was to a certain extent reminiscent of those found by MITCHELL (1966) in hypophysectomized birds treated with avian pituitary extract. In hens treated with PMSG, the endogenous oestrogen level — though not measured directly — was apparently high as the birds' left oviducts were as highly differentiated as those of birds in the phase of reproductive activity. In some cases even the rudimentary right oviducts showed signs of development. One group of hens (PMSGp) was killed one week after the closing of the 8-day hormone treatment. On the basis of morphological changes it seemed to be feasible that the many mature stigmated follicles will bring about an ovulation and the hens start to produce eggs again. But in no case did ovulation occur; the follicles softened and wilted and the vascularization of the ovary tended to regress. This observation is in good correlation with NALBANDOV's (1961) finding that gonadotropic hormone alone was insufficient to elicit an ovulation. MITCHELL (1967) failed to observe ovulation either, though he treated hens with a homologous FSH-containing preparation.

The uptake of O_2 and glucose and the production of lactic acid by the tissues examined were determined in three dissimilar physiological states:

(1) in case of practically maximum levels of gonadotropic and endogenous oestrogen hormones; (2) in the phase of the decrease of hormone levels and (3) during involution of the reproductive organs, that is, at the "zero" point of reproduction when the follicles were atretic and the oviducts atrophied. The experimental data were most convincing in respect of changes of respiration rates in magnum and uterus. The elevated oestrogen level brought about a multiple increase of O_2 uptake by the uterus, but reduced notably the O_2 uptake by the magnum (reciprocal change). In the PMSGp group, though no apparent involution of the oviduct was observed, uptake of O_2 by uterus and magnum altered significantly; the change was reciprocal, like in the PMSG group, but in this case the respiration rate of the uterus decreased and the O_2 uptake of the magnum increased. These changes could be followed up until the state of complete involution set in. In accordance with earlier findings (MISRA and KEMÉNY, 1964), in the involutional state no difference was found between the respiration rates of the individual oviduct portions.

As far as we are informed no literary data have been available on the uptake of O_2 and glucose, and production of lactic acid, by the follicular wall. The QO_2 values measured were even lower than those found in the magnum. Surprisingly, there was no difference between the above values measured in specimens from small, medium and large follicles.

It appears that in the background of the ion-translocation mechanism there is an intensive respiration at whose appropriate levels the macroergic

phosphates, among others ATP, are formed. Protein synthesis and secretion are taking place at lower levels of respiration. Final evidence for these implications would be by performing the assays on uterus and magnum specimens removed in the phase of maximum egg-shell- and albumen-producing activity, respectively. But the available results are sufficient to support BROWN and BADMAN's (1962) finding that the respiration rate of the uterus reaches maximum while the ovum is in the isthmus, that is, shortly before the deposition of calcium. The same authors observed minimum respiration rate in the magnum while the ovum was in it; they found high values in those birds whose oviducts did not contain ova but had large mature follicles in the ovary. They believe that the rate of energy metabolism is higher during the accumulation of protein than during the actual secretory phase. The low respiratory rate of the magnum has been attributed by BROWN and BADMAN (1962) to the large amount of "non-functional" protein contained in the magnum.

Interestingly, the PMSG-induced nearly threefold increase of O_2 uptake by the uterus was not accompanied by a rise of glucose uptake. It may be supposed that the hormonal treatment effected an increase of intracellular carbohydrate reserves which then provided the substrate required for the increased respiration.

In the PMSG group, production of lactic acid by the magnum tissue fell by 70% as compared to the controls. It seems likely that the massive dose of PMSG may have had an inhibitory effect on some essential enzyme involved in glycolysis. According to NALBANDOV (1959), lasting treatment with steroid PMSG may effect an enzyme depression in the oviduct of layers.

SUMMARY

QO_2 values, glucose uptake and lactic acid production by the tissues of follicle, magnum and uterus were examined on two-year-old hens with inactive atretic ovaries and oviducts.

Thirty-five hens with lasting reproductive inactivity, but otherwise healthy, were divided into experimental and control groups. The experimental birds were treated with 500 I. U. of pregnant mare serum gonadotropin (PMSG) (Gestyl, Organon) for 8 days. The inactivity of the reproductive organs was checked by palpation through the abdominal incision made to enable direct infiltration of the ovary with the first hormone dose. The further doses were injected intramuscularly at regular daily intervals. The control groups received saline on the same schedule.

Birds killed immediately after the last PMSG-treatment had fully mature follicles of 20—30 mm diameter as contrasted to the original diameter of 2—3 mm. The average weight of the ovaries rose from 4.5 $\pm$ 0.57 g to 72.6 $\pm$ 11.40 g, as contrasted to the controls. Each ovary contained 8—12 stigmated follicles of nutmeg size. The abnormal hierarchy of the follicles and the observation that in several cases also the right rudimentary oviduct showed signs of development have suggested that PMSG had a greater influence on the reproductive organs than the normal physiological stimuli.

Birds killed one week after the 8-day PMSG-treatment (PMSGp) had wilting follicles, but the oviduct still showed no signs of involution.

Changes in the QO_2 values of the examined organs are presented in Table I.

In the PMSG group, uptake of glucose by the uterus was unchanged, while uptake by the magnum was lower, as compared to the controls ($p < 0.01$). In the PMSG and PMSGp groups, the corresponding values of the follicles were significantly lower than in the control group ($p < 0.01$).

Production of lactic acid by the magnum was by 70% lower in the experimental than in the control group.

The findings are discussed in relation to the structural development of, and alteration of metabolic processes in, the oviduct.

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HOST—PARASITE RELATIONSHIP BETWEEN FISH NEMATODES (GENUS: THWAITIA) AND THEIR HOSTS

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Studies on the incidence and developmental cycle of *Thwaitia* (Philometra) species in Hungary (MOLNÁR, 1966) have disclosed certain correlations providing information on the wide variety of host—parasite relationships within one and the same genus.

It was found that in some hosts the sexual maturation of *Thwaitia* females was preconditioned by factors in the absence of which their development came to a halt even if the intensity of infestation was high.

The *Thwaitia* species are particularly suited for the study of host—parasite relationship for the following reasons: (1) their development takes place through a one-year cycle in well-defineable season-dependent stages; (2) normally developed and retarded parasites can be regularly encountered in most hosts and even the dead worms are retained; (3) after the relatively short period of fertilization, the development of worms takes a long time; the length of females is 3—3.5 mm at fertilization and 3—12 cm at the discharge of larvae; (4) in the developmental cycle two phases, one beneath the air bladder serosa and another outside it, can be clearly differentiated.

Our examinations were performed in the course of faunistic studies on fishes found to be infested with *Thwaitia* species; accordingly, the material examined originated from different habitats in Hungary.

Up to now the occurrence of four *Thwaitia* species has been noted: *Thwaitia ovata* (Zeder, 1803), *Th. rischta* (Skrjabin, 1917), *Th. abdominalis* (Nybelin, 1928) and *Th. kotlani* (Molnár, 1969). Out of them *Th. ovata*, *Th. rischta* and *Th. kotlani* occur primarily in the host population of large stagnant waters and rivers, whereas *Thwaitia abdominalis* occurs in brook fishes. The fish species harbouring *Thwaitia* and the number of hosts examined are shown in Table I.

In some cases complete parasitological sectioning was carried out, but mostly only the air bladder and other specific localizations were examined. The usually hardly visible but vigorously moving parasites were recovered under stereomicroscope from beneath the bladder serosa and were placed in saline.

Table I

Species and hosts of *Thwaitia*

Thwaitia ovata		*Thwaitia rischta*		*Thwaitia abdominalis*	
Hosts	Number of fishes examined	Hosts	Number of fishes examined	Hosts	Number of fishes examined
*Abramis brama**	1500	*Abramis brama**	1500	*Phoxinus phoxinus*	210
Abramis ballerus	40	*Abramis ballerus*	40	*Gobio gobio*	40
*Rutilus rutilus**	600	*Alburnus alburnus*	600	*Leuciscus*	36
		Blicca bjoerkna	60	*Leuciscus leuciscus*	6
		Scardinius erythrophthalmus	90		

* Exclusively in fishes from the Lake Balaton

It should be noted that a much greater number of hosts was examined than shown in Table I, but percentual evaluation was limited to those which were studied exclusively with the aim to obtain information on the occurrence and developmental cycle of *Thwaitia*. For example, fishes younger than two summers, so far not known to harbour *Thwaitia*, were not included in the evaluation.

Previous studies (MOLNÁR, 1966b, 1967, 1969) have shown that the developmental cycles of the four *Thwaitia* species in question are essentially the same. The main features of the cycle are briefly outlined as follows.

1. The developmental cycle of the *Thwaitia* parasites studied lasts one year. The females reach full maturity in the host by the end of May or early in June; at this stage the body cavities of the worms are filled with larvae. The discharge of larvae to the outside world can take place in two ways: (a) the females either migrate to the external cloacal region where they release the larvae by bursting in the hypotonic medium, or (b) they disintegrate while in the abdominal cavity and the larvae migrate into the water through the cloaca.

2. The further development of *Thwaitia* larvae takes place in cyclopses where they undergo two moults.

3. Fishes become infected during June or early July.

4. The parasites ingested by the fishes with the cyclopodes migrate from the intestine to the air bladder and settle beneath its serous membrane. Also copulation takes place in that location.

5. In the appropriate host the fertilized females migrate in July, or August to specific locations (abdominal cavity, fins, gill cover), where they slowly but steadily reach larval maturation by the next summer.

occurring in Hungary

Thwaitia kotlani			
Hosts	Number of fishes examined	Species not harbouring *Thwaitia* as assessed by examination of 30 specimens	
Aspius aspius	41	*Cyprinus carpio*	*Rhodeus sericeus amarus*
		Carassius carassius	*Chondrostoma nasus*
		Barbus barbus	*Barbus meriodionalis Petényi*
		Tinca tinca	*Leuciscus idus*
		Gobio albipinnatus	*Alburnoides bipunctatus*
		Vimba vimba	*Pelecus cultratus*
		Abramis sapa	

6. The males and, in inappropriate hosts, also the females are retained under the bladder serosa throughout the year. Occasionally numerous living, but retarded worms, 2 to 3.5 mm in length are encountered in that location. (*Thwaitia rischta* is an exception as also the males migrate from the bladder subserosa.)

I. Host—parasite relationship in fishes infested by Thwaitia ovata

Thwaitia ovata parasites have been found in three fish species: *Rutilus rutilus, Abramis brama, Abramis ballerus.*

1. Fully developed, or developing abdominally localized females were encountered in

(a) 100% of *Abramis brama* and *Rutilus rutilus* fishes with ligulosis;*

* Some authors reporting simultaneous occurrence of *Ligula* and *Thwaitia (Philometra)* infestation in lakes have noted that in spite of the massive infestation with *Th. ovata*, part of the fishes were infected with *Ligula* alone.

MARKOV and KOSAREVA (1962) and DONTSOV (1968) found simultaneous occurrence of *Ligula* and *Thwaitia* in the majority of *Abramis brama* fishes, but the minority of them had ligulosis but no *Thwaitia* infection.

This phenomenon might be explained by the following reasons: 1. The authors, if not familiar with the seasonal cycle of *Thwaitiae*, may have examined the fishes in a period (June) when the mature worms had been eliminated and reinfection had not yet taken place. 2. The authors might have disregarded that part of the developmental cycle of *Thwaitiae* takes place beneath the bladder serosa and that particular period (July, August) may not be suited for statistical evaluation. 3. Further to this, no developing *Thwaitia* females are encountered in fishes with ligulosis if *Ligula* infection took place after the invasion of *Thwaitia* larvae. In the course of our faunistic studies we have ourselves encountered some young *Ligula*-infected *Abramis brama* and *Rutilus rutilus* hosts in which *Thwaitia* infection was in the latent (subserous) phase even in autumn and in spring. But aimed examinations for *Thwaitiae* of fishes with manifest ligulosis invariably revealed a 100% incidence of mature *Thwaitiae* females, indicating that in those cases ligulosis had preceded the invasion of *Thwaitia* larvae.

With regard to the above considerations we examined the host—parasite relationship exclusively in fishes collected during the fall and spring season.

(b) 2.5% of *Rutilus rutilus* hosts not infected with ***Ligulae.***

2. Males localized under the bladder serosa and retarded females were encountered in

(a) 100% and 67% of *Ligula*-free *Abramis brama* and *Rutilus rutilus* fishes, respectively, from the Lake Balaton;

(b) approximately 50% of *Abramis brama, Abramis ballerus* and *Rutilus rutilus* fishes from rivers.

