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ONTOGENETIC DIFFERENCES IN THE ERYTHROGRAMS OF WHITE LEGHORN AND CORNISH CHICKENS LIVING UNDER TROPICAL CONDITIONS

By

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(Received May 7, 1971)

The purpose of the present study was to examine the values of the red blood picture in chickens in the course of development, and to establish the differences in these values between the WL and CO breeds. Another aim was to find whether there are any differences in the values for chickens kept under tropical conditions from those reported by other authors for the European moderate zone.

The effect of age on different blood parameters in chickens kept in the European zone has been studied by a number workers. Some of them treated all hematological values as a whole (JOVANOVIĆ and VAPA, 1959; 1960; JANTOŠOVIČ, 1965; VRBENSKÁ et al., 1967; RUSOV and PETROVIĆ, 1968). Others examined only some of these values, such as the hemoglobin level (TANAKA and ROSENBERG, 1953), hematocrit value and sedimentation rate (BIERER et al., 1964), erythrocyte, leucocyte counts and differential leucocyte count (PROCHÁZKA, 1965), hematocrit values (MEDWAY and KARE, 1959; BEST, 1966), etc.

A number of authors have studied seasonal effects and the influence of environmental temperatures on the hematological values both in chickens and mature poultry. For instance, DUKES and SCHWARTE (1931) and OLSON (1937) claim that the lowest values of hemoglobin and erythrocytes were obtained in the late summer and in autumn, while the highest values were found in winter. WINTROBE (1934) also reported higher hemoglobin levels in colder weather. JANTOŠOVIČ's data (1965) indicate that the hemoglobin level is higher in summer, while the erythrocyte count tends to decrease slightly. HUSTON (1960) found that not only were the erythrocyte count and hematocrit value higher at lower temperatures, but also the thyroid gland mass and O_2 intake. Also MOYE et al. (1969) examined the differences in hematocrit values, red cell mass and mean corpuscular volume, and demonstrated that all three values were higher at low temperatures.

Material and methods

The studies were undertaken in Cuba on 163 chickens of the White Leghorn (WL) breed and 160 chickens of the Cornish (CO) breed, from 1 day after hatching to the 28th day of age. The chickens came from two poultry breeding stocks which had been established in tropical conditions of this country for several generations. They were kept in open-sided sheds on deep wood-chip litter, with infra-red lamps used at night. The chickens were taken directly from the batteries at one day of age and fed suitable commercial mixtures according to their age. The health condition of the birds was good.

Table I

Meteorological data for the investigated period

Month	Temperature °C	Water precipitation mm	Relat. humidity %
January	21.7	60.7	77.0
February	22.1	41.4	75.0
March	23.1	47.8	75.0
April	24.4	50.2	75.0
May	25.5	102.3	78.0
June	26.4	145.6	81.0

The trials were carried out from January to June. Average monthly temperatures, water precipitation and relative humidity for this period are indicated in Table I. Hemoglobin content, packed cell volume, the total red blood cell count, the mean corpuscular volume, the mean corpuscular hemoglobin concentration and the mean corpuscular hemoglobin were determined in all birds at 1, 3, 5, 6, 8, 9, 12, 17, 19, 22, 23 and 26 days of age. For the purposes of statistical analysis the birds were divided into five age-groups, as follows: one-day-old, 2- to 7-day-old, 8- to 14-day-old, 15- to 21-day-old and 22- to 28-day-old. Blood samples were collected from young birds directly from the a. carotis, in larger birds from the v. ulnaris, into a syringe previously rinsed with a heparin solution.

Hemoglobin content was determined by the cyanhemoglobin method (SKÁLA and MATYSKOVÁ, 1965). The precipitate of nuclear substances formed after the addition of blood to the solution was separated by means of centrifugation for 3 minutes at 1500 r.p.m.

The total erythrocyte count was calculated in Natt—Herrick's solution (CELER, 1960). The packed cell volume was determined in Wintrobe's test-tubes. The mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated from the above mentioned values using the appropriate formulae.

The dispersion analysis method with multiple classification in odd numbers of replications was employed for the mathematical and statistical evaluation. The test characteristic F was evaluated at the 1% and 5% levels of reliability.

Results and discussion

The hemoglobin content (Tables II, III, IV) was comparatively high in chickens of both breeds on the first day after hatching (8.0—8.8 g%), but fell on the 3rd, 6th and 17th—19th days. In the WL chickens this low level persisted

Table II

Hematological values in WL and CO chickens according to age groups

Age in days	Breed	*n*	HB — g%	PCV — %	RBC — mm³	MCH — pg	MCV — /μ³	MCHC — %
1		32	8.0±1.1	30.4±2.7	2.1±0.2	37.7±4.9	142.3±15.9	26.3±2.9
2— 7		32	7.2±0.8	29.6±3.0	2.2±0.3	35.2±4.9	135.5±16.4	24.3±3.1
8—14	WL	32	8.3±0.5	30.4±2.9	2.2±0.3	37.5±4.0	138.2±23.2	27.1±3.3
15—21		32	7.1±0.7	25.9±2.4	1.9±0.3	37.7±4.1	138.1±12.7	27.3±4.1
22—28		35	6.9±0.9	27.3±2.8	2.0±0.3	34.4±3.4	134.9±15.9	25.6±2.2
1		33	8.8±1.1	31.2±2.6	2.3±0.3	39.2±6.7	139.1±16.5	28.4±3.3
2— 7		32	7.2±0.5	29.3±2.5	2.2±0.3	31.9±4.9	132.7±16.6	24.5±2.7
7—14	CO	32	8.1±0.7	29.6±2.7	2.2±0.3	37.7±4.9	137.9±14.7	27.5±2.3
15—21		32	7.3±0.7	28.4±2.9	2.1±0.3	36.7±3.7	139.6±19.9	26.6±2.7
22—28		31	8.1±0.7	29.9±2.7	2.2±0.3	37.1±4.2	139.3±18.0	26.6±2.5

until the 25th day, whereas in the chickens of the CO breed an increase occurred as early as the 22nd day.

Highly significant differences were found between the following groups: 1—2, 1—4, 1—5, 2—3 and 3—4. A significant difference ($p < 0.05$) was observed also between the groups 3 and 5. The WL chickens were found to show statistically significantly lower values than those of the CO breed.

Table III

Hematological parameters in WL chickens according to age

Age in days	*n*	Hb — g%	PCV — %	RBC — mm³	MCH — pg	MCV — /μ³	MCHC — %
1	32	8.0	30.4	2 199 000	37.7	142.3	26.3
3	18	7.0	28.3	2 093 000	33.4	135.2	24.7
5	8	8.1	31.3	2 237 000	37.6	143.2	24.1
6	8	7.2	31.8	2 650 000	34.3	121.2	22.7
8	9	8.4	30.9	2 138 000	39.1	146.3	26.4
9	13	8.2	32.1	2 170 000	37.1	145.6	25.2
12	11	8.3	28.4	2 360 000	35.8	121.2	29.6
17	20	7.2	26.0	1 860 000	38.9	140.9	27.4
19	6	6.3	23.7	1 725 000	36.1	139.2	26.6
22	8	6.7	25.5	1 935 000	34.4	131.5	26.2
23	14	6.4	26.3	1 870 000	34.3	141.8	24.3
26	12	7.4	28.8	2 203 000	33.9	132.5	25.6

Table IV

Hematological parameters in CO chickens according to age

Age in days	*n*	Hb — g%	PCV — %	RBC — mm^3	MCH — pg	MCV — $/\mu^3$	MCHC — %
1	33	8.8	31.2	2 265 000	39.2	139.1	28.4
3	10	7.2	30.3	2 346 000	31.4	130.3	24.1
5	12	7.6	28.9	2 243 000	33.8	129.5	25.8
6	12	6.9	30.6	2 260 000	30.2	137.3	22.4
8	17	8.0	29.5	2 129 000	37.4	139.0	27.1
9	7	8.3	29.0	2 020 000	38.2	144.2	28.7
12	7	8.1	31.5	2 530 000	37.1	124.9	26.0
17	10	7.5	29.9	2 080 000	36.4	146.0	25.0
19	12	6.9	27.3	2 038 000	36.0	135.6	26.8
22	6	7.5	30.5	1 980 000	37.0	155.0	23.8
23	10	7.7	27.8	2 048 000	37.3	137.1	26.8
26	12	8.6	31.5	2 400 000	36.0	132.1	27.3

VRBENSKÁ et al. (1967) obtained similar values in 1-day-old cockerels; however, they found that the maximum decrease occurred on the 10th day. Other authors, such as RUSOV and PETROVIĆ (1968), claim values exceeding 10.0 g% for chickens in early age.

The marked decrease in the hemoglobin content on the 3rd and 6th day may justify the assumption that embryonal hemoglobin is replaced by adult hemoglobin, as claimed by ROMANOFF (1960), who says that the progressive decrease in oxygen affinity from the 10th day of incubation persists until the 14th day of post-embryonal life by which time the "definite" form of hemoglobin has completely replaced the embryonal form. SOVA et al. (1968) found that from the 4th—7th day after hatching the absolute Fe reserve in chicken livers showed no changes, which results in a marked decrease of Fe in the indices expressing Fe in mg% of dry matter.

The packed cell volume as well as the amount of hemoglobin was quite high (30.4—31.2%) in one-day-old chickens of both breeds. In WL chickens it fell on the 3rd day but in the CO's only on the 5th day, but this was consolidated on the subsequent days. Minimum values were obtained from the 17th to the 22nd day and on the 19th day in WL and CO chickens, respectively.

The differences in the packed cell volume of the two breeds were statistically highly significant ($p < 0.01$) in all age categories. As far as the age groups are concerned, highly significant differences were obtained among the one-day-old and 15-day-old and older chickens, and also between groups two and four, and three and four.

A similar decrease was described by BEST (1966) for 4-day-old New Hampshire chickens, by VRBENSKÁ et al. (1967) for 5-day-old WL cockerels, and by RUSOV and PETROVIĆ (1968) on the 10th, 40th and 60th days. Recent authors, however, claim much higher values than those obtained in our study. MEDWAY and KARE (1959), on the other hand, assert that packed cell volume measured in chickens in weekly intervals shows practically no changes in the course of aging.