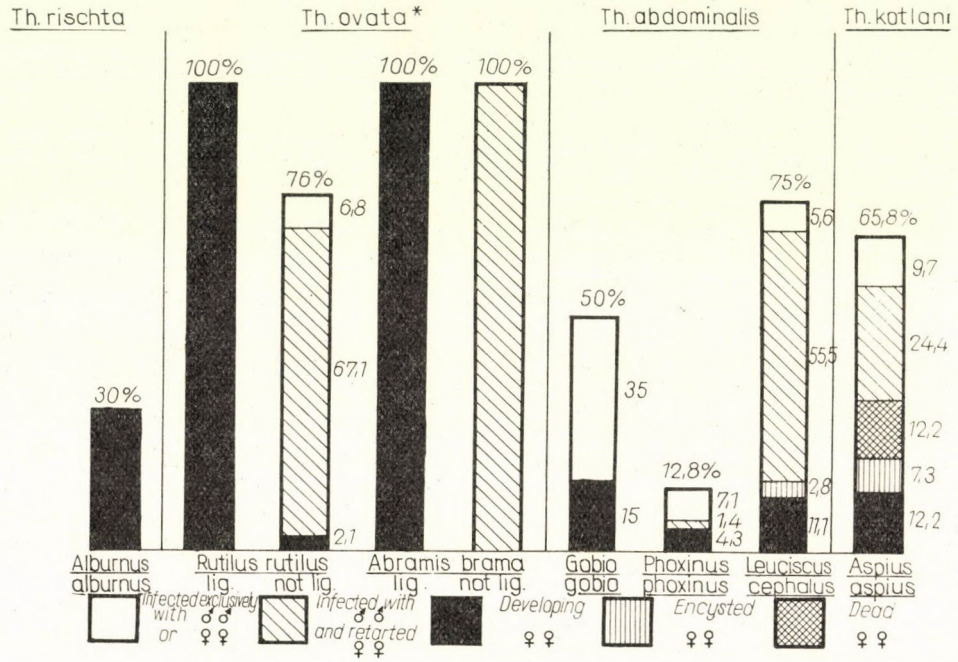

Fig. 1. Type and extensity of infestation with *Thwaitia* of different fish species

II. Host—parasite relationship in fishes infested by Thwaitia abdominalis

Thwaitia abdominalis was found in four fish species: *Gobio gobio, Phoxinus phoxinus, Leuciscus cephalus, Leuciscus leuciscus.*

1. Fully developed or developing, abdominally localized females were found in

(a) *Gobio gobio* fishes which contained at least one male and one female parasite as well as in the majority of *Phoxinus phoxinus* fishes infested by *Thwaitia abdominalis;*

(b) approximately 15% of *Leuciscus cephalus* and *Leuciscus leuciscus* fishes infested by *Thwaitia abdominalis.*

2. Males parasitizing the bladder serosa or retarded females were found in

(a) hosts harbouring exclusively males or females;

(b) the majority of *Leuciscus cephalus* and *Leuciscus leuciscus* hosts infested by *Thwaitia abdominalis.*

3. Further to this, in the abdominal cavity of a *Leuciscus cephalus* specimen 2 developing female parasites were found encapsulated in thick connective tissue; their lengths were only one half compared to the rest of parasites which at the same time measured 40—50 mm.

III. Host—parasite relationship in fishes infested by Thwaitia kotlani

1. Fully developed or normally developing abdominally localized females were found in 12% of *Aspius aspius* fishes.

2. Abdominally localized, developing, but slightly retarded females encapsulated in connective tissue were encountered in 7.3% of the examined *Aspius aspius* specimens.

3. Abdominally localized females which died after having reached a length of 10—25 mm (in April) and had dry cuticles overgrown by connective tissue were found in 12.7% of *Aspius aspius* fishes.

4. Males and females localized under the bladder serosa were found in 24.4% of *Aspius aspius.*

5. Living males or females localized under the bladder serosa were found in 9.7% of *Aspius aspius.*

IV. Thwaitia rischta

Though it has a wide host range, it is unsuitable for the study of host—parasite relationship as after fertilization both the males and females leave the bladder serosa and, accordingly, the factors limiting their development can hardly be identified.

Discussion

An analysis of the host—parasite relationship between the various *Thwaitia* parasites and their hosts has shown that undisturbed development of a given parasite takes place in only some host specimens, implying that *Thwaitia* parasites have more and less favourable hosts and even in the case of *Thwaitiae* having a single known host, the adaptation of the parasite may vary widely (Fig. 1).

From this angle, exclusively the adaptation of *Th. abdominalis* can be regarded perfect; of the range of hosts *Gobio gobio* and *Phoxinus phoxinus*

appear to provide optimum conditions for practically undisturbed development if both males and females are present.*

In contrast, for *Th. abdominalis Leuciscus cephalus*, for *Th. ovata*, *Ligula*-free *Rutilus rutilus* and for *Th. kotlani*, *Aspius aspius* represent the host species in which full development takes place only in the minority of the specimens, even if the intensity of infestation is high. Only few of the settled parasites start to develop and reach full maturity. The often limited development of *Thwaitia* in the above fish species suggests that the latter are less favourable hosts. The scanty data available on *Leuciscus leuciscus* permit no definitive conclusion.

A particular type of host is represented by the *Ligula*-infested *Rutilus rutilus* and *Abramis brama* fishes, as the synergistic effect of *Ligula* infection on *Thwaitiae* results in a complete parasite development in both species. Considering that a *Ligula*-infested *Abramis* or *Rutilus* may harbour 50—300 females in the abdominal cavity, while a *Ligula*-free *Rutilus rutilus* only 1 or 2, if any, there is reason to suppose that the *Ligula*-infested fishes making 4.5% of the *Rutilus* and *Abramis* population play a more important role in the maintenance of the often conspicuously high *Thwaitia* incidence in the Lake Balaton than the 2.1% of *Thwaitia*-infested *Ligula*-free *Rutilus* hosts, in which complete development of *Thwaitia* may occur.

The variability of adaptation to the host is exemplified by the relation of *Th. kotlani* to *Aspius aspius*, in which some of the parasites seem to complete an undisturbed life cycle, others show retardation of development in various stages.

It appears that in the case of all species studied, the most decisive phase of the developmental cycle is the migration from the bladder subserosa, as usually in all hosts but *Gobio gobio* and *Phoxinus phoxinus*, the parasite's development comes to a halt in that phase.

A general feature of *Thwaitia* infestation is the phenomenon practically unknown with other nematodes, that the development of females takes place in two well-defined — subserous and extraserous — stages. In certain hosts a practically undisturbed development of the parasite is invariably provided, *viz.*, the host—parasite relationship is brought to an issue in favour of the

* It should be noted that in Hungary, the incidence of *Th. abdominalis* and *Th. kotlani* is much lower than that of *Th. ovata* and the former are inferior to the latter also in respect of the extensity and intensity of infestation. Intensity is closely related to nutrition, that is, to the amount of cyclopses ingested. This may offer an explanation for the fact that the intensity of infestation is higher in fishes with ligulosis which remained plankton-feeders as compared to benthophagous ones. Accordingly, in the same habitat, the comparatively large-sized *Leuciscus* fishes which need more food, are more intensively infested as compared to the smaller *Gobio* and *Phoxinus* fishes. Thus the latter hosts are often either free of *Thwaitia* or contain a single worm or exclusively male or female worms. This, however, had no influence on the classification of the hosts as I grouped them on the basis of the proportion developing to retarded worms rather than on the basis of the percentage of absolute infestation.

latter. In contrast, in other hosts migration to, or development in, the abdominal cavity would seldom take an undisturbed course in which case the contest is decided in favour on the host. The *Thwaitiae* are either unable to migrate from the bladder subserosa into the abdominal cavity or, though having migrated there, are stopped in their development by being encapsulated in connective tissue. The connective tissue capsule interferes with the nutrition and growth of the worm and often causes its death; in any case, it prevents larva production, stopping thereby the reproduction of the "trapped" parasites.

In the latter type of hosts the normal development of the parasite is dependent on certain factors. In a notable proportion of *Abramis* and*Rutilus* fishes, ligulosis is such a factor. It remains to be clarified whether the *Ligulae* produce this effect by improving the growth conditions of *Thwaitiae* through the abdominal exudate due to the mechanical irritation or through reducing the general resistance of the fish host.

The available information is insufficient to decide whether the occasional normal development of *Thwaitiae* in less favourable hosts is due to ligulosis-like factors such as other diseases, or malnutrition. Nevertheless, it seems likely that the limitation of development outlined above is not due to acquired immunity. In fact, two summer fishes did not harbour a larger number of normally developed parasites than their elder companions, though in case of an immunity a greater degree of protection of the elder fishes would be clearly apparent. Anyway, the marked influence of ligulosis on the extensity and intensity of infestation with mature *Thwaitia* females points to the role of predisposing factors in the normal development of the latter.

SUMMARY

Host—parasite relationship between four *Thwaitia* (Nematoda) species — *Th. ovata*, *Th. rischta*, *Th. abdominalis* and *Th. kotlani* — occurring in Hungary, and their fish hosts was studied.

In certain fish species these nematodes reach full maturation in only part of the infested specimens.

Retarded development may be due either to the inability of females to migrate from the bladder subserosa, or to their impairment during further development in the abdominal cavity.

In some cases of *Thwaitia* infection the full maturation of females needs some preconditioning factor(s). Such a factor appears to be concurrent ligulosis, which provides 100% development of *Th. ovata* females.

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BEHAVIOUR OF TURKEY POX VIRUS DURING ADAPTATION TO EMBRYONIC PIG KIDNEY EPITHELIAL CELL CULTURES

By

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The turkey pox virus strain used in the present experiments was applied for immunization first of turkeys, then also of chickens, after serial transfers in the chorioallantoic membrane (CAM) of embryonated hen's eggs (BAMBERGER, SZAKMÁRY and KAKUK, 1957). The strain has been used extensively with good results ever since. Some of its properties have been studied by MALICKI (1964), MAYR (1963) and GELENCZEI and LASHER (1968). The strain was adapted to embryonic chick fibroblast culture by BAMBERGER and MARKOVITS in 1965, and used for the immunization of chickens. BAMBERGER (1967) succeeded in propagating the strain in embryonic pig kidney cells (EPKC), but the titre was too low to enable the preparation of a potent vaccine.

In the present paper observations made during further passages of the virus in EPKC and the immunogenic properties of the adapted strain are described.

Materials and methods

The virus strain used, titration methods and preparation of EPKC were the same as described in a previous paper (BAMBERGER, 1967). Evaluation of immunogenicity on the basis of pox reaction in chickens was carried out by BAMBERGER and SZAKMÁRY's method (1963) as described also by BAMBERGER and MARKOVITS (1965).

Results

Initially serial transfers of the turkey-pox strain to EPKC had often failed. In such cases the next tissue culture was inoculated with a material from the CAM passage of the previous EPKC transfer step. This implies that the alternation of passages in EPKC and CAM invariably depended on necessity.

The sequence of serial and alternate passages is shown in Table I.

From the 99th passage on, alternate transfers in CAM were no longer needed: the strain became adapted to EPKC.

In the EPKC cultures the virus titre was low. In only few cultures did the joint virus content of the tissue culture fluid and of the cells, disintegrated

by freezing and thawing reach a titre of $10^{-4.5}$ (calculated for 1 ml) as titrated on the CAM. The maximum titre of the fluid medium was in general $10^{-3.5}$

Direct transfer from EPKC to EPKC were often successful also prior to the 99th passage. But the maximum titres given above were demonstrable through only a few passages; in subsequent transfers the titre tended to de-

Table I

Sequence of alternative serial passages

The plain figures represent serial numbers of passages in EPKC, those printed in italics serial numbers of alternate transfers to CAM

1	2	3	4	5	6	7	*1*	*2*	8	9	10	*3*	*4*	11	*5*	12
13	*6*	*7*	14	15	*8*	16	*9*	17	18	*10*	19	20	21	*11*	22	*12*
23	24	*13*	25	*14*	26	*15*	27	28	*16*	29	*17*	30	*18*	31	*19*	32
33	34	*20*	35	*21*	36	*22*	37	*23*	38	39	*24*	40	*25*	41	42	*26*
43	*27*	44	45	*28*	46	47	*29*	48	*30*	49	*31*	50	51	52	*32*	53
33	54	*34*	55	*35*	56	57	58	*36*	59	60	*37*	61	62	*38*	63	64
65	66	67	68	69	70	71	72	*39*	*40*	73	*41*	74	75	*42*	76	77
78	*43*	79	80	*44*	81	82	83	*45*	84	85	86	*46*	87	88	89	*47*
90	*48*	91	92	93	94	95	*49*	96	97	98	*50*	99	100	101	102	103
104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120

crease and after further 3—6 direct transfers the virus disappeared. In the 99th passage, the maximum titre of the first CAM-to-EPKC transfer was higher than the average values given above. The $10^{-4.5}$ dilution of the tissue culture fluid was still able to produce pox lesions on the CAM. This titre usually decreased in the next two EPKC passages, but subsequently remained at the same level — $10^{-3.5}$ — through 20 direct passages. Fluctuations in the virus content of the tissue culture fluid are shown in Fig. 1.

Virus titres of the tissue culture fluids were determined daily following inoculation. It was found already prior to the critical 99th passage that on

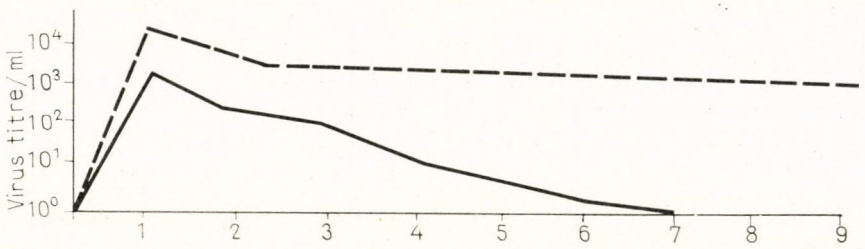

Fig. 1. Fluctuations of maximum virus titre in tissue culture fluids from serial EPKC passages. Remark: ——— between the 40th and 90th passages; — — — after the 99th passage

the day succeeding inoculation, the virus content of the tissue culture fluid was notably less than the input virus. On the subsequent days the titre tended to increase, reaching maximum concentration sometimes by the 2nd day, sometimes by the 4th, 5th or 7th day, a variation apparently independent of the number of passages.

Fluctuations of the virus content of the tissue culture fluid after the 99th passage are shown in Fig. 2.

To assess their virus content and immunogenicity, tissue culture fluids harvested before and after the 99th passage were, in addition to titration on the CAM, regularly inoculated into chickens. Under experimental conditions, tissue culture fluids of at least 10^{-2} CAM titres had invariably positive effect,

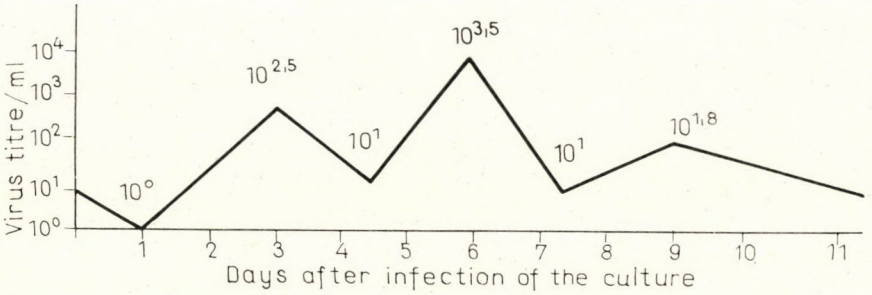

Fig. 2. Daily fluctuation of virus titre of culture fluids from passages succeeding the 99th transfer

producing appropriate follicle reactions and immunity to challenge. But under field conditions, even materials with $10^{-3.5}$ CAM titre failed to produce 100% follicle reaction and only 30—40% of the birds developed an appropriate immunity. The reason apparently was that in the field the amount of the rubbed in material may not have always reached 0.1 ml, and also the time of rubbing may have been abbreviated.

As, owing to its low virus content, the tissue culture fluid did not seem suitable for fowl-pox vaccine preparation, attempt was made to propagate the EPKC-adapted virus on the CAM to obtain higher cencentrations. This enabled a comparison of the adapted strain's properties to those of the routinely used vaccine strains, using roughly identical concentrations.

The 119th, *i.e.*, the 20th direct EPKC passage was carried through 3 transfers in the CAM. Membranes from the 3rd CAM passage were used for preparation of a vaccine in the usual way. This vaccine (S, from "Swine") was compared to the commercial pigeon-pox (P) and turkey-pox (T) vaccines, partly by the follicle method, partly by the wing web method. With each vaccine 20 chickens, 3 months old, were inoculated. The numerical values indicating pox reactions to vaccination and challenge on the 30th day afterwards

are shown in Table II. Challenge was made with a virulent fowl-pox virus strain (V), routinely used for immunogenicity testing in the Institute for Veterinary Vaccine Control.

Table III presents the data from Table II re-grouped in the sequence of numerical values, to facilitate comparison.

Table II

Reactions to vaccination and challenge of chickens immunized with various fowl-pox vaccines

Designation of vaccine		P		S		T		No immunization
Method of vaccination		foll.	w. web.	foll.	w. web.	foll.	w. web.	
Post-vaccination day	5	1.58	0.50	3.0	0.67	0.67	0.68	
	10	2.00	0.36	2.33	1.00	3.08	1.83	
	15	0.08	0.08	0.50	0.45	1.73	2.00	
	20	0.00	0.00	0.00	0.00	1.64	2.08	
Averages:		0.91	0.27	1.45	0.53	1.78	1.62	
Challenge with strain V by follicle method								
Post-challenge day	5	1.80	2.00	1.67	1.82	0.00	0.00	1.81
	10	2.00	3.27	1.33	2.73	0.00	0.00	3.47
	15	0.10	1.64	0.33	0.55	0.00	0.00	2.47
	20	0.00	1.73	1.25	0.55	0.00	0.00	1.73
Averages:		0.75	2.16	1.14	1.41	0.00	0.00	2.72

Explanation. P, pigeon-pox vaccine; S, vaccine prepared from the EPKC-adapted strain; T, turkey-pox vaccine; V, virulent fowl-pox virus.

In unvaccinated chickens the most severe reaction was produced by the virulent strain V. Highest reactivity was found with vaccine T on administration by the follicle method and hardly less severe by the wing web method. Difference between the reactivity of the two methods was more pronounced in the case of vaccines S and P. Lowest reactivity was found with vaccine P when given by the wing web method. Taking the methods of vaccination into consideration, the reactivity of vaccine S was intermediate between that of vaccines T and P.

Table III also shows that, of the preparations tested, vaccine T had the highest immunogenicity. The birds inoculated with it remained symptom-free on challenge. Vaccine P conferred an appropriate immunity by the follicle method, but proved hardly immunogenic by the wing web method. Birds immunized with the latter method behaved on challenge similarly to unvaccinated controls. Vaccine S conferred a practically identical immunity by the follicle and wing web method.

Table III

Sequence of severity of pox reactions to vaccination and challenge

Immunization		Challenge		Average numerical value of pox reaction
Vaccine strain	Method	Strain	Method	
No immunization		V	follicle	2.72
		T	follicle	1.78
		T	wing web	1.62
		S	follicle	1.45
		P	follicle	0.91
		S	wing web	0.53
		P	follicle	0.27
P	wing web			2.16
S	wing web			1.41
S	follicle	V	follicle	1.14
P	follicle			0.75
T	follicle			0.00
T	wing web			0.00

Explanations as in Table II

Discussion

A turkey-pox virus strain which after serial passages in CAM or chick embryo fibroblast cell culture has been used as vaccine strain, was adapted to EPKC.

The EPKC-adapted strain had a low titre: the average virus titre of the tissue culture fluid together with the virus released by disintegration of the cultured cells was as low as $10^{-3.5}$ (calculated for ml), as assessed by pox lesions on the CAM.

In the tissue culture, viral growth was cyclic. On the 3rd, 6th and 9th days the virus content was higher than on the days between. Maximum titre was reached on the 6th day.

Immunization of chickens with the virus-containing tissue culture fluid had equivocal results under field conditions. Only 30—40% of the birds developed an appropriate immunity. This indicates the unsuitability of the EPKC-adapted strain for vaccine preparation.

In order to compare the adapted strain to other vaccine strains, it was carried through three passages in the CAM to appropriately increase its titre. This was undertaken to dispel the eventual erroneous judgement of its pathogenicity and immunogenicity resulting from the low concentration obtained in EPKC.

In the CAM, the adapted strain reached a titre similar to that of the pigeon- and turkey-pox virus strains routinely used for fowl-pox vaccine production in Hungary. Comparing similar concentrations of the three strains, it was found that adaptation to EPKC decreased not only the pathogenicity, but also the immunogenicity, of the strain. As to its properties, the adapted strain was as a rule intermediate between the pigeon- and turkey-pox vaccine strains. According to experimental evidence, it retained only one typical property, *viz.*, identical immunogenicity by the follicle and wing web method. In this respect it differed from the pigeon-pox virus strain, as the latter was highly immunogenic by the follicle method, but hardly immunogenic by the wing web method.

SUMMARY

A turkey-pox virus strain was adapted to embryonic pig kidney cells (EPKC) by alternate passages in the chorioallantoic membrane (CAM) of the embryonated hen's eggs. From the 99th EPKC passage on, alternate transfers to CAM were no longer necessary; the strain grew well through the subsequent 20 direct EPKC passages.

The adapted strain showed a cyclic growth in the culture; its titre, reactivity, and immunogenicity were lower as compared to the original strain.

This implies that EPKC-adapted turkey pox virus is unsuitable for fowl-pox vaccine preparation, both directly and by transfer to CAM.

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LABORATORY DIAGNOSIS OF RABIES BY THE FLUORESCENT ANTIBODY TECHNIQUE

DIAGNOSTIC VALUE OF THE METHOD

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It is known that the presence of Negri bodies in the brain proves, while their absence does not disprove, the existence of rabies. In herbivores Negri bodies may be absent in the early stage of the disease, and in swine even later. The infection experiment, which has been considered the most sensitive method of rabies diagnosis, is very time-consuming; it takes several weeks to read the results even when albino mice are infected intracerebrally.

In some countries, above all in the United States (GOLDWASSER and KISSLING, 1958; GOLDWASSER *et al.*, 1959; MCQUEEN, 1959; SCHNEIDER and FURUSHO, 1966), Canada (BEAUREGARD *et al.*, 1965), German Democratic Republic (JENTZSCH, 1964; JENTZSCH and PITZSCHKE, 1965) and German Federal Republic (SCHNEIDER, 1964a, b; SCHNEIDER and WECHENDÖRFER, 1964), fluorescent antibody (FA) techniques have been employed for rabies diagnosis for nearly a decade. This method has proved considerably more sensitive than the diachromic staining procedures applied for the demonstration of Negri bodies and has the additional advantages of being rapid and specific.

Initiated by the favourable experiences abroad, the diagnosis of rabies by the FA technique was introduced also in this Institute in 1967. In the present paper the results obtained with this method over a period of 18 months are reported.

Material and method

The direct method of the FA technique was applied. The fluorescein-isothiocyanate (FITC) labelled antirabies immunglobulins (conjugates) used initially were supplied by the Institute of Animal Health, Jena (GDR) and the Institute of Poliomyelitis and Viral Encephalitides, U.S.S.R. Academy of Medical Sciences, Moscow; later our own preparation was used. Several immune sera were tested for this purpose. Out of them the serum prepared in the Control Institute for Veterinary Vaccines, Budapest, proved to be the most suitable, as in addition to the demonstration of Negri bodies it was also apt to demonstrate smaller viral aggregations. Conjugation was carried out according to JENTZSCH (1963), (1967), but to enhance the binding of FITC to proteins all phases were performed at 25° C (MCKINNEY *et al.*, 1964).

Thin impressions were prepared from fresh cross-sections of 4 brain areas, the two Ammon's horns, cerebellum and medulla oblongata. The preparations were dried at room temperature and fixed by flaming (JENTZSCH, 1965, 1966). The fixed preparations were covered each with one drop of conjugate, which was previously checked for specificity (MOCSÁRI and SZEMERÉDI, 1968) and diluted as assessed by preceding titration. To prevent desiccation, the preparations were then incubated in wet chamber for 30 minutes at 37° C. Subsequently they were washed for 10 minutes in a PBS of pH 7.4, rinsed with distilled water and air-dried without blotting. A known positive and negative preparation served as controls with each staining series. In doubtful cases, inhibition test was carried out with a non-labelled homologous immune serum (MOCSÁRI and SZEMERÉDI, 1968).

Parallel to staining with the FA technique, all brain specimens were examined for the presence of Negri bodies with the Gerlach staining method routinely used in this Institute. Impressions from the brain areas listed above were fixed for 5 minutes in methanol and subsequently stained according to Gerlach.

Initially infection experiments were carried out parallel to the two microscopic methods. In every case 6 albino mice, 10 to 15 g in weight were inoculated intracerebrally with 0.03 ml of a 1 : 10 suspension of the suspect brains (DEAN, 1966). The brains from mice died spontaneously during, or killed after, the 28-day period of observation were examined both by Gerlach's and FA staining.

Later on, when the results had convinced us of the specificity and high sensitivity of the FA technique, infection experiments were set up exclusively in those cases where the results of the two microscopic methods were inconsistent. Yet, regardless of the consistently negative results of the latter two methods, infection experiments were always performed when there was a history of biting of man, or a strong suspicion of rabies.

Results

The central nervous systems of 1600 animals of various species were examined. The results are summarized in Table I. The results obtained with the applied three methods of examination are compared in Table II, and the diagnostic efficiencies of the FA technique and Gerlach's staining technique in Table III. As shown in the Tables, in every case where the impressions stained according to Gerlach showed Negri bodies, FA staining gave invariably positive results. Of the 530 cases found positive by the FA test, Negri bodies were demonstrable in only 448 (84.5%). To decide whether the cases found negative by Gerlach's staining, but positive by the FA staining, were actually specific,

Table I

Species distribution of the examined and positive samples

Species	Cases examined	Positive cases
Dog	352	5
Cat	348	23
Swine	10	2
Sheep	12	3
Goat	1	1
Cattle	2	
Fox	752	490
Red deer	31	4
Wild cat	16	
Wild boar	8	
Badger	9	2
Hare	18	
Ondatra	9	
Ferret	6	
Polecat	6	
Hamster	1	
Beach marten	1	
Gopher	1	
Rat	8	
Squirrel	1	
Ground squirrel	1	
Field mouse	1	
Hedgehog	2	
Bat	4	
Total	1600	530

Table II

Comparative results obtained with the three methods of examination

Method	Total number of cases examined	Results	
		Positive	Negative
Fluorescent antibody technique	1600	530	1070
Gerlach's staining	1600	443	1152
Experimental infection	348	87	261

Table III

Comparison of the results obtained with fluorescent antibody technique and Gerlach's method in 1600 cases

Fluorescent antibody technique		Gerlach's staining method			
		positive		negative	
Result	Number of cases	No. of cases	per cent	No. of cases	per cent
Positive	530	448	84.5	82	15.5
Negative	1070	—	0.0	1070	100.0

infection experiments were carried out with the brain material. The positive diagnosis of rabies was always confirmed by the infection experiments.