The total erythrocyte count was relatively high in 1-day-old chickens of both breeds, which corresponds with the data reported by all authors (JOVANOVIĆ and VAPA, 1959; PROCHÁZKA, 1968; VRBENSKÁ et al., 1967; RUSOV and PETROVIĆ, 1968). However, the data on the further changes in the count show some differences; thus only VRBENSKÁ et al. (1967) and JANTOŠOVIĆ (1965) assert that the minimum values were obtained on the 7th day. In our trials the erythrocyte count was comparatively balanced up to the 17th day, when there was a sudden decrease which was maintained until the 23rd day. Although on the 1st day after hatching a reduction of the erythrocyte number was not directly demonstrated, this is suggested by the decreases in the hemoglobin level on the 3rd and 6th days and in the packed cell volume on the 6th and 12th days, by the fluctuating decrease in mean corpuscular hemoglobin from the 3rd to the 6th day, and by the relative hypochromia occurring from the 3rd day after hatching and balanced only on the 8th day.

Statistical treatment revealed that the number of erythrocytes was highly significantly lower in the WL than in the CO breed. Highly significant differences were observed among the one-day-old, 2- to 7-day-old, 8- to 14-day-old, and the fourth age group of chickens. No statistically significant differences were revealed among the other age groups.

The falling values of most of the indices of the erythrogram justify the assumption that in this period erythrocyte destruction occurs also in poultry, as described by MORROS SARDÁ (1960) for new-born children. Furthermore, as SCHALM (1964) has described for a number of mammals (calves, piglets, kittens, etc.), shortly after birth the fetal erythrocytes are replaced by corpuscles better suited to serve the developing young. This is corroborated also by SOVA et al. (1969), who found increased bilirubinemia in 4- to 5-day-old chickens.

Erythropenia occurring from the 17th day is particularly apparent in the chickens of the WL breed; it was also observed by RUSOV and PETROVIĆ (1968) in the New Hampshire breed on the 20th day. Examining the effect of external temperature on the total erythrocyte count MOYE et al. (1969) found that in chickens kept from the age of 7 days to 42 days at a temperature of 30 °C the erythrocyte count dropped to 2.78 million. It seems probable therefore that the reduced erythrocyte counts in the present experiment, as compared with values reported by other authors, and the erythropenia observed between days 17 and 23 are connected with the tropical climatic conditions.

The mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were rather high in one-day-old chickens of both breeds. The values of the MCV indicate a relative microcytosis in WL chickens on the 12th and to a smaller extent on the 22nd day. There was a similar small microcytosis in the CO chickens on the 5th, 12th and 26th days, but the change on the 22nd day was very marked. In 4-week-old chickens of both breeds, however, the MCV is less than the values measured after hatching. The present results coincide with those of VRBENSKÁ et al. (1967), who also demonstrated a considerable decrease in MCV on the 5th day. The values reported by RUSOV and PETROVIĆ (1968) are high in comparison with the results obtained during this study throughout the development of chickens; on the other hand, MOYE et al. (1969) claim lower values.

The mean corpuscular hemoglobin shows some fluctuating decrease from the 3rd to the 6th day, and from the latter day onwards slightly lower values than for chickens after hatching. VRBENSKÁ et al. (1967) assert the minimum MCH only in 8-day-old chickens, and RUSOV and PETROVIĆ (1968) mention much higher values.

Relative hypochromia occurred also on the 3rd day after hatching in both groups of chickens, and MCHC started to rise from the 8th day. Further falls were observed on the 23rd and 22nd day in the WL and CO chickens, respectively. VRBENSKÁ et al. (1967) found that the minimum values reached on the 8th—10th day returned to the values obtained just after hatching only on the 14th day.

The differences between breeds in these three values were not significant. No significant differences were observed in the MCV values in the single age groups, but in MCHC and MCH highly significant differences occurred between groups 1 and 2, 2 and 3, and 2 and 4; further there was a significant difference in MCHC between groups 2 and 5, and in MCH between groups 1 and 5.

The above comparisons show that the values of all the parameters of the erythrogram determined in this study, with the exception of the mean corpuscular volume, are lower than those reported by most European authors.

SUMMARY

In a study carried out in Cuba, the erythrograms of 163 White Leghorn and 160 Cornish chickens were determined between one and 28 days of age. Hemoglobin content, packed cell volume and erythrocyte count for both breeds moved within the lower range of values obtained by most workers in the European moderate zone, and were significantly lower for the WL chickens than for the CO breed. No significant differences between breeds were observed in the MCV, MCHC and MCH values.

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STUDIES ON GILL PARASITOSIS OF THE GRASSCARP (CTENOPHARYNGODON IDELLA) CAUSED BY DACTYLOGYRUS LAMELLATUS ACHMEROV, 1952

IV. HISTOPATHOLOGICAL CHANGES

By

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(Received May 10, 1971)

Investigation of microscopic lesions of fish gills is often hampered by the inadequacy of existing information about the anatomy and histology of the normal gill. In order to facilitate an understanding of the pathohistology of *Dactylogyrus lamellatus* infections in grasscarp *(Ctenopharyngodon idella)*, the first part of this paper is devoted to a description of the gill structure determined in healthy specimens of the species.

The gill anatomy of cyprinids has been studied by PLEHN (1901), SCHÄPERCLAUS (1954), BYCZKOWSKA-SMYK (1959) and HARDER (1964). Histological details have been dealt with by fewer authors (e.g. BINET and VERNE, 1931) and have usually been examined in connection with pathological changes, although KUHN and KOECKE (1956) and LANG (1968) studied the normal histology of the gill lamellae of *Carassius carassius* for diagnostic purposes. The acidophilic cell elements of the gills have been observed by LEINER (1937) and HOLLIDAY and PARRY (1962).

Microscopic gill lesions have chiefly been studied in connection with carp dactylogyrosis; WUNDER (1926, 1929). KULWIEC (1929), SPICZAKOW (1930), IVASIK (1953), SCHÄPERCLAUS (1954), BAUER (1959) and PAPERNA (1964) have dealt with the damages of *D. vastator* and BAUER and NIKOLSKAYA (1954) and PROST (1963) with those of *D. extensus*.

WILDE (1937) investigated gill lesions caused in the tench by *D. macracanthus*.

Observations on gill parasitosis of herbivorous fishes have as yet been reported only by BAUER et al. (1959).

Materials and methods

Grasscarps of various ages and body dimensions were used in the examinations. The majority were procured from pond farms, the rest originated from a laboratory stock. On extermination of the fish, the gills were divided into two parts: one half was examined in untreated state for gross and microscopic lesions, the other half was fixed immediately in 10% formalin for histological processing. The fixing solution was diluted to 4% after 4—24 hours and the fixed specimen was embedded in paraffin after 1—8 weeks. The sections were stained with haematoxylin and eosin or by Farkas—Mallory's technique. Most sections were cut longitudinally so that the branchial lamellae were sectioned parallel to their longitudinal axis (Fig. 2). Exceptionally, sections were cut in the plane of the lamellae or transversally.

Results

I. Anatomical structure of the gill lamella of grasscarp

A row of branchial lamellae is attached to either side of the gill arch. A cartilaginous ray runs along each lamella; this consists of a thick axis and two lateral plate-like processes that extend on either side toward the edge of the lamella (Fig. 1). The axis lies closer to the inner than to the outer edge of the lamella; the inner edge is understood as that near the contralateral lamella. In transversal section the inner platelike process is shorter than the outer one

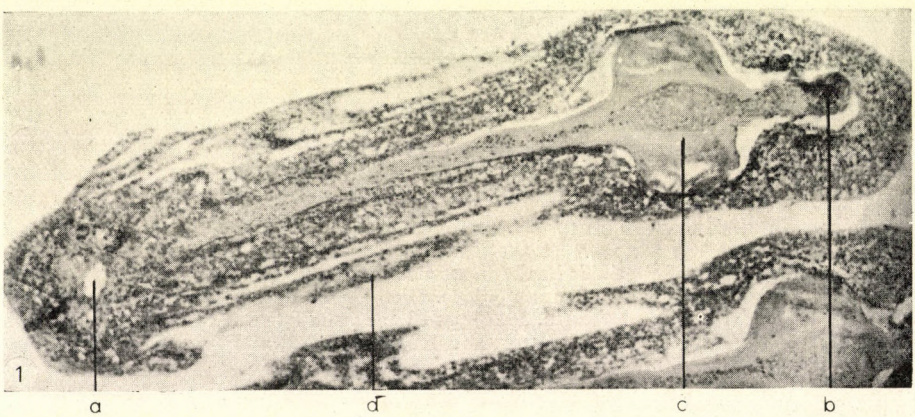

Fig. 1. Branchial lamellae of a 3-summer grasscarp in cross section: a, arteria laminae branchialis efferens; b, arteria laminae branchialis afferens; c, axis of cartilaginous ray; d, respiratory plates in cross section

and its length corresponds with the diameter of the axis, whereas the outer process is 6—7 times longer. The arteria laminae branchialis efferens passes along the edge of the outer plate, and is continuous with the arteria laminae branchialis afferens running along the edge of the inner plate.

The respiratory plates (foliae) are perpendicular to the plane of the branchial lamellae; they extend from the axis of the cartilaginous ray to the a. laminae branchialis efferens and are not more than one-third as thick as they are long. The edges of the lamellae are free of respiratory plates on both sides.

II. Normal histology of the gill lamella of grasscarp

The cell types found in the gill lamella of the grasscarp correspond, for the most part, with those observed by KUHN and KOECKE (1956) in the gills of *Carassius carassius.*

The cartilaginous supporting structure of the branchial lamellae is surrounded by connective tissue. This forms a single layer in young fishes but is multilayered and fibrous in older ones. The constituent cells and their nuclei are elongated. Fibrous connective tissue surrounds the afferent and efferent arteries and a few cells are present along the arterioles passing into the respiratory plates.

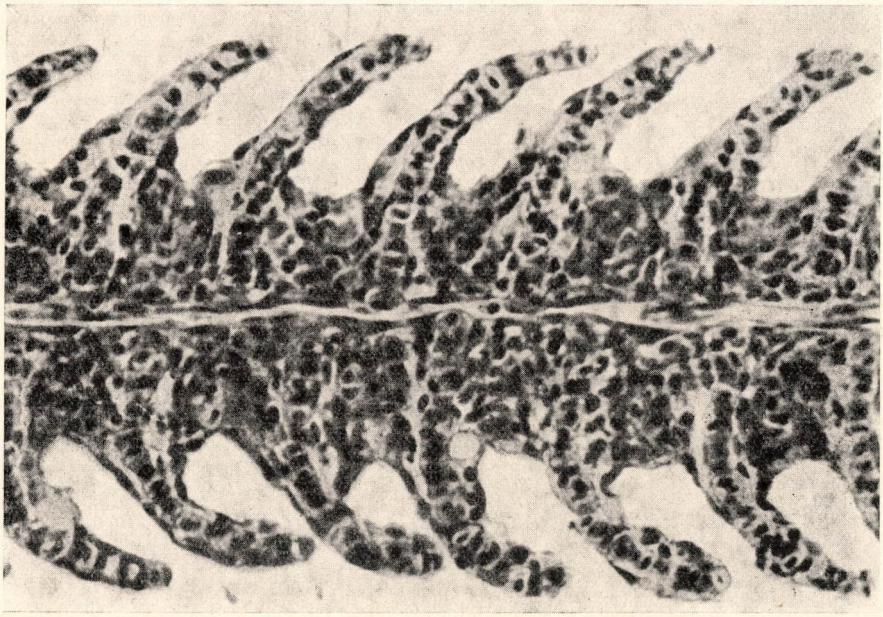

Fig. 2. Branchial lamella of a one-summer grasscarp in longitudinal section

The branchial lamellae are covered by a stratified epithelium which is continuous over the edges but interrupted where the respiratory plates arise from the lamella (Fig. 2). The epithelium is composed chiefly of cuboidal cells, which, although of common origin, represent two different morphological types (Fig. 3). One cell type forming the bulk of the branchial epithelium in adult fishes, but appearing only in the superficial epithelial layers in young ones, is characterized by large, round or oval nuclei which stain lightly and have 1—3 chromatin granules. The cytoplasm, also large and pale-staining, encloses vacuoles of various sizes and has confluent margins. The other cells form the bulk of the branchial epithelium in young fishes, but in older ones are seen only in the deep layers; they are relatively small and, like their nuclei, irregularly polygonal. The nuclear chromatin stains easily and encloses one or two nucleoli. The cytoplasm is dense, with confluent margins. Several transitory forms exist between the two cell types.

The acidophilic cells described by LEINER (1937) in *Carassius* are regularly encountered in the deep layers of the stratified epithelium. These are very large and have a disc-like or elongated nucleus at one end. The cytoplasm contains small eosinophilic granules and many small non-staining vacuoles. In some cells the granules are clumped.

Other normal components of the stratified branchial epithelium are the goblet cells, most of which are found at the surface or at the edges of the branchial lamella, although some between the respiratory plates. An additional

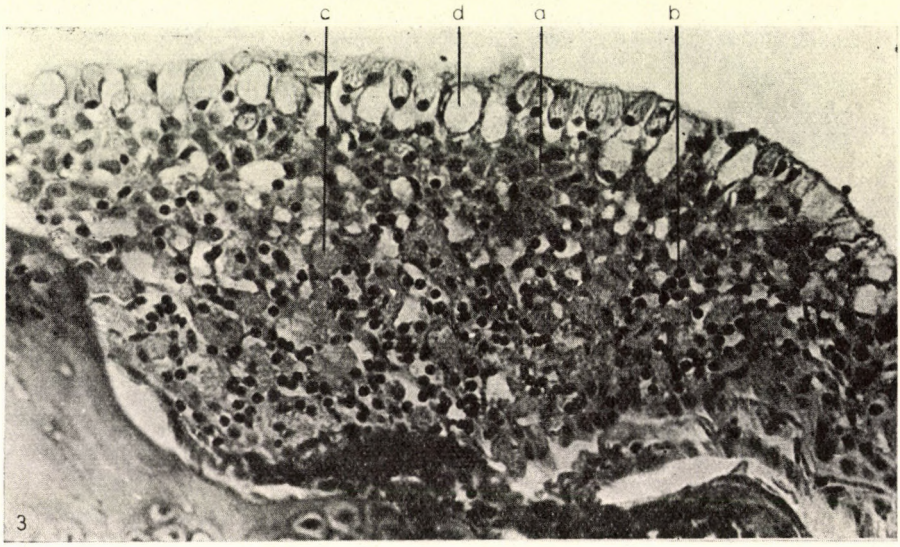

Fig. 3. Stratified gill epithelium of the grasscarp: a, type 1 cuboidal cells; b, type 2 cuboida cells; c, acidophilic cells; d, goblet cells

cell type occurring at the edges of the lamellae is oval in shape and one-third of the size of goblet cells. The nucleus of these cells lies on the epithelial side and eosinophilic stripes pass from the nucleus to the cell surface. Nothing is known about the function of these cells, but similar ones occur in the urinary tract mucosa (Figs 3, 4).

The respiratory plates attach to the lateral surfaces of the branchial lamellae. The supporting structure of the plates is composed of a meshwork of modified endothelial cells, the so-called pilaster cells. The respiratory plates are covered by respiratory epithelium. The pilaster cells, which form endothelial walls of the capillary network, are oval or round endothelial cells with flattened nuclei (Figs 5, 6).

The epithelial cells coating the respiratory plates are probably of the same origin as the cuboidal cells of the branchial epithelium, but both they and their nuclei are flatter, particularly in younger fish and they attach to the basement

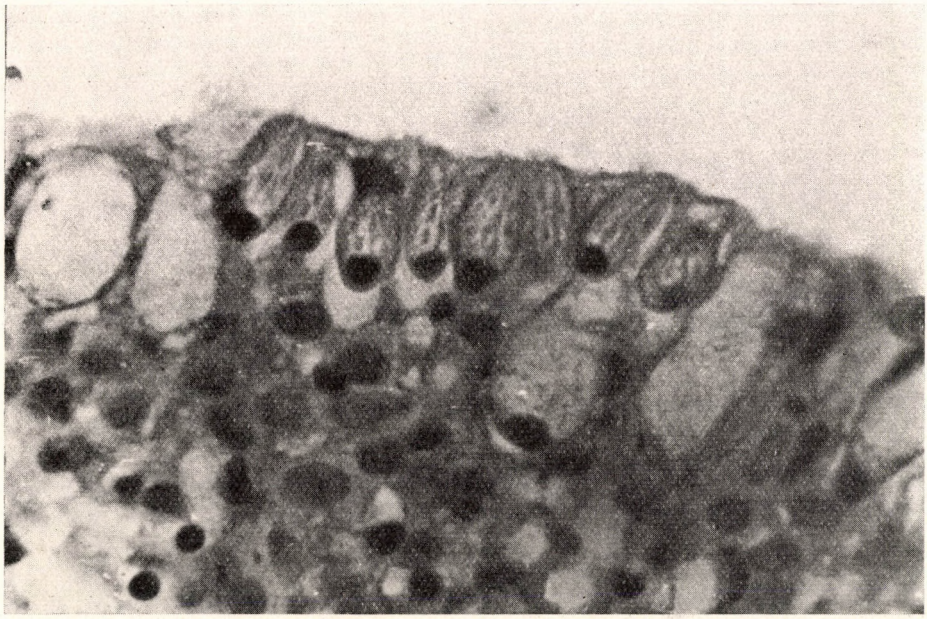

Fig. 4. Cells with eosinophilic striation and of an unknown function between goblet cells

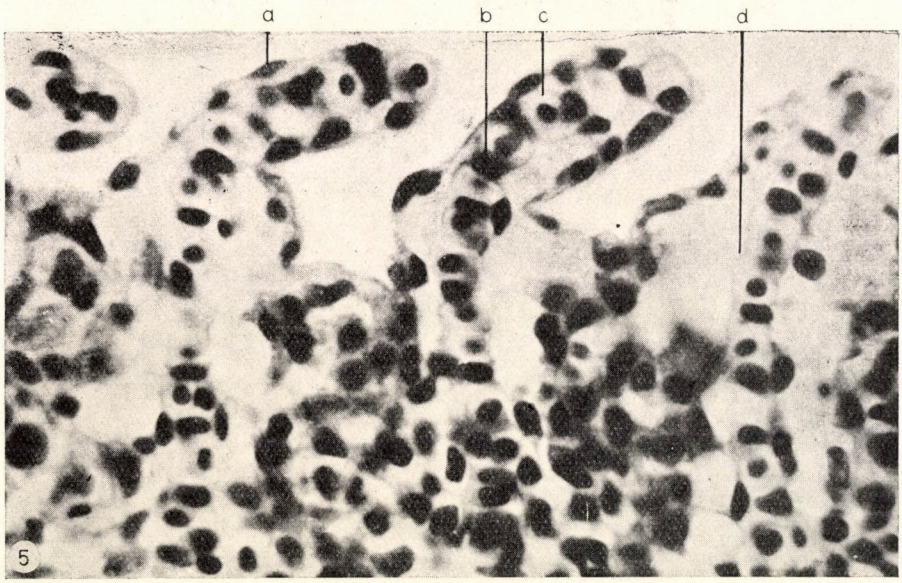

Fig. 5. Respiratory plates in transversal section: a, epithelial cells; b, endothelial cells; c, red blood cells; d, basement membrane

membrane overlying the endothelium. Occasionally there are small, round cells with large nuclei, which are probably lymphocytes.

In interpreting sections for routine examination cut parallel to the gill arch (Fig. 2) care must be taken not to confuse the picture that results from any inaccuracy in the plane of section with that presented by true "denudation" of the tips of the branchial lamellae, as in both cases the respiratory plates are absent in whole or in part.