The results of animal experiments undertaken to appraise the diagnostic value of the FA technique are shown in Table IV. In only a single case was the animal experiment negative when both Gerlach's and FITC staining showed positive results. But in 4 cases where FA staining was negative, the infection experiment confirmed the diagnosis of rabies.

Table IV

Comparison of the results obtained by fluorescent antibody technique and experimental infection in 348 cases

Experimental infection		Fluorescent antibody technique	
Result	Number of cases	Positive	Negative
Positive	87	83 (98.8%)	4 (1.5%)
Negative	261	1 (1.2%)	260 (98.5%)

Discussion

The evaluation of the examinations revealed a 99% diagnostic reliability for the FA technique, whereas the Gerlach staining technique showed Negri bodies in only 85% of the obviously infected brains.

False negative results were found with the FA technique practically only during the first four weeks after its introduction. Later on, when we became familiar with the various sources of error inherent in the technique, the initial errors could be explained.

The efficiency of the immunofluorescence tests depends largely on the quality of the conjugate. A poor quality of the conjugate seems to be the most frequent source of error in appropriately performed and controlled experiments. Examinations by JENTZSCH and WINKLER (1967) have shown that not all high-titre anti-rabies sera are suitable for the demonstration of small viral aggregations by the FA technique.

Another frequent source of error is the non-uniform distribution of the viral antigen in the brain. False conclusions stemming from this circumstance may be reduced or eliminated by examining simultaneously several — at least four — brain areas.

Since with the FA technique exclusively the unequivocally positive results are diagnostically relevant, in doubtful cases the performance of infection experiments is imperative.

SUMMARY

Brains from a total of 1600 animals of different species were examined for rabies parallel with Gerlach's staining method and direct fluorescent antibody (FA) technique. In 348 cases also infection experiments were performed. Of 530 brains found positive with the FA technique Negri bodies were demonstrable with Gerlach's staining in 448 (84.5%). Parallel examinations by FA technique and infection experiments carried out on 87 infected and 261 non-infected brains yielded consistent results in 98.8 and 98.5% of the cases, respectively.

Consideration of the mostly technical sources of error — poor quality of the conjugate, non-uniform distribution of viral antigen in the brain — involved in the FA technique have led to the conclusion that the latter's negative results are not exclusive of the presence of rabies, thus in doubtful cases infection experiments must not be omitted.

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ANTHELMINTIC EFFICACY OF 2,6-DIHYDROXYBENZOIC ACID-4'-BROMANILIDE (HOE 296 V) AGAINST MATURE AND IMMATURE PARAMPHISTOMUM MICROBOTHRIUM IN GOATS

By

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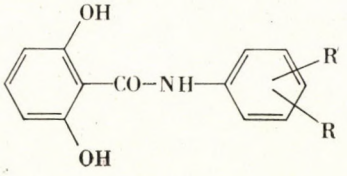
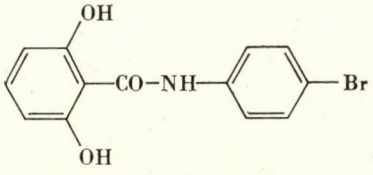
Introduction

In search of new anthelmintics with cestodicidal activity, extensive experimental investigations were carried out by DÜWEL with a series of 2,6-dihydroxybenzoic acid-anilides,* synthesized by H. RUSCHIG and J. KÖNIG. The compounds which showed high efficacy against tapeworm infections are described with the general formula I (KEELING, 1968). R = H or alkyl; R' = Halogen



In early experimental studies on sheep and cattle infected with paramphistomes, several anthelmintics proved to be effective against mature or immature stages. The cestodicidal drug niclosamide (Lintex,® Mansonil®) and bithionol appeard to be the most effective compounds against immature paramphistomes in the abomasum and intestines of sheep (LÄMMLER, 1968).

In the present study, 2,6-dihydroxybenzoic acid-4'-bromanilide, Resorcylam (Hoe 296 V) (II), one of the most active cestodicidal drugs of the series mentioned above was tested in preliminary trials against the *Paramphistomum microbothrium* infection in goats.



* South African patent No. 67/0007, Farbwerke Hoechst AG, Frankfurt/M.—Hoechst, Germany.

Materials and methods

Experimental animals

Three adult African dwarf goats, artificially infected with metacercariae of *P. microbothrium* and passing paramphistome eggs in the faeces were selected for treatment of mature parasites. Six 3—4 months old kids (bred in the institute) were infected for the treatment of immature parasites.

Infection:

Paramphistome eggs were collected from donor animals and cultured at 30° C for 10 days. The hatched miracidia were exposed to 3 different species of tropical snails, *Physopsis globosus* (Strain of Johannesburg, South Africa), *Bulinus tropicus* (Strain of Onderstepoort, South Africa) and *B. contortus* (Strain of Khemis-el-Kachena, Algeria). The snails, bred in the laboratory were examined periodically for discharge of cercariae. The snails started discharging cercariae 40—45 days after infection. The infected snails were maintained in dark aquaria. They were allowed to discharge cercariae twice a week on filter paper in a funnel. Later, the encysted cercariae (metacercariae) still on filter paper were counted microscopically and fed to kids within 14 days after harvest.

Anthelmintic and dose

A suspension of Hoe 296 V (2,6-dihydroxybenzoic acid-4'-bromanilide) was prepared using 9 g of the powder in 100 ml water. This gave a total volume of 105 ml. The suspension was given orally in one dose of 1 ml/kg body weight (about 65 mg/ml active substance since 1 g powder contained 750 mg active substance).

Criteria of therapeutic activity

Therapeutic activity was based in mature paramphistomosis on the presence or absence of parasite eggs in the faeces and parasites in the gastro-intestinal tract. In immature paramphistomiasis it was based on the presence or absence of parasites in the gastro-intestinal tract.

Results

Experiment I

Mature *Paramphistomum microbothrium* infection: Three goats infected with *P. microbothrium* were examined and faecal egg counts were made 2 times before treatment. Thereafter 2 goats were treated and one remained as untreated control. Faecal egg counts were made during 30 days after treatment. The number of eggs in the treated goats decreased considerably 3 days post

Table I

Efficacy of Hoe 296 V (2,6-dihydroxybenzoic acid-4'-bromanilide) against mature *Paramphistomum microbothrium* infection in goats
Dose: 65 mg/kg body weight per os

Goats	Treated/ Control	Pre- and post-treatment egg count per 2 g faeces* (in days)									Duration from treatment to autopsy (in days)	No. of worms recovered
		−7	−5	+3	+4	+7	+10	+14	+17	+30		
1	Control	602	757	761	690	709	410	298	305	609	—	—
2	Treated	1065	891	8	1	0	0	0	0	0	325	0
3	Treated	1085	979	6	2	0	0	0	0	0	36	0

* Sedimentation technique

Table II

Efficacy of Hoe 296 V (2,6-dihydrobenzoic acid-4'-bromanilide) against immature *Paramphistomum microbothrium* infection in goats
Dose: 65 mg/kg body weight per os

Kid Nos.	No. of meta-cercariae fed	Duration from infection to *Paramphistomes recovered* (in days)								Total	% Efficacy
		Treatment	Autopsy	Rumen	Reticulum	Omasum	Abomasum	Small intes-tine	Large intestine		
1	1600	Control	40	397	59	0	0	26	0	482	—
2	1600	30	40	0	0	0	0	0	0	0	100
3	1600	30	40	0	0	0	0	0	0	0	100
4	1800	Control	35	872	190	0	0	15	0	1077	—
5	1800	20	35	0	0	0	0	0	0	0	100
6	1800	20	35	0	0	0	0	0	0	0	100

medication and no eggs were demonstrable 1 week after treatment. Then, the treated goats were sacrificed and examined for parasites. The control goat, on the other hand, continued to pass paramphistome eggs in the faeces (Table I).

Experiment II

Immature *Paramphistomum microbothrium* infection: 3 kids received 1600 metacercariae. 2 of them were treated 30 days after infection and autopsied 10 days later. The remaining kid was kept as the untreated control and autopsied with the other treated kids.

3 additional kids were infected with 1800 metacercariae. 2 of them were treated 20 days after infection and autopsied 15 days later. The third kid, the untreated control, was sacrificed with the other 2 treated kids (Table II).

Discussion

In the present study, a new cestodicidal compound, 2,6-dihydroxy-benzoic acid-4'-bromanilide (Hoe 296 V), at about 65 mg/ml suspension per kg body weight (9 g powder = 6.75 g active substance in 100 ml water), showed a remarkable efficacy against toh mature and immature *P. microbothrium* in goats. It is, therefore, possiblebot remove both mature and immature paramphistomes with a single oral administration.

The present results suggest that the new compound is most effective against 20 day-old, 30 day-old and adult *P. microbothrium* in goats. HORAK (1962) tested Freon® 112, hexachlorophene, Promintic® and niclosamide (Lintex) against *P. microbothrium* infection in sheep. He found Freon 112 ineffective, but single doses of hexachlorophene and Promintic were 43—45% effective against immature paramphistomes. In repeated doses the efficacy of these drugs was increased above 99%. A single oral administration of niclosamide at 50 mg/kg was more than 94% effective against paramphistomes in the abomasum and intestines, whereas an oral administration of as much as 75 mg/kg only was 15.9% effective against these flukes in the rumen. An oral dose of 50 mg/kg showed more than 95% efficacy against 20—22-day-old paramphistomes. Again in 1964, he reported that niclosamide at 50 mg/kg being highly effective against 7, 14 and 20-day-old paramphistomes in sheep, but ineffective against metacercariae. He found 50 mg/kg ineffective against immature paramphistomes in cattle and observed variable results at 100 mg/kg. 150 mg/kg proved ineffective against adult flukes.

Although, these studies were confined to only *P. microbothrium* infection in goats, the present compound appears to be more effective than niclosamide, since it is active against both mature and immature *P. microbothrium*. It is

suggested that further studies should be undertaken to evaluate its activity against various stages of different species of paramphistomes in sheep and cattle.

SUMMARY

In preliminary trials a new cestodicidal compound, 2,6-dihydroxybenzoic acid-4'-bromanilide, Resorcylam (Hoe 296 V) has been tested against mature and immature *Paramphistomum microbothrium* infection in goats. At about 65 mg active substance/kg body weight (using a suspension of 9 g powder = 6.75 g active substance in 100 ml water), the compound was highly effective against 20- and 30- day-old, as well as adult paramphistomes in goats. The compound had no apparent side effects.

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THE SUSCEPTIBILITY OF SUCKLING RABBITS TO INFECTION WITH COCCIDIA

By

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Parasitologists concerned with the biology of rabbit coccidiosis generally agree that rabbits of different ages respond differently to identical doses of infection. Most severe liver lesions are developed only by animals, approximately 1.5—2 months old, which have been weaned at least for one or two weeks; later on susceptibility tends to decrease.

This age-dependent susceptibility of rabbits to coccidial infestation is best known in relation to *Eimeria stiedai* infections. According to the experimental data of KOTLÁN and PELLÉRDY (1937), suckling rabbits resist infections with *E. stiedai* oocysts for at least the first two weeks of their life, while at the age of 20 days they are almost fully susceptible. Similar observations were made by ROSE (1958). KOTLÁN and PELLÉRDY (1937) failed to offer an explanation for the resistance of suckling rabbits to *E. stiedai* infection. They tentatively attributed it on one hand to maternal immunity transferred intrauterinely or through the milk to the offsprings, on the other, to special biochemical functions or the anatomical structure of the young organism, above all of the young liver, which somehow do not favour the settlement of coccidia.

The information on resistance to intestinal coccidiosis of suckling rabbits is also scanty, and PELLÉRDY (1965) summarized the relevant data.

The present experiments were undertaken to clarify whether suckling rabbits are actually resistant to coccidial infestation and if they are, to what extent.

Newborn rabbits belonging to one and the same litter were infected once or repeatedly at different intervals after birth to examine whether a single infection or repeated infections would cause liver coccidiosis. Another experimental series was set up to check the resistance of suckling rabbits to intestinal coccidia. In all experiments, the basic principle was to attempt the suppression of resistance by extremely massive doses of oocysts.

Experiment 1

The susceptibility of suckling rabbits was checked with the parenteral dose which was known to cause massive infestation of older animals (PELLÉRDY, 1969). From a litter, one 9-day-old rabbit (a) was infected intraperitoneally with fresh merozoites recovered from a donor rabbit which had been infected

14 days earlier. Two of its litter mates were infected orally (b) and intraperitoneally (c) with 200 000 *E. stiedai* oocysts each at the age of 13 days. A fourth litter mate (d), 14 days old, received intraperitoneally 1 ml of a mixture of sporocysts and excysted sporozoites. Artificial excystation was allowed to take place in pancreatic solution. A fifth litter mate (f), 18 days old, was given 200 000 *E. stiedai* oocysts orally.

The rabbit infected with merozoites (a) was killed on the 5th day after infection (p. i.). In its liver there was neither gross nor microscopic evidence of the settlement of coccidia. Both 13-day-old rabbits were killed on the 20th day p.i. In the liver of the orally infected rabbit (b) there were developmental stages and oocysts, which were also seen in unstained smears, and in the bile oocysts alone. In the liver of the parenterally infected rabbit (c) there was neither gross nor microscopic evidence of coccidiosis. The rabbit infected with excysted oocysts (d) showed thickening of the bile ducts and the presence of oocysts in the liver and bile when killed on the 19th day p.i. The fifth rabbit (f), killed on the 18th day p.i. showed proliferation of the bile ducts and inflammatory cellular reaction around them, but these lesions were much milder as compared to those usually seen in older animals infected with similar doses of oocysts. The presence of various endogenous stages of *E. stiedai* was also stated in this animal.

Experiment 2

Suckling rabbits from another litter were infected orally with about one million oocysts of *E. stiedai* when 2 days old. The infection was repeated after 4 days. One rabbit died at the age of 7 days and in its intestine empty oocyst shells were found. The survivors were reinfected with the same massive doses of oocysts on the 8th, 13th and 15th day of life.

The rabbits were killed successively on the 19th, 20th, 22nd, 25th, 27th and 29th day after the first infection. All of them had considerably enlarged livers with pale areas of no sharp contours on the surface. Many oocysts were found in these areas, as well as in the enlarged bile ducts and gall bladder. Taking the 16—17-day endogenous developmental cycle of *E. stiedai* into consideration, it is obvious that at least the oocysts found in the rabbit killed on the 19th day after the first infection developed as a result of this first infection at the age of 2 days.

On microscopic examination of the liver on the 19th day p. primam i. (p.p.i.) a larger amount of coccidia was seen to parasitize the walls of the larger bile ducts compared to the smaller ones. Different endogenous stages as well as oocysts were encountered, but the former forms were hardly distinguishable owing to the different times of infections. Though the proliferations of the bile ducts were distinct, they never reached the cauliflower-like density found in

older animals. Invaginations were less deep, less frequent and the proliferation less arborized.

The liver of the rabbit on the 20th day p.p.i. was considerably enlarged and large pale areas with no distinct contours were seen over its surface. Here and there on the diaphragmal surface of the liver sharply demarcated coccidian

Fig. 1. Diaphragmal surface of a 25-day-old rabbit infected with one million oocysts of *Eimeria stiedai*, each on the 2nd, 4th, 8th 13th and 15th day of life. The enlargement of the bile passages with vesicle-like protrusions are clearly seen. About the half of the natural size

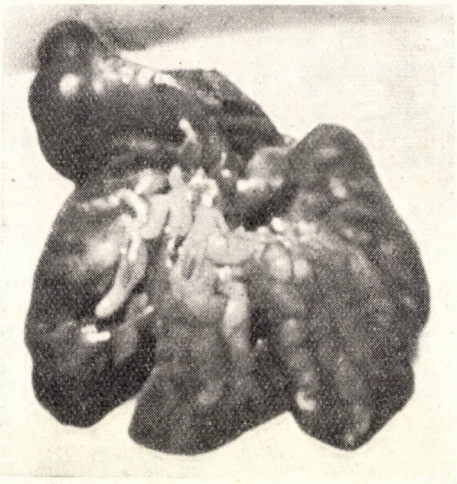


Fig. 2. Portal surface of the liver shown in Fig. 1. The distended bile passages are of pencil-thickness and show many bubble-like extensions. About two thirds of the natural size

foci occurred. At the portal surface many thin-walled bile ducts of pencil thickness packed with *E. stiedai* oocysts were apparent. Histologically, the proliferation was more distinct as compared to that found in the rabbit killed on the preceding day, but the liver lesions were less pronounced than they used to be in the older rabbits.

The liver of the rabbit killed on the 22nd day p.p.i. showed a still more distinct distension of the bile ducts at the portal surface. In histological cross-sections the dilated bile ducts appeared as enormously enlarged cavities. There was a more distinct proliferation of the bile duct walls than in the rabbits killed previously, yet it was still moderate as compared to the picture common in older hosts. Endogenous asexual and sexual developmental stages as well as oocysts and debris did not fill up the lumen entirely as in the older rabbits after the same time p.i.

By the 23rd day p.p.i. the bile ducts were found to have distended almost to finger thickness (Figs 1, 2) which contained detached macrogametes, mature oocysts, and debris of parasites and tissue. These observations were made in unstained smears as the decomposing liver of the spontaneously died rabbit was unsuitable for histological examination.

By the 25th day p.p.i. the liver had enlarged enormously. The periportal bile ducts were of finger thickness and showed many protuberations. At the diaphragmal surface of the liver, the enlargement of the bile ducts was signified by vesicle-like protrusions. In their yellowish-purulent contents there were masses of oocysts. Extremely numerous *E. stiedai* oocysts were encountered also in the intestine. Microscopic examination showed that the coccidia hardly invaded the smallest bile ducts, while in the dilated large ones many developmental stages could be seen in the epithelium, sometimes two in one cell.

The lesions found in the liver from the animal autopsied on the 27th day p.p.i. were roughly of the above type. Maximum enlargement of the liver was found on the 29th day p.p.i.. At that time, in addition to the distension of the bile ducts many focal lesions, typical of coccidiosis, were present. Microscopically, the greyish-white sharply demarcated foci contained many oocysts and showed a distinct proliferation of the epithelium which hardly differed from the conventional coccidial lesions of older rabbits.

Experiment 3

The foregoing experiment was repeated essentially on the same schedule with rabbits from 3 litters. The 9 rabbits from the first litter (a) were infected once at the age of 0.5 days, 9 rabbits of the second litter (b) thrice at 3-hourly intervals at the age of 1 day, and 6 rabbits of the third litter (c) on two occasions at 5 hourly intervals when 3 days old. Each dose consisted of several millions of *E. stiedai* oocysts, using a paste-like centrifugal sediment of the infectious material which consisted exclusively of oocysts. This infectious material was administered orally with a spatula and rinsed down with a little water.

(a) Rabbits from the first litter were autopsied successively on the 4th, 5th, 6th, 11th, 12th, 13th, 14th, 15th and 16th day p.i. Macroscopically a yellowish discoloration of the liver surface was not seen until the 12th or 13th day, when also the surface texture appeared more pronounced. Apart from this, the livers appeared roughly normal. Histologically, sporadic schizogonic

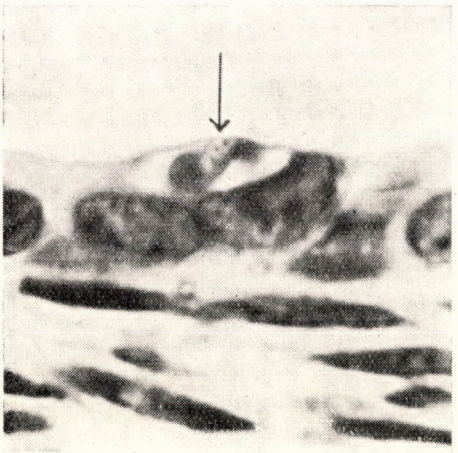


Fig. 3

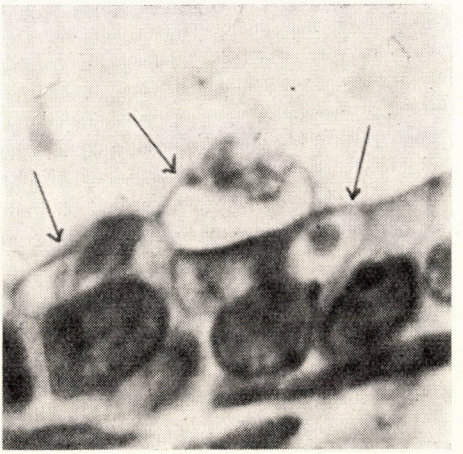


Fig. 4

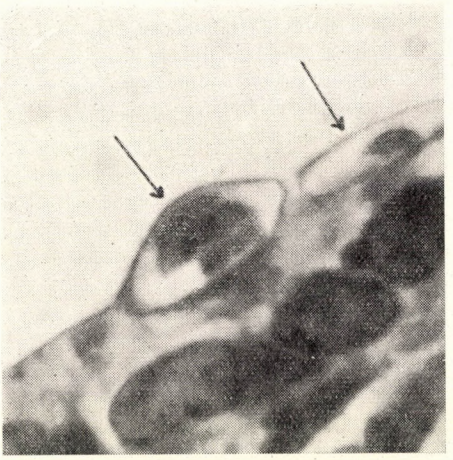


Fig. 5

Fig. 6

Figs 3—6. Young and premature schizonts in epithelial cells of the bile ducts in the liver of a rabbit infected when 1 day old. 8th day of infection. H. E. × approx. 1000

stages were found as early as the 12th day while gametogonic stages appeared on the 16th day. No oocysts were found in the liver tissues. The small bile ducts were usually not involved, the few parasites found in the livers occurred chiefly in the large or medium sized ducts. Proliferation, first noted on the

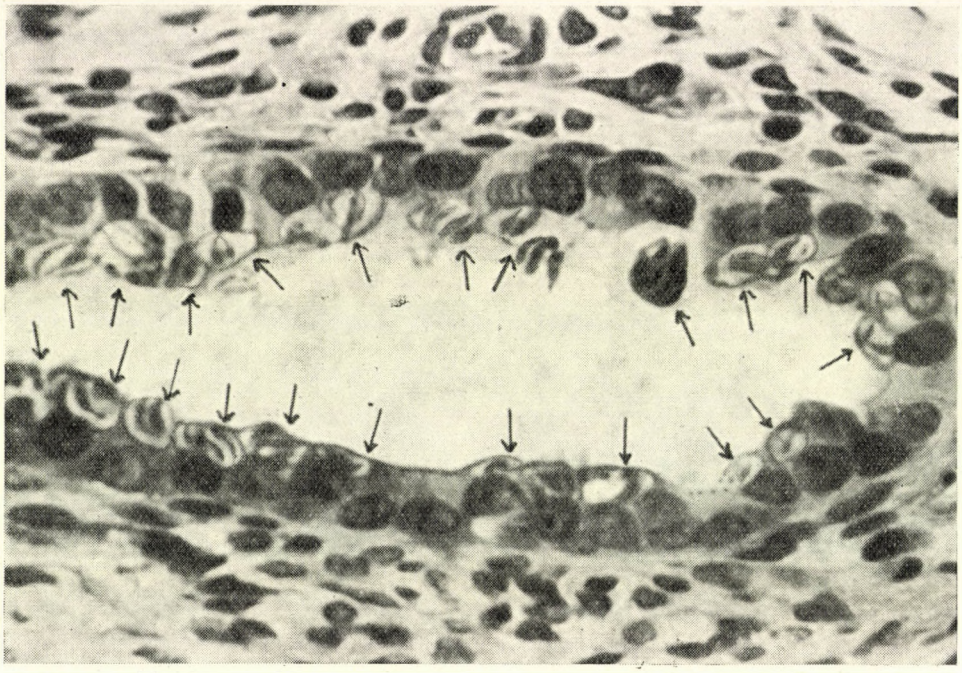


Fig. 7. Middle-sized bile duct on the 10th day p.i. in the liver of a suckling rabbit infected when 1 day old with one or two millions of *E. stiedai* oocysts. Developing schizonts attach loosely to the epithelial lining. H.E. × approx. 500