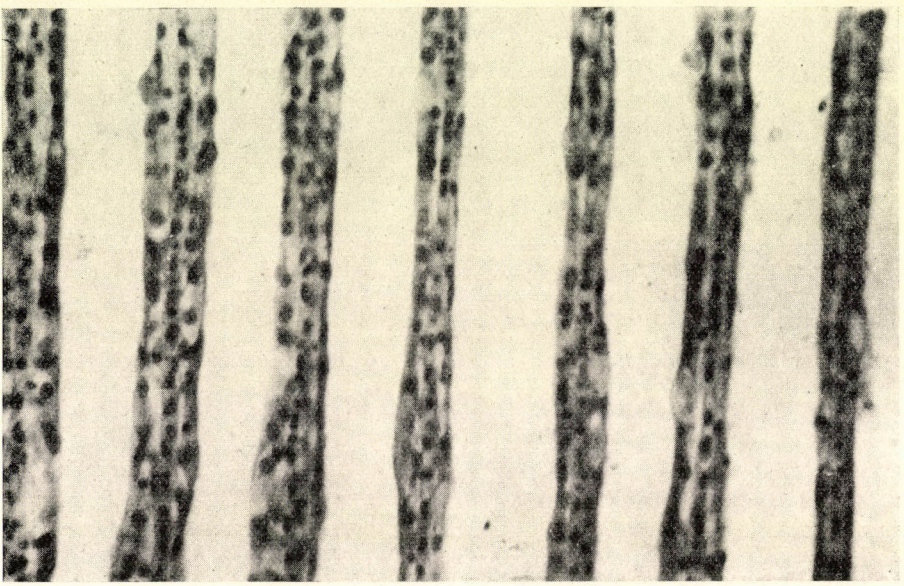

Fig. 6. Respiratory plates in longitudinal section

III. Histological examination of gills from grasscarps infected with D. lamellatus

The sections of gill specimens from grasscarps with dactylogyrosis contained many parasites and showed two kinds of changes: local lesions developing in the immediate surroundings of the parasite, and general lesions involving either the entire gill or its greater part.

Local lesions

These are relatively slight, as PAPERNA (1964) and PROST (1963) also observed in dactylogyrosis of the common carp. The parasite usually settles in the space between two respiratory plates, sinking its central hooks into the base of one plate and its marginal hooks into the surface of the other one. The tip of the central hook either penetrates the cells or pierces an artery; it may

even pass completely through the plate (Fig. 7). The marginal hooks sink into the cytoplasm of the coating epithelial cells and tear it. The central hooks cause epithelial erosions and minor haemorrhages, but no tissue proliferation.

General gill lesions

These comprise degeneration, tissue defects, haemorrhages, necrosis, atrophy and cell proliferation. The nature of the lesions depends on the duration of the disease, on the number of parasites established on the gills and on the extent of regeneration.

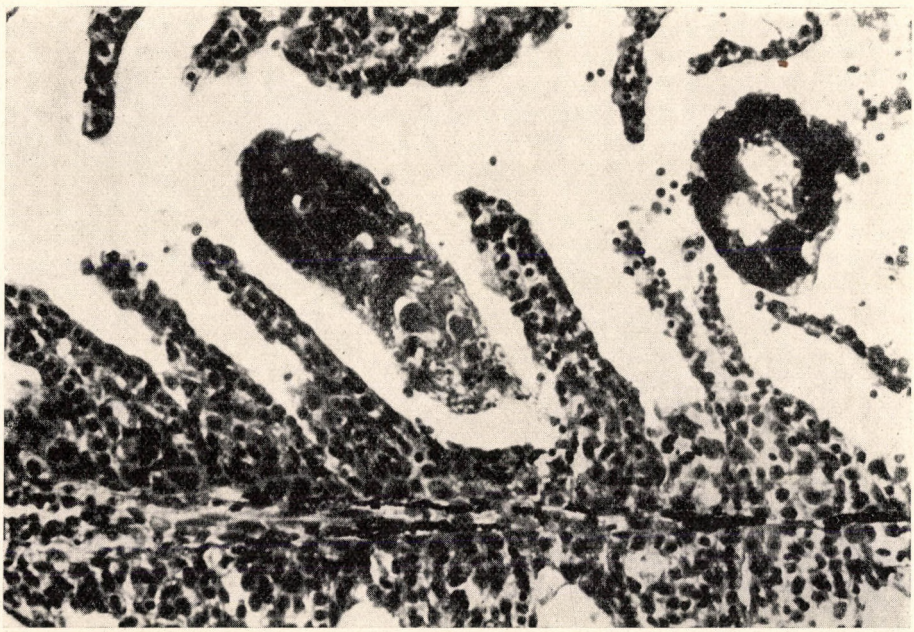

Fig. 7. D. lamellatus sinking its hooks into the base of the respiratory plate

1. An abrupt invasion of the host by many *D. lamellatus* parasites results in a rapid course of dactylogyrosis. This form of the disease ends fatally before the characteristic symptoms and lesions can develop.

Grossly, the gills are pale and anaemic and examination under the stereomicroscope reveals the presence of many parasites and loss of definition of the gill structure. Microscopically, degenerative changes appear to be predominant. The epithelial cells of the respiratory plates are damaged or may be entirely absent in places, leaving the endothelial surface exposed. The remaining epithelial cells are ruptured or degenerated (Fig. 8). The type 1 cuboidal cells of the stratified epithelium between the plates also degenerate or necrotize.

Extravasated red blood cells and eosinophilic patches of plasma are seen between the branchial lamellae and respiratory plates.

2. Typical acute dactylogyrosis is manifested 2—3 weeks after invasion of the parasites. By this time the gill lesions become grossly visible. The massively infested gills are paler than normal and may assume a mosaic-like appearance if there are haemorrhages. Copious mucus covers the lamellae. The latter appear deformed and carry thick greyish bodies and processes of various sizes which may form adhesions by fusion. Some break off, leaving visible defects.

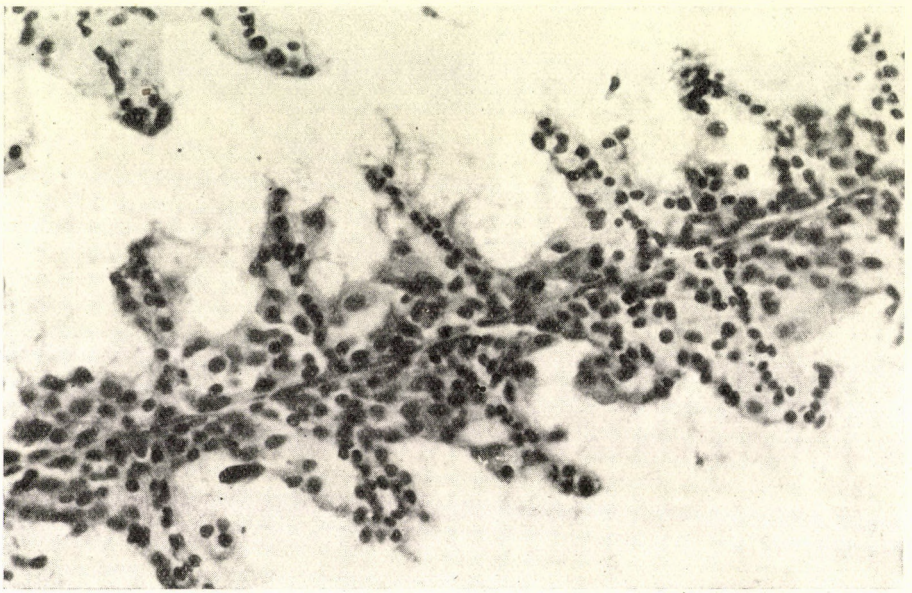

Fig. 8. Degeneration and necrosis of the respiratory plate epithelium

This form of dactylogyrosis is characterized by proliferation of the gill epithelium. The proliferation originates from the cuboidal cells, which grow larger and increase in number, causing a thickening of the lamella. The epithelial cells between the respiratory plates enlarge, bulging above the level of the plates and eventually producing adhesions between the lamellae.

The cells of the proliferative tissue have a foamy cytoplasm and a large, round, clear nucleus which encloses one or two lightly staining nucleoli. The cytoplasmic vacuoles are small and granular in the deeply seated cells, large and vesicular in cells of the upper layers. The cell margins are indistinct. Some of the superficially localized cells necrotize and undergo cytoplasmic disintegration and karyolysis (Fig. 9).

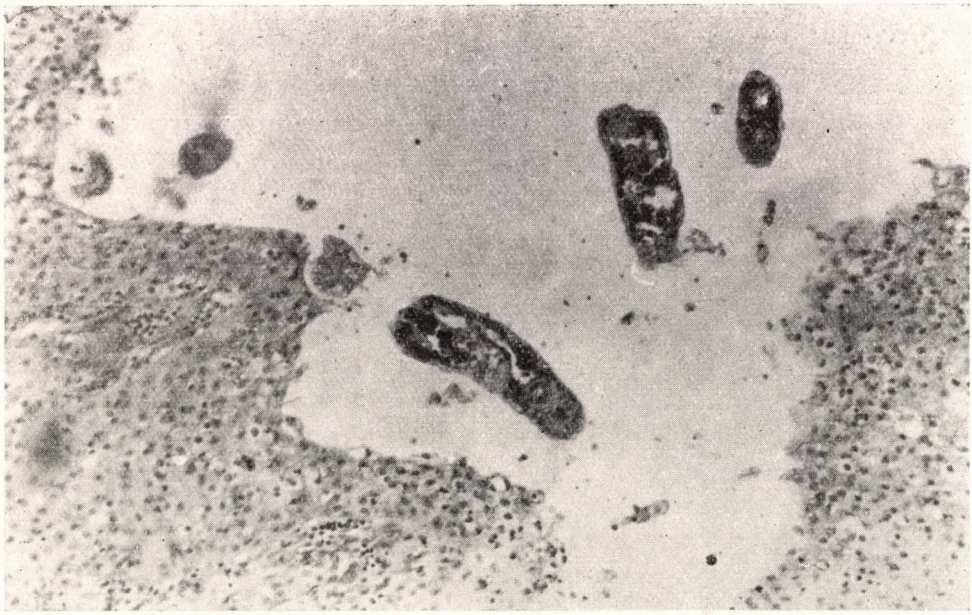

Fig. 9. Ruptured epithelial cells around the site of attachment of the parasite

Fig. 10. Adhesion of branchial lamellae and lacunar haemorrhages

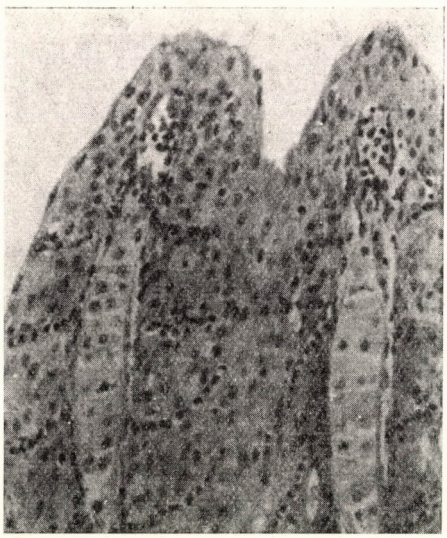

Fig. 11. Fusion of branchial lamellae as a result of cell proliferation

In moderately severe cases of dactylogyrosis, the normal structure can be recognized at the base of the lamellae, but in the central part of the swollen lamella degenerated cuboidal cells fill the spaces between the respiratory plates, pushing the plate epithelium toward, and the capillary net downward, deep into the proliferative tissue. At the tip of the lamellae, several layers of proliferative tissue overgrow the remains of the capillaries, thus giving rise to the respiratory-plate-free "lamellar processes" observed by WUNDER (1929).