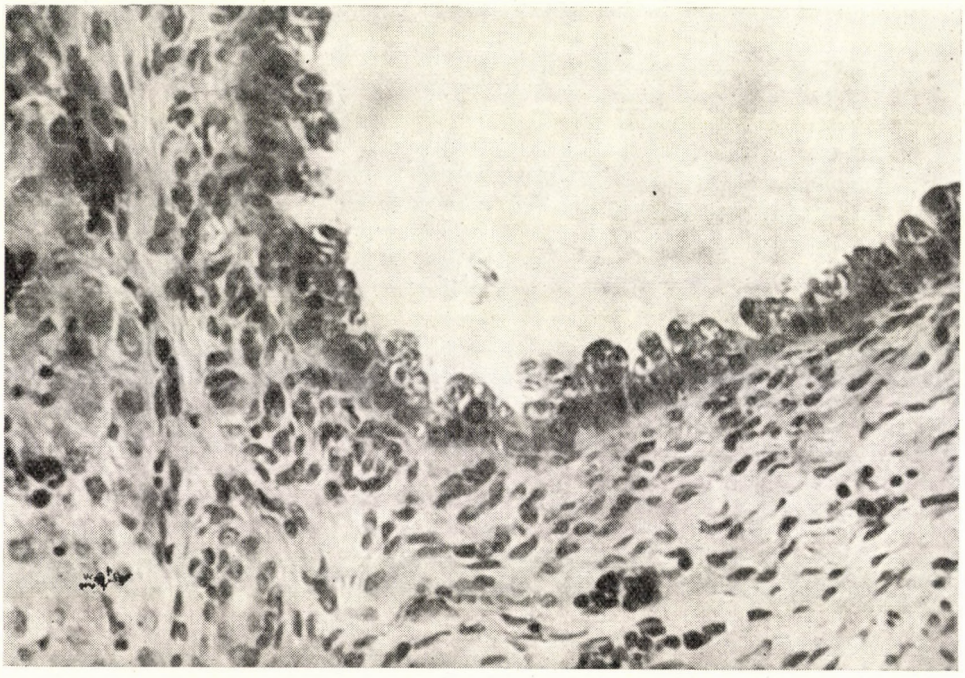


Fig. 8. Moderate proliferation of the wall in the bile duct of a 1-day-old suckling rabbit infected with several millions of *E. stiedai* oocysts. 10th day after infection. H. E. × approx. 250

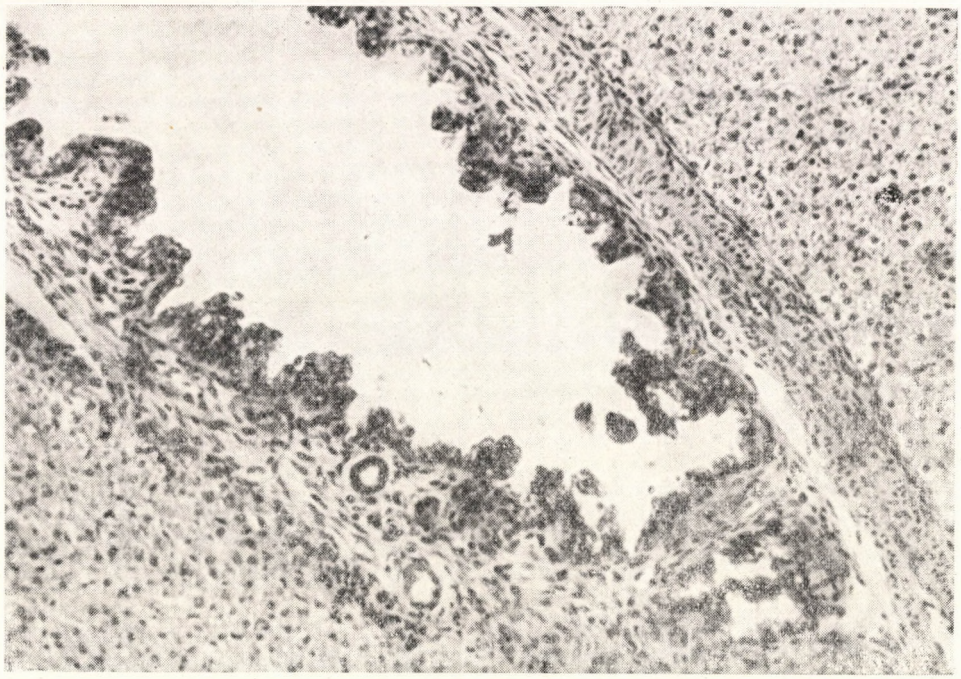
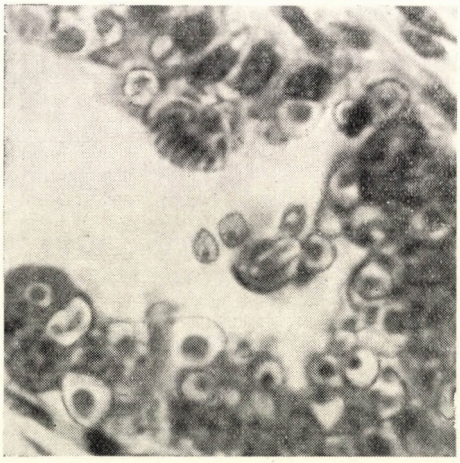
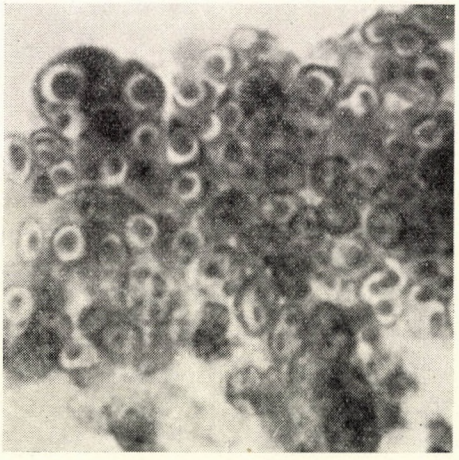


Fig. 9. The same type of lesion in the liver of a litter-mate 3 days later. The proliferation is more pronounced, though the protuberances of the bile duct wall are short and sporadic. H.E. × approx. 150





Figs 10—11. Schizogonic stages (Fig. 10) and (pre)gamonts (Fig. 11) on the 16th day p.i. in the liver of suckling rabbit infected when 3 day old. H. E. × approx. 500

15th day, was slightly more pronounced on the 16th day, but by far not to such an extent as is common in older rabbits after similar infections.

(b) The nine rabbits from the second litter were examined successively on the 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th and 13th day after infection. Infestation appeared to be more pronounced than in the previous group though there was no notable distension or proliferation of bile ducts. From the 4th

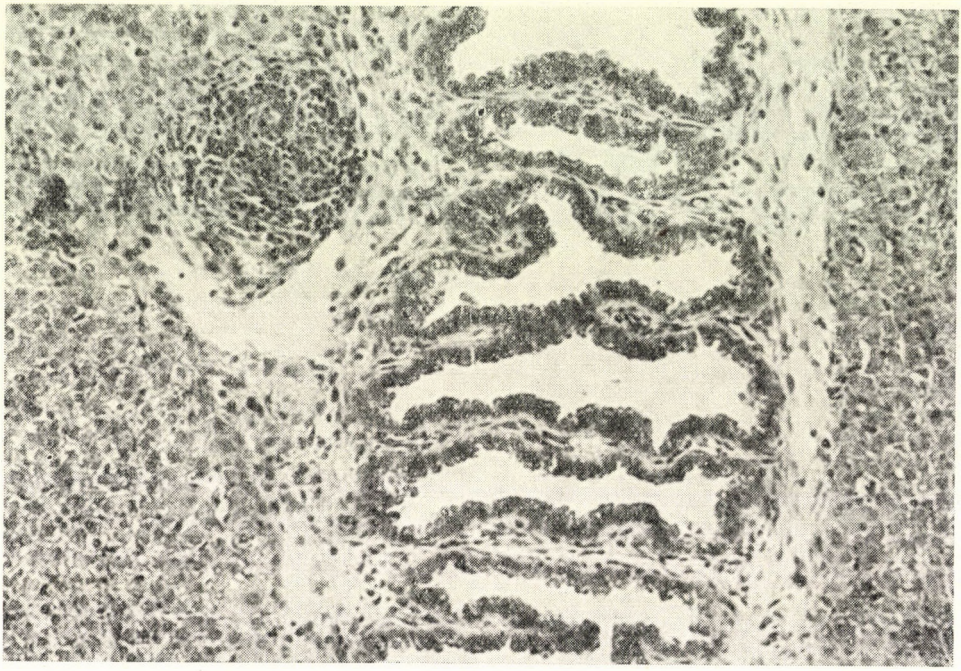


Fig. 12. Queer pattern of proliferation on the 16th day p.i. Bile duct of a suckling rabbit infected with massive dose of *E. stiedai* oocysts when 3 days old. The epithelium is packed with various endogenous stages, first of all gamonts. H.E. × approx. 150

day p.i. on, sporozoites and trophozoites could be seen at the bases of the epithelial cells of the bile ducts. From the 7th day on, schizonts were seen here and there in the medium sized ducts (Figs 3, 4, 5, 6). The schizonts were attached loosely to the practically normal epithelium (Fig. 7). A moderate proliferation appeared on the 10th day p.i. (Fig. 8) and it was more pronounced on the 13th day p.i. (Fig. 9).

(c) The six rabbits from the third litter were examined post mortem on the 2nd, 4th, 7th, 12th, 14th and 16th day p.i. Sporozoites were first seen around the bile ducts on the 4th day. From the 12th to 16th day, schizogonic stages up to gamonts (Figs 10, 11) occurred in increasing numbers. No oocysts were seen in this case either. Proliferation started on the 12th day and became fairly pronounced by the 16th day (Fig. 12).

Experiment 4

Due to the successful infection experiments with *E. stiedai,* attempts were made to prove the susceptibility of suckling rabbits to intestinal coccidiosis. From a litter of 6 rabbits, 3 were infected at 4 days (a) and the other 3 animals at 9 days of age (b) with a mixture of oocysts which contained practically all species of intestinal coccidia of the rabbit.

From both groups, one rabbit received untreated oocysts, the second partially excysted oocysts with free sporocysts and the third oocysts which were excysted so as to release a substantial amount of the sporozoites.

(a) In the group infected when 4 days old, on the 8th day p.i. the infection was most pronounced in the rabbit given the oocysts excysted to release sporozoites. One drop from the surface of glycerine-flotation of the intestinal contents from this animal contained about 10 oocysts of intestinal coccidia, while similar material from the rabbit given partially excysted oocysts contained only 1 or 2. In the third rabbit, infected with intact oocysts, no oocysts were seen.

(b) The 3 suckling rabbits, infected at the age of 9 days, were examined also 8 days p.i. In this case, too, the infection with oocysts excysted to release sporozoites was the most successful. In the intestinal contents of this rabbit averagely 10 oocysts were seen in each field of vision after flotation, while in samples from the other two rabbits only 2 or 3 oocysts were found with the same method.

Some short segments of the small intestine of the rabbit infected with the oocysts excysted to release sporozoites had a thickened wall which was of a paler colour as compared to the other parts of the intestine. In the altered intestinal regions, about 1—2 cm in length, schizonts were apparent in the scrapings and histological examination revealed the presence of many developmental stages. In the other parts of the intestine, as well as in the middle portion of the intestine of the rabbit infected with less excysted oocysts, the number of developmental stages was much lower and in the intestine of the rabbit given intact oocysts no coccidia could be detected histologically.

Discussion

The results of the present experiments appear to disprove the earlier statements that suckling rabbits are resistant to coccidian infestation.

The previous findings by KOTLÁN and PELLÉRDY (1937), and ROSE (1958), indicating the resistance of suckling rabbits to some thousands of *E. stiedai* oocysts is not incongruent to the present findings as we used extremely massive doses of infective material.

It appears that the resistance of rabbits to intestinal coccidia and to *E. stiedai* is most pronounced but not complete at birth and tends to decrease

afterwards. This is supported by the notably lower susceptibility of the younger rabbits as compared to the older ones and by the distinctness of gross lesions encountered in the livers of serially infected rabbits when 2—3 weeks old. This correlates well with our practical experience that at weaning at 18 days of age, rabbits are less susceptible to coccidian infection with normal doses of oocysts than 1 or 2 weeks later when resistance has fully declined. Our experiments have also shown that suckling rabbits can be infected with *E. stiedai* parenterally though not to such an extent as the 1—2 months old weaned rabbits.

Based on the above considerations, successful attempts have been made to clarify whether "natural" resistance to coccidiosis follows a similar course in other mammalian hosts (SCHRECKE, personal communication).

SUMMARY

Infection experiments on a total of 42 suckling rabbits of different ages were performed with *E. stiedai* and intestinal coccidia of the rabbit. Given extremely massive doses (e.g. several millions oocysts per animal), the rabbits were susceptible from birth on to both oral and with *E. stiedai* parenteral infection. The resistance of suckling rabbits to all species of rabbit coccidia was strongest at birth and subsequently tended to decline. Experimental infection was considerably more successful with oocysts which were excysted to release sporozoites than with intact oocysts. One of the rabbits given excysted oocysts of intestinal coccidia at the age of 9 days had typical coccidial lesions in 1 to 2 cm long segments of the small intestine.

In the livers of rabbits infected serially with *E. stiedai* up to 2—3 weeks of age there was a marked dilation of the bile ducts on the portal surface. The dilated bile passages protruded sharply from the liver tissue.

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LÉPINE, P.—GAMET, A.: *La rage* (Rabies). Collection de monographies. Les maladies animales à virus. L'Expansion Editeur. Paris. 1968. Price 26 Francs.

This volume of the series of emerging virus diseases of farm animals is especially opportune, as rabies is expanding in Europe and in other parts of the world. The volume contains up-to-date data on sylvatic rabies spred mainly by foxes and advancing steadily in Europe from north-east to south-west.