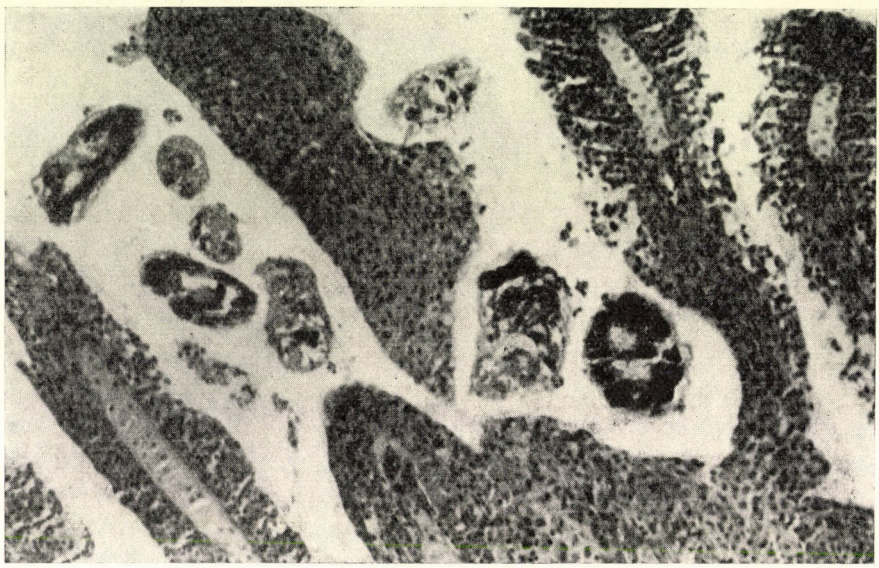

Fig. 12. Parasites established at the tips of the branchial lamellae

In severe cases, 3—5 or even more gill lamellae may coalesce with one another (Figs 10, 11), usually at the tips, but occasionally along their entire length. The original structure of the lamellae is hardly perceptible within the intricate pattern of the proliferative tissue. The capillary network, pushed into the deep epithelium by proliferation, can be recognized in places, but elsewhere it is seen only in the form of lacunas consisting of endothelial and red blood cells. The proliferative tissue occasionally contains mononuclear cells. Connective tissue cells are seen only around the vessels or along the deformed cartilaginous supporting structure of the gill lamellae.

The cells of the proliferative tissue degenerate and necrotize on severely deformed gill lamellae. In the softened, necrotic tissue the endothelial network is retained in a relatively intact state until the epithelial cells disappear. Necrosis later involves the cartilaginous parts and arteries and affected lamellae break off, leaving grossly visible gill defects.