After a general introduction and history of the disease the properties of the virus showing similarities to myxoviruses are described, but the classification of rabies virus in the separate group of Rhabdoviruses seems to be motivated. The discussion of differences between virulent street virus and fix virus strains is important in both theoretical and practical respect. Clinical symptoms and pathological lesions of the disease are treated briefly. In the chapter on the pathogenesis of the disease emphasis is layed on the possibilities and importance of various ways of infection.

Among diagnostic methods of rabies interest is focussed to methods which enable a rapid demonstration of the virus. The great importance of the immunfluorescent technique is stressed, but limitations of this method are also discussed in the light of the great responsibility in deciding on the necessity of vaccination of subjects in contact with suspect animals. In the chapter on the postinfectious treatment the necessity of wound treatment as well as the production and indications of the application of vaccine and immune serum are discussed. Disagreeable side effects of the immuno prophylactic methods are described in detail. The last two chapters on the epizootiology and prophylaxis of the disease are restricted to listing generalities of basic importance.

The majority of the 230 publications listed in the bibliography are in French and English, only few other papers are mentioned. (The 10 German titles included are written rather faulty.) This restriction of the literature used may be an explanation for shortcomings of the text, especially concerning some topics of practical veterinary interest. For instance veterinary surgeons acting in infected countries, but probably also those of immediately threatened countries like France, will miss details concerning the results of prophylactic vaccination of dogs and other animal species. There will not be full agreement with the authors' proposition to vaccinate persons exposed professionally to the infection (laboratory research workers and veterinary surgeons of endemic regions) by yearly vaccination. Chapters on the virology and pathology of the disease as well as those on laboratory diagnostic methods and treatment are well compiled sources of valuable information for all specialists engaged with rabies problems both in the laboratory and in the field.

T. SZENT-IVÁNYI

MORNET, P.—GILBERT, Y.: *La peste equine.* (African horse sickness.) Collection des monographies. Les maladies animales à virus. L'Expansion Éditeur. Paris. 1968. 140 pages. Price: 39 Francs.

The actuality of this new volume of the French series on virus diseases of animals is given by the fact that African horse sickness is one of the so called exotic virus diseases which, due to increasing trade relations with African countries, crossed the border of formerly enzootic areas of southern and equatorial Africa and caused disastrous outbreaks in South Asia, North Africa and appeared once also in Europe. Besides economical losses caused by the disease, the results of recent research work on the disease and its virus are reported.

In the sequence of chapters on the history, virology, clinical symptoms and pathological lesions, epizootiological and etiological features, diagnosis, immunity and sanitary regulations applied against the disease, the authors follow the structure of former volumes of the same series (on African swine fever, rinderpest, infectious anaemia of horses and Aujeszky's disease). The introductory chapter describes the history of the disease in the originally infected areas of Africa south of the Equator, and disastrous outbreaks in Egypt and Palestine in 1943—44, in South Asia in 1959—60, and in North Africa in 1965—66. Though the horse has lost most of its former importance as a drought animal in developed countries, race and sport horses represent an important and continuously increasing economical value. Efforts made by international bodies (FAO, OIE) to confront the danger of introduction of the virus into Europe, especially after an occasional outbreak of the disease at the southern coast of Spain in the autumn 1966 are discussed.

Properties of the etiological agent are described in detail and relations with reo- and arbovirus groups discussed. Theoretical and practical bearings of the certainly most important biological property of the virus, the occurrence of at least 9 serologically different types, is treated in detail. After concise chapters of about 20 pages each on clinical and pathological features as well as the epizootiological and etiological characteristics of the disease, an especial emphasis is layed on the description of diagnostic procedures and control measures, including vaccine production and application in the field. A supplementary chapter summarizes literary data on the susceptibility of the dog to the virus of African horse sickness and its eventual role in the spreading of the infection. The bibliography lists 224 publications.

The value of this concise monography, summing up all necessary and up- to- date informations on African horse sickness is increased by the fact that the authors are in possession of a long years' experience on the disease. This renders their monography not only a useful up-to-date information on this emerging exotic virus disease, but also a delightful piece of reading.

T. SZENT-IVÁNYI

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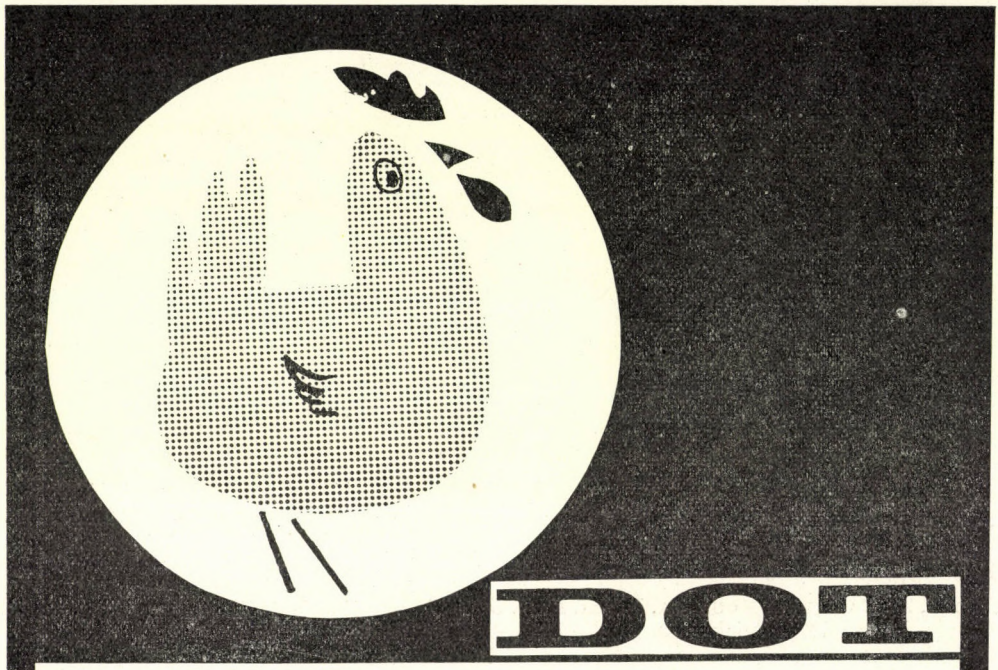

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РЕЗЮМЕ

ЗОБНАЯ ЖЕЛЕЗА КОСТИСТОЙ РЫБЫ НИЛЯ CLARIAS LAZERA

В. РИЗКАЛЛА

Зобная железа Clarias lazera сместилась немножко к зади с обычного места. Последнее имеется непосредственно позади жабр.

В малых индивидах длины 9 мм, этот орган не показывает дифференциации тканей. Это явление, то есть разделение на корковую и мозговую зоны, первый раз наблюдается в индивидах длины 12—15 мм. Добавочная, то есть третья зона, обнаруживается только в животных длины выше 17 до 150 мм. В крупных рыбах зобная железа подвергается инволюционным изменениям, что продолжается до ее полного исчезновения.

Основная масса зобной железы состоит главным образом из ярко окрашивающихся тимоцитов, среди которых имеются ретикулярные клетки, образующие остов. Некоторые ретикулярные клетки кажутся фагоцитами, тогда как другие дифференцируют в липоидные клетки.

Для зобной железы характерно наличие кист, которые с возрастом как по количеству, так и по размерам прибывают. Кисты представляют собой ареалы перерождения в этом органе. Больше того, кисты различных размеров, наполненные слизью, обнаруживаются в зобной железе рыб, несмотря на их возраст.

На ранней стадии гистогенеза зобной железы имеется связь между этим органом и лимфатической головкой почки при посредстве лимфоцитарных тяжей. Иногда обнаруживается даже полное сливание этих двух органов.

НАДПОЧЕЧНИКИ И СТАННИЕВЫ ТЕЛЬЦА КОСТНОЙ РЫБЫ НИЛЯ CLARIAS LAZERA

В. РИЗКАЛЛА

Надпочечники у Clarias lazera являются высокоорганизованной компактной тканью, окружающей переднюю часть задней кардиальной вены, где эта ткань внедряется в правую долю.

Надпочечная железа состоит из межпочечной ткани, образующей бóльшую часть железы, в которой имеются разбросанные группы хромафинных клеток.

Межпочечную ткань формируют клеточные тяжи железистых клеток, разделенных капиллярами. Клетки содержат аскорбиновую кислоту и разное количество жира.

Разбросанные группы хромафинной ткани кислыми красками окрашиваются плохо. Хромафинные клетки обычно закруглены, они больше ложнопочечных клеток, с нечеткими контурами.

Станниевы тельца представлены 3—7 малыми, круглыми тельцами, разбросанными и частично понуренными в центральную поверхность средней почки.

Снаружи тельца обладают соединительно-тканной капсулой, состоят из эпителиальных долек, разделенных друг от друга богатой сосудами соединительной тканью.

Станниевы тельца являются высокоорганизованными эпителиальными органами, четко отделяющимися от почечной ткани, в которой они находятся. Они вероятно представляют собой эндокринный орган с неизвестной функцией.

НАБЛЮДЕНИЯ О ГИСТОХИМИЧЕСКИХ СВОЙСТВАХ РАЗВИВАЮЩЕГОСЯ ФОЛЛИКУЛА И ЖЕЛТОГО ТЕЛА ЯИЧНИКА КОШКИ И СОБАКИ

С. С. ГУРАЯ

Изучены гистохимические свойства развивающегося фолликула и желтого тела яичника кошки и собаки. Клетки зернистого слоя развивающегося фолликула богаты цитоплазматической РНК. Среди клеток зернистого слоя тут-там наблюдаются липидные тельца, состоящие из фосфолипидов. Наиболее заметным гистохимическим изменением, ведущим к дифференциации (или лютэинизации) клетки зернистого слоя фолликула в клетку желтого тела является появление большого количества липопротеинов в цитоплазме. Липидные тельца, содержащие фосфолипиды, или фосфолипиды и триглицериды тоже образуются в цитоплазме клеток желтого тела. Как липидные включения, так и диффузные липопротеины видимо связаны с синтезом стероидных гормонов.

Элементы стромы **theca interna** тут-там тоже содержат фосфолипидные тельца. Однако наблюдающиеся в **theca interna** кошьячего яичника гипертрофированные клетки наполнены грубыми липидными тельцами, состоящими из фосфолипидов, холестэрола или холестэролевых эстеров и триглицеридов. Их нельзя наблюдать в желтом теле.

С регрессом клеток желтого тела грубые липидные включения, состоящие или из фосфолипидов и триглицеридов, или фосфолипидов, триглицеридов и холестэрола или эстеров холэстерола накопляются в больших количествах в цитоплазме. Некоторое количество пигмента, вероятно липофусцина, тоже образуется на поздних стадиях регресса желтого тела. Увеличение количества грубых липидных включений совпадает с уменьшением количества диффузного липопротеина, из чего можно судить, что первые могут образоваться путем патологических изменений из последнего. Регрессирующие клетки желтого тела, наполненные грубыми липидными тельцами, могут иметься в строме яичника в течение месяцев.

ОПРЕДЕЛЕНИЕ РЕЗЕРВА ЖИВЧИКОВ ВНЕ СЕМЕННИКОВ У БУЙВОЛА

А. М. ОСМАН и Е. ЭЛ-АЗАБ

Изучался резерв живчиков вне семенников у двух возрастных групп буйволов — 1,5-и 2,5-летних — с целью выявления возможной корреляции между количеством всех живчиков и весом и объемом семенников и их придатков. Для определения количества живчиков применялся новый метод, связанный с мацерацией и измерением при помощи цитометра. Данные и соотношения показаны в таблицах. У обеих возрастных групп сигнификантное соотношение обнаружено между количеством всех живчиков и весом придатков семенников (гр. I: $r = +0{,}973$, $P < 0{,}1\%$; гр. II: $r = +0{,}884$, $P < 0{,}27\%$). Сигнификантная корреляция между количеством всех живчиков и весом семенников обнаружено только в возрастной группе I ($r = +0{,}654$, $P < 5\%$).

ОПРЕДЕЛЕНИЕ МАРГАНЦА В ПЕЧЕНИ ЦЫПЛЯТ БЕЛЫЙ ЛЕГХОРН В РАННИЙ ПОСЛЕИНКУБАЦИОННЫЙ ПЕРИОД

З. СОВА, З. МАРЕК, К. КОУДЕЛА и Й. ХОУШКА

В возрасте 1—31 день изучалось содержание марганца в печени цыплят белый легхорн. Сразу после вылупления в печени обнаруживалось всего 1,33 μм марганца, то есть $0{,}34 \pm 0{,}086$ μг/100 г сухого вещества и 0,124 μг/100 г всей печени. За время первой недели вес печени увеличился в два раза, тогда как количество всего марганца в печени увеличилось только незначительно — до 1,92 μг. С 18. дня возраста наступает резкое увеличение количества марганца в этом органе, в результате чего при увеличении веса печени до 31-дневного возраста в 7 раз — содержание этого элемента в печени увеличивается в 17 раз.