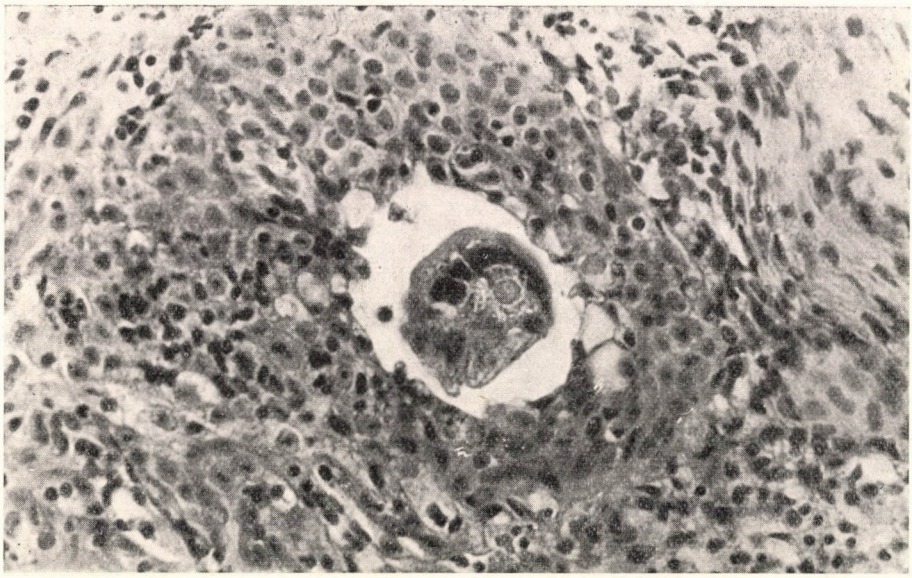

Fig. 13. Parasite encased by proliferative tissue in transversal section

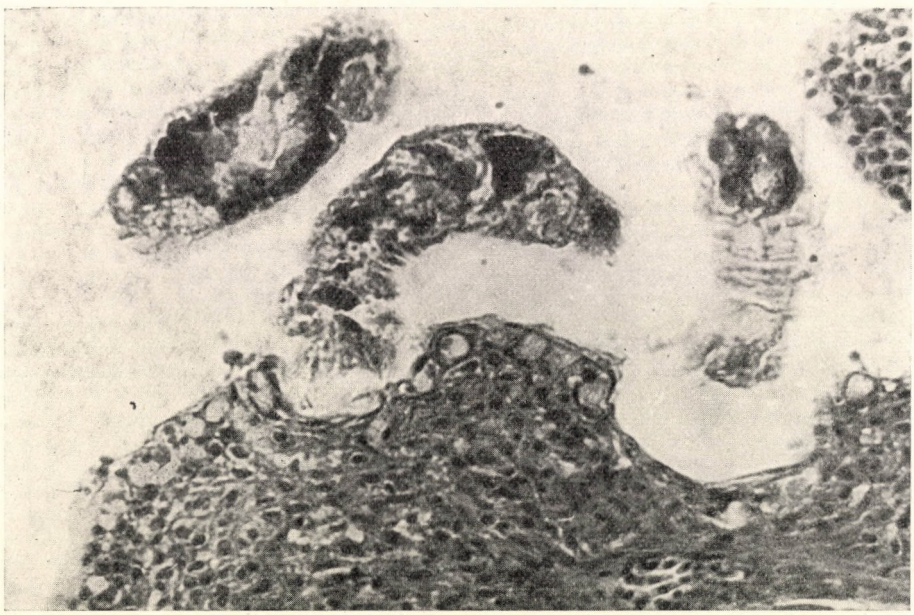

Fig. 14. Characteristic depressions at the attachment sites of the parasites

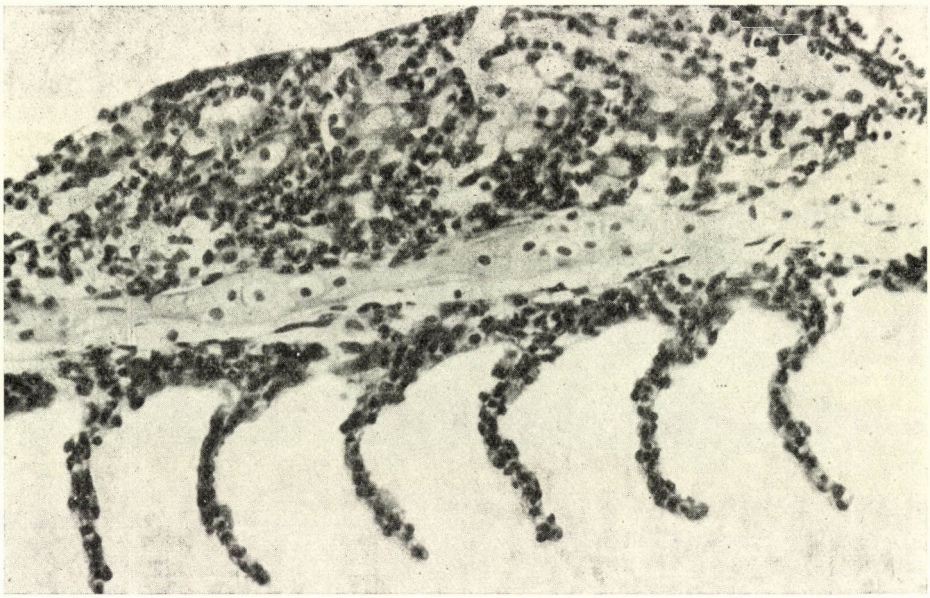

Fig. 15. Local tissue proliferation in chronic dactylogyrosis

Fig. 16. Epithelial atrophy in chronic dactylogyrosis

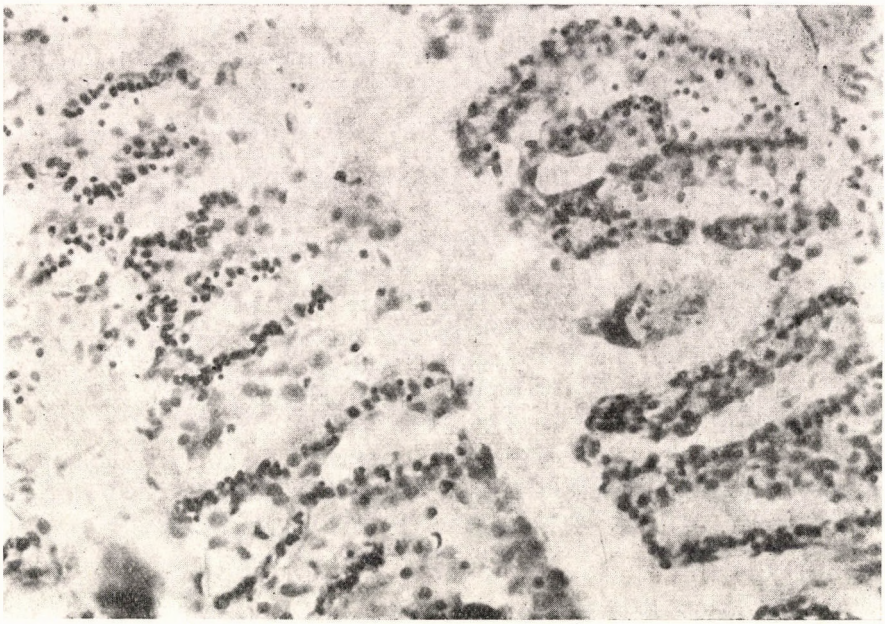

Fig. 17. Necrosis of cuboidal cells

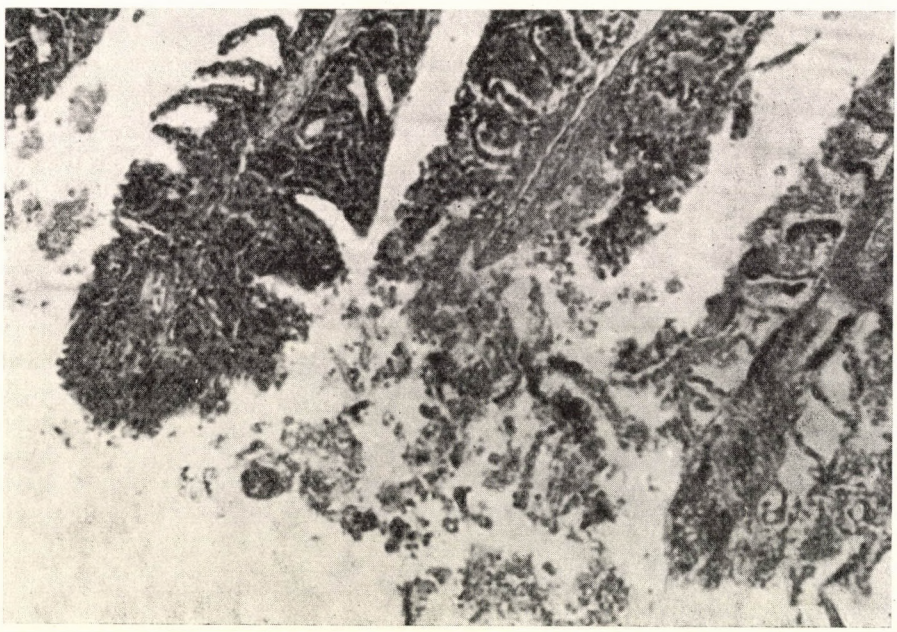

Fig. 18. Necrosis of the tips of the lamellae

The parasites expelled from their localizations by the proliferative tissue either reattach to the stratified epithelium at the tips and edges of the lamellae (Fig. 12) or are retained between the lamellae and become encased by proliferative tissue (Fig. 13). A characteristic depression arises around the haptor of parasites fixing themselves to the stratified epithelium (Fig. 14).

The microscopic picture of chronic *D. lamellatus* infection is predominated by degenerative lesions. The epithelial coat of the respiratory plates disappears, leaving them either denuded or fringed with cell fragments hanging from the basement membrane. In less affected areas, the proliferative tissue fills the spaces between the respiratory plates but without causing adhesions (Fig. 15). A more characteristic change is the reduction of epithelium between the plates; only 2—3 layers cover the underlying cartilage and connective tissue and the upper layer is damaged (Fig. 16). In certain cases the greater part of the gills becomes necrotic; necrotic lamellae held together only by the cartilaginous part of the gill arches alternate with sound ones. In such areas, cell necrosis takes place by both karyolysis and karyorrhexis (Figs 17 and 18).

Discussion

The gross and microscopic lesions caused by *D. lamellatus* resemble the damages of *D. vastator* (WUNDER, 1929; PAPERNA, 1964; USPENSKAYA, 1961), *D. macracanthus* (WILDE, 1937) and *D. extensus* (BAUER and NIKOLSKAYA, 1954; PROST, 1963). Lesions caused by *D. vastator* and *D. macracanthus* are characterized by tissue proliferation and those by *D. extensus* by superficial epithelial damage.

The type of the predominant microscopic lesion depends on the body dimensions of the host, on the number of the invading parasites and on the duration of invasion. If the fish is small, fewer parasites are required to cause a gill dysfunction than in a larger fish. If the infestation is massive, symptoms and gill damages appear earlier than after a mild invasion. While the size of the host and the number of the parasites determine the pathogenicity and the severity of the degenerative processes, the duration of invasion has a decisive influence on the nature of the microscopic lesions.

An abrupt, massive larval invasion chiefly affects the respiratory epithelium. Degeneration or desquamation of the epithelium diminishes the oxygen intake, but more damage is done by extravasation of blood plasma across the denuded endothelium as in young 2—3 cm long grasscarps this results in an osmotic dysbalance of the blood.

If larval invasion takes place over a longer period, the host organism tries to compensate the degenerative changes by regenerative phenomena. Tissue proliferation is typical of acute dactylogyrosis. Proliferation involves

the cuboidal epithelium (PAPERNA, 1964) but not connective tissue cells. The initial phase of regeneration is characterized by swelling of the cuboidal cells and their nucleus proliferation begins only later with the enlarged cells rising above the level of the respiratory plates and overgrowing them. Cells at the surface gradually degenerate and mucus accumulates in their cytoplasm.

Epithelial cell proliferation prevents denudation of the endothelium and hence extravasation of blood plasma, but it isolates the capillaries from their surroundings thereby inhibiting oxygen exchange. In mild cases of dactylogyrosis, when the unimpaired gill area is large enough to provide for sufficient oxygen uptake, cell proliferation can compensate the degenerative lesions caused by the parasite. If, however, the gill lesions are extensive, respiratory function ceases and the fish dies of asphyxia. A certain amount of mucus is present on normal gills, through the activity of the goblet cells localized on the surface of the epithelium and between respiratory plates, but copious secretion of mucus by impaired gill cells interferes with respiration. The increased secretion of mucus is not, however, related to hyperfunction or multiplication of goblet cells (PAPERNA, 1964) because most of these deteriorate along with the superficial epithelium. In all probability it originates from cuboidal cells which undergo a mucoid degeneration prior to disruption. This process takes place primarily in the superficial layers of the gill epithelium.

Interestingly, the acidophilic cells of the stratified epithelium do not notably multiply during the gill disease, although this is usual in other pathologic processes. Aggregation of the cytoplasmic granules is, however, more common than the normal finely granular appearance.

In chronic dactylogyrosis, the compensatory mechanism is limited to the substitution of the deteriorated tissues and no cell proliferation takes place. General weight loss due to parasitosis results in atrophy of the gill tissues, and degenerative phenomena are seen at the sites where the parasites attach, as well as in the superficial cell layers.

Necrosis of certain gill areas takes place both in acute and chronic dactylogyrosis. After the acute condition has subsided, the lamellar tips which have become fused by tissue proliferation necrotize and fall off. In chronic dactylogyrosis, necrosis of the lamellae very likely takes place in consequence of the impairment and obliteration of the supplying vessels. After detachment of the necrotic parts a complete restitution is possible.

SUMMARY

Gills of healthy grasscarps *(Ctenopharyngodon idella)* and specimens infected with *Dactylogyrus lamellatus* were examined. The anatomical and histological structure of normal branchial lamellae is described to aid diagnostic comparison.

Establishment of *D. lamellatus* parasites on the gill lamellae gives rise to local and general lesions. The local lesions comprise erosions, endothelial impairment and minor areas of cell degeneration around the site of attachment of the parasite. The general gill lesions include

degeneration, tissue damage, haemorrhages, necrosis, atrophy and cell proliferation. The nature of the lesions depends on the duration of the disease, the number of parasites established on the gills and the extent of regeneration.

If the infection takes a rapid course, the typical lesion is degeneration of the respiratory plate epithelium. In acute dactylogyrosis this is compensated by proliferation of the cuboidal cells of the lamellar epithelium which results in the disappearance of respiratory plates, process-like transformation and adhesion of the lamellae. If the condition is chronic, the respiratory epithelium becomes damaged, the epithelium atrophies and the lamellae necrotize but there is only a local degeneration.

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RENAL PHOSPHORUS EXCRETION OF CATTLE AND ITS RELATIONSHIP WITH CALCIUM SUPPLY

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The phosphorus supply of animals is usually checked by chemical analysis of the rations fed to them and their organs (blood, bone, hair). Recently, analysis of phosphorus excretion has been proposed as a new approach to the problem (MÁRKUS and TÖLGYESI, 1972). The connected questions of urinary phosphorus excretion and factors influencing it, however, are dealt with only incidentally by textbooks of mineral metabolism.

The phosphorus content of bovine urine has been assessed by direct or indirect measurements as 11 ppm (MANSTON, 1964; MANSTON and VAGG, 1970), 10—70 ppm (BERTZBACH et al., 1965), 1.3—1.5 ppm (BLOSSER, 1950), etc., although much higher values, such as 400 ppm (SCHREIBER, 1952; GERICKE, 1958), 440 ppm (SAUERLANDT, 1951) and 4000 ppm (BOEHNCKE and TIEWS, 1971) have also been reported.

The present studies on renal phosphorus elimination of cattle were carried out on one hand to reconcile the above contradictory observations, on the other to find an explanation for the low urinary phosphorus levels observed in infertile cows on a state farm (TÖLGYESI and ANTAL, 1967). Since low urinary phosphorus excretion in ruminants has often been attributed to chronic P-depletion (VOGEL, 1962) and excess calcium has been shown to depress both the utilization and renal elimination of phosphorus (GUEGUEN and RERAT, 1967), these interrelationships were examined to throw more light on the problem.

Materials and methods

Urine and faecal samples were collected for preliminary examinations in 1967 from a total of 14 cattle farms in areas with different soil composition. Usually spontaneously excreted urine and faeces were used, but occasional samples were taken by bladder catheter or from the rectum. Diurnal fluctuations were disregarded because they proved immaterial compared with individual variations. At each sampling the excreta of 5—8 animals per herd were analysed individually, because preliminary examinations showed that this is the minimum number of samples from which an average for the herd can be determined.

Phosphorus content was determined by the method of URBÁNYI (1931), calcium with a Zeiss flame photometer, and net base excretion (JÖRGENSEN, 1957) and urine pH were assessed by means of a Radelkis 204 pH meter.

The phosphorus balance of cattle was established by a method elaborated in this Department (TÖLGYESI, 1971).

Results

1. Daily renal phosphorus elimination of cattle

Quantitative relationships were determined according to the guideline norms widely used in this country (URBÁNYI, 1969). Five cows weighing about 500 kg and producing about 10 litre milk daily were given a daily phosphorus

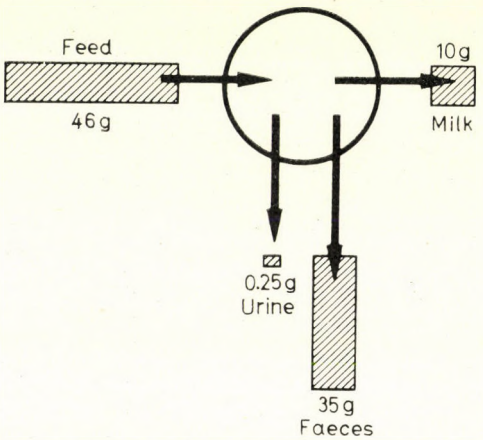

Fig. 1. Phosphorus balance of cow

supply of 46 g. The Ca/P ratio of the feed was 4. The average amounts of phosphorus excreted with faeces, milk and urine were about 35, 10 and 0.25 g, respectively, under the given conditions (Fig. 1). Of these values, only the amount eliminated with the milk was significant; the urinary level amounted only to about 0.5% of the quantity supplied, contrasted to 76% in the faeces and 22% in the milk. In this light, the urinary excretion of phosphorus appears negligible with regard to phosphorus balance.

2. Phosphorus level of bovine urine

In the autumn feeding period, the average concentration was 14 ± 15 ppm (= mg/litre), as determined by three replica measurements on 150 cows from 8 herds. The average for the winter feeding period was determined as

18±14 ppm, for urine samples of 95 cows from 12 herds. Individual variations may be higher than interherd variations and may attain one order of magnitude within one and the same herd. Thus the assessment of a herd average requires the analysis of urine samples from at least 6—8 cows. The extreme limits of herd averages were 4 and 67 ppm.

3. Relationship between urinary phosphorus level and Ca/P ratio of the faeces

Since calcium influences the transport of phosphorus in any biological system and since the Ca/P ratio can be ascertained by chemical analysis of the faeces (MÁRKUS and TÖLGYESI, 1972), the relationship between this ratio and renal phosphorus elimination was analysed by statistical methods.

a) The rank correlation coefficient ($p = 0.1$) was -0.71 for the autumn feeding period, suggesting a fall of urinary phosphorus excretion with the rise of the Ca/P ratio; the latter varied between 3.2 and 10.3 and the former between 7 and 67 ppm.

b) The Ca/P ratio determined for 12 herds in the winter feeding period was 3.2—10.3 and urinary phosphorus level simultaneously varied from 4 to 37 ppm. A significant inverse relationship between the two parameters is suggested by the rank correlation coefficient of -0.72 (at a $p = 0.01$ probability level, because of the greater number of samples analysed).

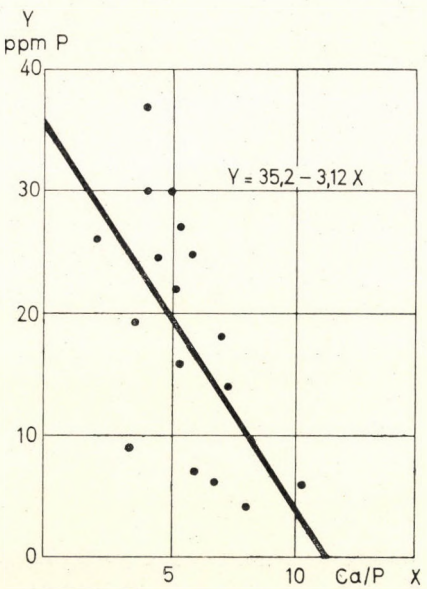

Fig. 2. Relationship between faecal Ca/P ratio and urinary P concentration

A linear regression equation was then formulated for the two variables determined in the winter period:

$$y = 35.2 - 3.12x$$

From this for a given Ca/P ratio x, the corresponding urinary phosphorus level, y, can be derived at a probability level of 0.01. The graphical representation of the equation (Fig. 2) clearly shows the fall of urinary phosphorus level with the rise of the Ca/P ratio in the faeces (and feed).

c) Rank correlation between the phosphorus contents of urine and faeces was 0.48, and so statistically not significant.

4. Relationship between urine pH and phosphorus elimination

The rank correlation coefficient obtained from a complex investigation was −0.44 (viz. statistically not significant), but there was a tendency suggesting that urinary phosphorus level is lower at alkaline pH.

5. Relationship between the excretion of base and phosphorus

The acid–base balance of the organism has several connections with phosphorus elimination. A relationship was therefore sought between net urinary acid–base excretion and urinary phosphorus level.

a) The urinary phosphorus concentration and the Jörgensen numerical values for acid–base excretion (range: +10 and +307 mEq.) are not significantly related ($r = -0.22$), but there is a slight tendency for lower phosphorus levels to be coupled to greater base excretion.

b) There is no significant relationship either between faecal Ca/P ratio and urinary base excretion ($r = 0.23$), but there is a slight tendency for base excretion to increase with the rise of the calcium level.

c) Although not apparent on the individual level, a relationship between urinary pH (range: 7.22—8.75) and base excretion could be inferred from the farm averages. The corresponding rank correlation coefficient was 0.85 ($p = 0.01$).

Discussion

It follows from the measurements that under conventional feeding conditions in Hungary, the average phosphorus level of bovine urine is about 16 ppm, which represents about 0.5% of the daily phosphorus supply. Standard

deviations between herd averages amount to 100%, while intraherd variations may attain a whole order of magnitude. Phosphorus elimination with milk and faeces is about 40 and 140 times greater, respectively, than the quantity excreted with the urine. Herd averages for the Ca/P ratio and urinary P level are inversely related: the higher the ratio, the less phosphorus is eliminated with the urine.

Other observations on metabolism of phosphorus (URBÁNYI, 1969; MANSTON and VAGG, 1970) and other elements permit the conclusion that the urinary phosphorus level varies with the efficiency of phosphorus supply. The fact that fluctuation of the urinary phosphorus level is more closely related with the change of the faecal Ca/P ratio ($r = 0.72$) than with faecal phosphorus content ($r = 0.48$) suggests that more attention than hitherto should be paid to the avoidance of calcium excess. Dietary factors, including calcium excess, affects more the urinary than the blood concentration of phosphorus. This follows from the role of the kidney in the maintenance of homeostasis. The observations described in this paper were intended only to establish the general nature of this effect, so that complementary laboratory examinations are always required to confirm whether extreme urinary phosphorus levels signify only an extreme dietary Ca/P ratio or the presence of some metabolic disorder (acidosis, alkalosis) or organic disease (nephritis, nephrosis).

SUMMARY

Cows fed 46 g phosphorus daily in a dietary Ca/P ratio of 4 excreted about 35, 10 and 0.25 g phosphorus with faeces, milk and urine, respectively. The average urinary phosphorus level was assessed as 16 ppm, with a standard deviation of $\pm 100\%$. Limit values obtained in 245 individual measurements were 4 and 67 ppm. The urinary phosphorus level is inversely related with the faecal Ca/P ratio ($r = -0.71$ or -0.72) for the winter feeding period. This relationship can be expressed with the linear regression equation $y = 35.2 - 3.12\,x$ from which urinary phosphorus level (x) can be calculated at a significance level better than $p = 0.1$ for any given faecal Ca/P ratio (y). Phosphorus contents of faeces and urine are not significantly related.

Urinary phosphorus determination can be used as a complementary assay to detect feeding errors resulting from an abnormal Ca/P ratio.

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BIOCHEMICAL CHANGES IN THE CERVICO-VAGINAL MUCUS OF INFERTILE COWS

By

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When artificial insemination is applied in a cattle herd, it is a common observation that although all cows are inseminated with good-quality semen, and the majority subsequently conceive, conception fails in a number of cases, despite the apparent normality and good health of the animal. The cows which do not conceive prove to be free from any infectious or venereal disease and return again into heat. For this reason they are usually called "repeat breeders". CASIDA (1951) in fact suggested that a repeat breeder is any cow which requires more than one service to conceive. It has been estimated that failure of fertilization, and the consequent breeding inefficiency, accounts for about 25% of infertility among cattle (TANABE and CASIDA, 1949). Subinfertility of this type is undoubtedly responsible for considerable economic losses to both farmers and breeders all over the world.

The functions of the cervico-vaginal mucus in assisting the passage of spermatozoa through the female genital tract — by providing nutrients for spermatozoal metabolism and offering optimum conditions for fertilization — can not be neglected in this respect. Numerous reports in the literature suggest that in cows which fail to conceive the cervical mucus may have undergone biochemical alterations that render it deleterious to spermatozoa and hinder their progress.

The present investigation was undertaken in an attempt to throw more light upon the biochemical properties of cervical mucus from repeat breeders and from cows which secrete an abnormal "watery" mucus, and to compare the findings with those determined for normal easy-breeding cows.