КОБАЛЬТ В ПЕЧЕНИ ЦЫПЛЯТ БЕЛЫЙ ЛЕГХОРН В РАННИЙ ПОСЛЕИНКУБАЦИОННЫЙ ПЕРИОД

З. МАРЕК, З. СОВА, К. КОУДЕЛА и Й. ХОУШКА

Изучено количество кобальта в печени цыплят белый легхорн в возрасте 1—31 день. Непосредственно после вылупления печень содержала 18,2 μг/100 г кобальта, то есть всего 196 μг этого элемента. Позже количество кобальта начинает увеличиваться и, таким образом, к концу первого месяца возраста значения кобальта в этом органе колебались в пределах 15,6 — 46,3 μ/100 г печени, то есть 60 $\pm$ 56 μг и 180 $\pm$ 40 μ/100 г сухого вещества. В течение 31 дня вес печени увеличился в 7 раз, тогда как количество кобальта в ней — в 13,6 раза. Таким образом печень месячных цыплят содержала 2660 μг кобальта.

НЕКОТОРЫЕ ГЕМАТОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ У КРУПНОГО РОГАТОГО СКОТА ПРИ НОРМАЛЬНОЙ И ПОЛУСИНТЕТИЧЕСКОЙ ДИЕТЕ

Й. ПАВЕЛ

Проводился анализ крови у трех козлов, содержавшихся на нормальной диете (луговое сено и ячмень) и двух козлов при содержании их на полусинтетической диете (пшеничная солома, крахмал, мочевина, подкормка нужными минеральными веществами). Проводились следующие анализы крови: определение всего белка, остаточного азота, фильтрация сывороточных белков на жели Сефадекс 5—200, бумажно-электрофоретическое фракционирование сывороточных белков, липопротеинов и гликопротеинов, определение активности сывороточной молочнокислой, малатовой и сорбитной дегидрогеназ, щелочной фосфатазы и амилазы.

При анализе данных обнаружено, что у животных на полусинтетической диете сигнификантными являются снижение остаточного азота в сыворотке, понижение активности сывороточной сорбит-дегидрогеназы и щелочной фосфатазы.

БЫСТРЫЙ МЕТОД ДИАГНОСЦИРОВАНИЯ ТРИХОМОНАДОЗА У БЫКОВ МИКРОСКОПИЧЕСКИМ ИЗУЧЕНИЕМ СОСКОБА СЛИЗИСТОЙ ПРЕПУЦИЯ

П. ШУТКА и Л. КАТАИ

Хотя широкое внедрение в практику искусственного осеменения существенно сократило заболеваемость быков трихомонадозом, паразитов все же обнаруживается среди быков, применяющихся главным образом в естественной случке. Поэтому данный диагноз является важным средством в профилактике этого паразитоза. Обычный метод диагноза — изолирование паразита из полоскательной жидкости препуция — не удовлетворяет этой цели. Под импульсом подобных начинаний литературы авторы описывают быстрый эффективный метод обнаружения **Trichomenas foetus** микроскопическим изучением нативного соскоба слизистой препуция, полученного специальным инструментом. Результаты изучения 522 соскобов проверены и методом культивации.

РЕАКЦИЯ ОСЕДАНИЯ ЭРИТРОЦИТОВ И ПОКАЗАТЕЛЬ ГЕМАТОКРИТА У ЗДОРОВЫХ УТОК

Й. ЯНТОШОВИЧ и К. ФРИД

Изучались РОЭ и показатели гематокрита у здоровых взрослых и молодых двухмесячник уток. РОЭ изучалась у 46 уток-самок, у 21 самца и 10 утят при помощи пипеток Вестергрин просвета 2,5 мм при наклоне 60°. Разница в этих показателях двух поголовьев была незначительной (1—2 мм) и несигнификантной. Возраст влияет на РОЭ в том смысле, что у молодых животных эта реакция проходила быстрее.

Показатели гематокрита изучены у 47 уток-самок, у 20 самцов и 10 утят обычным методом. У разных полов обнаружены незначительные, несигнификантные отличия. Меньшие показатели обнаруживались у животных молодого возраста.

ЕТСАНЭКСПЕРТИЗА СТРАДАЮЩЕГО РЕТИКУЛОПЕРИТОНИТОМ КРУПНОГО РОГАТОГО СКОТА С ОСОБЫМ ВНИМАНИЕМ НА ОБНАРУЖИВАЕМОСТЬ

А. ФОРРАИ и И. САЗАДОШ

Проведено патолого-анатомическое исследование 139 голов крупного рогатого скота, болевшего до убоя перфоративным ретикуло-перитонитом и среди них 125 изучено и бактериологически. В боенском материале г. Печ среди 20 257 нормальных убойных животных у 0,32% обнаружен ретикуло-перитонит, а среди вынужденно убитых — у 9,3%. В 83 (59,7%) случаях среди 139 перфорацию сетки сопровождало острое или хроническое воспаление брюшины. В 56 случаях инородное тело поступило дальше и повредило легкие (14,4%), сердце (10%), селезенку (7,9%) и печень (7,1%).

На основании данных своих наблюдений авторы считают обоснованным проводить бактериологическое исследование материала при незначительном и хроническом воспалениях брюшины. При помощи бактериологических исследований в 49,6%-ах случаев выявлено наличие **Corynebacterium pyogenes**. Сепсис от упомянутой микробы диагносцирован в 27 случаях. На основании патолого-анатомической и бактериологической оценок 139 случаев мясо 58 голов скота декларировано безусловно годным для потребления, 19 голов — слабого качества, 15 животных — условно годным и, наконец, мясо 47 голов оказалось негодным для потребления.

ТОРМОЖЕНИЕ ВЫРАБОТКИ ПЕРВИЧНОГО АНТИБАКТЕРИАЛЬНОГО ИММУНИТЕТА ПРИ ПОМОЩИ ГЕТЕРОЛОГИЧЕСКОЙ АНТИЛИМФОЦИТАРНОЙ СЫВОРОТКИ

Л. РЕТИ и М. ПАДАНИ

Изучался эффект гетерологической антилимфоцитарной сыворотки (АЛС) на выработку первичного антибактериального иммунитета у мышей, иммунизированных адсорбированной к гидроокиси алюминия вакциной против рожи свиней и перезаражавшихся дозой 10^5 DLM вирулентного рожистого штамма. Обнаружены следующие факты.

Гетерологическая антилимфоцитарная сыворотка эффективно тормозила выработку иммунитета против бактерий рожи свиней.

Иммунитет подавляющая обработка АЛС была эффективной только на ранней. стадии иммунизации. Более эффективной была, если она применялась до иммунизации.

В продуктивной фазе иммунитета от АЛС не получено иммунитет подавляющего эффекта.

Изучалась роль АЛС, как гетерологического белка в механизме подавления иммунитета. Обнаружено, что в их системе интерференция имелась между изучаемым геном и антигенной стороной гетеропротеина АЛС. Эта интерференция однако не влияет существенно на специфическое иммунитет подавляющее действие АЛС.

ИЗУЧЕНИЕ ВЛИЯНИЯ ТРОФНОГО ГОРМОНА СЖК НА ПОЛОВЫЕ ОРГАНЫ НЕОТКЛАДЫВАЮЩИХ НЕСУШЕК. ПОТРЕБНОСТЬ В КИСЛОРОДЕ И САХАРЕ И ОТДАЧА МОЛОЧНОЙ КИСЛОТЫ ЯИЧНИКАМИ, МАГНУМОМ И УТЕРУСОМ

А. КЕМЕНЬ, ЕВА ФОДОР и ДЬ. ЛЕНЧЕШ

Изучалось значение QO_2 фолликулов, магнума и утеруса и степень поступления сахара в ткани этих органов и отдача ими молочной кислоты у двухлетних несушек с атретизированными яичниками и яйцеводом.

35 уже давно неактивные несушки разбиты в две группы — подопытную и контрольную. Животные первой группы в течение восьми дней ежедневно получали препарат гормона СЖК (Гестил Органон, 500 МЕ). Состояние (неактивность) половых органов проверяли наощупь на лапаратомизированных животных. Первой дозой инфильтровали яичник, после чего следовал курс внутримышечного введения препарата, ежедневно в одинаковое время при одинаковой дозе. Контрольным животным таким же способом вводили физраствор поваренной соли.

Под влиянием гормона СЖК из фолликулов промера 2—3 мм развились вполне зрелые таковые промера 20—30 мм. Вес яичников с 4,5 ± 0,57 повысился до 72,6 ± 11,40 г в среднем. В одном яичнике насчитывали по 8—12 фолликулов величины в орех. Ненормальное распределение фолликулов и повторное наблюдение, когда правосторонний атрофизированный яйцевод тоже стал развиваться, указывают на то, что обработка гормоном СЖК действует более энергично, чем физиологическое раздражение.

При убое несушек неделю спустя от обработки гормоном СЖК фолликулы были уже дряблыми, сморщенными, но яйцевод еще не показывал явлений инволюции.

Именения значений QO_2 в изученных органах показаны в табл. I.

В обработанной гонадотропным гормоном СЖК группе поступление глюкозы в ткани утеруса было без изменения, тогда как в ткани магнума поступило ее меньше, чем у контрольных животных ($p < 0{,}01$). Эти же показатели яичников обработанных животных были сигнификантно ($p > 0{,}01$) меньше, чем контрольных таковых.

Магнум обработанных животных гонадотропным гормоном СЖК отдавал на 70% меньше молочной кислоты, чем тот же орган контрольных животных ($p > 0{,}01$).

Данные дискутируются в связи со структурой, развитостью яйцевода и формированием обмена веществ в нем.

ФОРМИРОВАНИЕ ВЗАИМОСВЯЗИ ПАРАЗИТ—ХОЗЯИН МЕЖДУ ВИДАМИ РОДА THWAITIA И ИХ ХОЗЯЕВАМИ

К. МОЛНАР

Изучена взаимосвязь четырех видов Thwaitia — Th. ovata, Th. rischta, Th. abdominalis и Th. kotlani —, обнаруживающихся и в Венгрии с их хозяевами.

Развитие этих видов в некоторых видах хозяев не всегда проходит бесперебойно.

Перебои в развитии могут обнаруживаться в том, что самки задерживаются под серозной оболочкой плавательного пузыря, или они повреждаются в брюшной полости.

В некоторых случаях инвазии твайтиами для бесперебойного развития самок нужно наличие некоторых факторов. Одним среди них можно считать лигулезную инвазию у хозяев, которая обеспечивает 100%-ное развитие самок твайтий.

ПОВЕДЕНИЕ ВИРУСНОГО ШТАММА ОСПЫ ИНДЕЕК В ПЕРЕПРИВИВКАХ НА ТКАНЕВУЮ КУЛЬТУРУ ИЗ ПОЧКИ СВИНОГО ПЛОДА

К. БАМБЕРГЕР, П. МАРКОВИЧ и ПИРОШКА ВЕКЕШ

Венгерский штамм оспы индеек путем переменной привики на тканевую культуру почки свиного плода и хорио-аллантоис куриного зародыша удалось адаптировать к тканевой культуре почки свиного плода. С 39-й перепрививки на тканевую культуру почки свиного плода уже не нужно было перепрививать на хорио-аллантоис куриного зародыша. После этого в 20 пассажах штамм дружно размножался в этой культуре.

Привыкший к тканевой культуре почки свиного плода штамм размножается циклически, но титер низок, вызывает менее выраженную прививочную реакцию и обладает более слабым иммуногенным свойством, чем исходный вирусный штамм.

На этом основании штамм не удобен для производства вакцины против оспы птиц.

ЛАБОРАТОРНОЕ ДИАГНОСЦИРОВАНИЕ БЕШЕНСТВА МЕТОДОМ ИММУНОФЛУОРЕСЦЕНЦИИ; ДИАГНОСТИЧЕСКОЕ ЗНАЧЕНИЕ МЕТОДА

Е. МОЧАРИ и Д. КОВАЧ

За время 18 месяцев изучен головной мозг 1600 голов разных видов животных на бешенство методом окрашивания по Герлах и прямой иммунофлуоресценции и проведено 348 экспериментальных прививок. Среди 530 мозгов, обнаруженных методом иммунофлуоресценции неблагополучными, окрашиванием по Герлах только в 448 выявлены (84,5% тельца Негри. Метод иммунофлуоресценции и результаты экспериментального

заражения касательно 87 зараженных и 261 отрицательного мозгов совпадали на 98,8 и 98,5% соответственно.

В обсуждении отчасти технической причины погрешностей при исследованиях методом иммунофлуоресценции (слабое качество конъюгата, неравномерное распределение вируса в мозгу) авторы приходят к заключению, что отрицательный результат при употреблении этого диагностического метода не исключает наличия бешенства, поэтому в данном случае нельзя отказаться от применения экспериментального (диагностического) заражения.

АНТИГЕЛЬМИНТНАЯ ЭФФЕКТИВНОСТЬ 2,6-ДИГИДРОКСИ-БЕНЗОЙНАЯ КИСЛОТА-4'-БРОМАНИЛИД (HOE 296) ПРОТИВ ПОЛОВОЗРЕЛЫХ И МОЛОДЫХ ЭКЗЕМПЛЯРОВ PARAMPHISTOMUM MICROBOTHRIUM В КОЗЕ

Г. ЛЕМЛЕР, Б. Н. ШАХАИ и Х. ГЕРЦОГ

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