Method

Cervico-vaginal mucus samples were collected at the time of oestrus from 51 repeat breeder cows and 34 normal, easy-breeding cows on different farms near Budapest. All animals were free from infectious and venereal disease and had previously been in heat more than once at regular intervals. In addition, 52 samples of watery mucus were also examined. Most of these were obtained from repeat breeders, but were grouped separately on account of their very different physical properties (essentially the consistency and "Spinnbarkeit"). Gynaecological examination of the animals was carried out at the time the samples were collected. In the laboratory, samples were investigated with regard to:

1) physical properties, including colour, consistency, pH and "Spinnbarkeit";

2) the amount of reducing sugars (except fructose) and the fructose content, determined spectrophotometrically by the methods of EK and HULTMAN (1957) and ROE (1934), respectively;

3) the amount of chloride, determined by potentiometric titration;

4) calcium and magnesium contents, determined by complexometry, as described by BAKSAI (1970) and FLASCHKA and HOLASEK (1952), respectively;

5) the inorganic phosphate content, estimated by the method of FISKE and SUBBAROW (1925).

The results of all biochemical analyses were calculated in terms of mg/100 ml of mucus and the data evaluated statistically to determine mean values, standard deviation, variance and degree of significance (SNEDECOR and COCHRAN, 1967).

Results and discussion

The results of the biochemical assays of the different cervico-vaginal mucus samples are compared in Table 1.

The pH values of mucus from repeat breeder cows recorded in this investigation fall within the ranges reported for similar material by SMITH and ASDELL (1941), BROWN (1944) and ROARK and HERMAN (1950). Although mucus from these animals appears to be slightly more acidic than that from the normal cows (average pH 6.9 ± 0.4), the difference is not significant. Watery mucus samples, on the other hand, were significantly ($p < 0.05$) more alkaline than normal mucus. These samples did not form threads (i.e. did not exhibit the Spinnbarkeit property), and their consistency was too low to be measured by consistometer.

Table I

Biochemical composition of

Reproductive condition	No. of animals	pH	Reducing sugars	Fructose
Repeat breeder	51	6.7 ± 0.8 (6.1—7.2)	13 ± 10.1 (9—23)	8 ± 6.7 (traces —12)
Watery mucus	52	× 7.3 ± 0.5 (6.8—7.8)	× 7 ± 12.7 (traces — 15)	× 4 ± 3.0 (traces — 8)
Normal	34	6.9 ± 0.4 (6.6—7.2)	20.1 ± 25.8 (7—32)	7.1 ± 11.6 (2—25)

× Significantly different from values of normal oestrous mucus.

The alkalinity of watery-type cervical mucus, which was also reported by CSEH and GÁSPÁR (1963), seems to exert an injurious effect on spermatozoa. The pH of freshly ejaculated semen is usually on the acidic side of neutrality, varying from 6.5 to 6.9, with a mean 6.75 (ANDERSON, 1942). PATTABIRAMAN et al. (1967) found that spermatozoal motility is greatly affected by the pH of the cervical mucus: as the mucus becomes more alkaline the percentage of viable spermatozoa decreases. Spermatozoal progress through the female genital tract is, moreover, greatly affected by the lack of Spinnbarkeit of watery mucus. In explaining comparable observations on human cervical mucus, ODEBLAD (1959) assumed that during mid-cycle the mucus is assembled in a micellar arrangement in such a way that sperm migration is allowed in the spaces between the micelles. In the absence of Spinnbarkeit, therefore, spermatozoal movement will clearly be retarded. CSEH and GÁSPÁR (1963) and PANIGRAHI (1964) found that cows secreting mucus of high Spinnbarkeit usually conceived and were only exceptionally returned for a second insemination, while most repeat breeders had mucus of a very low Spinnbarkeit. The same findings were reported for human beings by COHEN et al. (1952) and MARCUS and MARCUS (1963). According to both groups of workers, the longevity of spermatozoa within the cervix is proportional to the degree of Spinnbarkeit, and insemination is most successful where the mucus shows the greatest ability to form threads.

The amount of the total reducing sugars (expressed as glucose) in cervical mucus from the repeat breeder animals is little different from the normal value, while in the watery secretions it is less than half this value. The amount of fructose in the mucus from the repeat breeders is likewise nearly the same as in the normal animals, but only traces were found in most samples of watery

different types of oestrous mucus

Total sugars	Chlorides (as NaCl)	Calcium	Magnesium	Phosphorus (inorganic)
		mg/100 ml		
21 ±17.6 (11—35)	759.9± 24.6 (855.5— 919.4)	× 19.9± 7.9 (10.8—37.5)	× 4.5±4.9 (2.5—6.7)	0.64±0.11 (traces — 0.96)
× 11 ±25.7 (traces — 23)	× 996.5± 28.5 (842.6—1425.6)	12.5± 8.4 (5.9—18.4)	× 9.9± 4.6 (4.7—11.3)	0.25±0.20 (traces — 0.4)
27.2±38.0 (9—57)	869.2± 105 (730.6—1220.0)	10.1± 5.5 (2.0—19.7)	2.7± 2.4 (0.4—10.3)	< 0.50

± Standard deviation.

mucus. The importance of the sugar content of the cervical mucus in contributing to the survival and nutrition of spermatozoa during their passage along the female genital tract has been discussed elsewhere (EL-NAGGAR and BAKSAI, 1971). GASSNER et al. (1952) showed that adding fructose to fructose-poor ejaculates may prolonge the longevity of semen. MACLEOD (1941, 1947) established that spermatozoa require certain carbohydrates — including glucose, mannose, maltose, fructose and glycogen — to provide energy for motility. In the absence of these sugars sperm motility decreases but can be restored by addition of these sugars to the solutions.

As found in a previous investigation (EL-NAGGAR et al., 1971), the chloride content of the cervical mucus from repeat breeder animals lies within the limits for the normal cows at oestrous time, while the values determined for samples of watery mucus are significantly ($p < 0.05$) higher than those of normal oestrous mucus. Other investigations on cattle (GUPTA et al., 1962, VESER, 1969) have produced similar results. For the human cervical mucus also, HERZBERG et al. (1964) found that there is a distinct difference in the sodim chloride content of the dried material between normal and pathological cases. It is suggested that the high chlorid content of watery mucus may account for the decreased consistency and the absence of Spinnbarkeit and it undoubtedly has a direct effect on the viability of the spermatozoa in the female genital tract.

Greater than normal concentrations of calcium, magnesium and phosphate ions were present both in the cervical secretions of the repeat breeder cows and especially in the watery mucus samples, in which the high concentration of magnesium ions is conspicuous. Increased amounts of inorganic ions compared to the physiological norms therefore accompany a decrease in the amount of monosaccharides.

It should be noted that there are increases in both acidic (phosphate and chloride) and alkaline (calcium and magnesium) ions in watery mucus, but these do not take place to the same extent: while the amount of the acidic substances increases by 14%, we found that alkaline substances increase by 75%. This presumably explains the alkalinity of such mucus compared to the slightly acidic physiologically normal mucus.

In summarizing, we may say that the biological balance of organic and inorganic substances is disturbed in both groups of pathological mucus, consequently physical and chemical properties are changed. The cyclic variation in the secretion of minerals and organic materials under hormonal control will be discussed in detail in another paper.

SUMMARY

The pH, total reducing sugars, fructose, chloride, calcium, magnesium and inorganic phosphate contents of 51 cervico-vaginal mucus samples obtained from repeat breeder cows and 52 samples from cows that gave a watery type of mucus at oestrus were compared with

values determined for 34 samples of normal oestral mucus. Watery mucus was found to have an alkaline pH 7.3, significantly low amounts of reducing sugars, fructose and inorganic phosphate, but a high level of chloride and magnesium, compared with normal oestral mucus. There was little difference in pH, reducing sugars, fructose, chloride and inorganic phosphate contents between the secretions of repeat breeders and normal cows, but highly significant differences in calcium and magnesium contents.

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РЕЗЮМЕ

ОНТОГЕНЕТИЧЕСКИЕ РАЗЛИЧИЯ В ЭРИТРОГРАММЕ У ЦЫПЛЯТ БЕЛЫЙ ЛЕГХОРН И КОРНИШ В ТРОПИЧЕСКИХ УСЛОВИЯХ

ЕВА ДОБШИНСКА

В кубинских условиях изучалась эритрограмма у 163 цыплят белый легхорн и 160 цыплят корниш с 1-дневного по 28-дневный возраст. Содержание гемоглобина, packed cell volume и количество эритроцитов у обоих пород колебалось в нижних пределах показателей, обнаруженных специалистами в умеренной европейской климатической зоне; зато эти показатели у цыплят белый легхорн были сигнификантно низшими, чем у цыплят корниш. Не обнаружено сигнификантных отличий у двух групп в показателях общего корпускулярного объема, общей концентрации корпускулярного гемоглобина и общего корпускулярного гемоглобина.

ИССЛЕДОВАНИЯ ПО ЖАБЕРНОМУ ГЕЛЬМИНТОЗУ У АМУРА (CTENOPHARYNGODON IDELLA), ВЫЗВАННОМУ DACTYLOGYRUS LAMELLATUS ACHMEROW, 1952

IV. Патогистологические исследования

К. МОЛНАР

Изучалась гистология жабр здоровых и инвазированных **Dactylogyrus lamellatus** экземпляров белого амура (**Ctenopharyngodon idella**). Автор детально описывает анатомию и гистологию жаберной пластинки здорового белого амура, ознакомляет с разными типами эпителиальных клеток, образующими жаберный эпителий.

При помощи гистологических методов на жаберной пластинке инвазированных **D. lamellatus** белых амуров можно различить местные и общие изменения.

Местные изменения образуются вокруг фиксации гельминтов и характеризуются десквамацией, повреждением эндотелиальных клеток и локальным клеточным перерождением.

Общие тканевые повреждения слагаются из регрессивных и прогрессивных явлений и носят разный характер, в зависимости от продолжительности и интенсивности инвазии, дальше, от степени регенерации.

Для быстрого течения инвазии характерно перерождение эпителия пластинок второго порядка. При типичном остром дактилогирозе регенерация компенсируется разрастанием кубовидных клеток многослойного эпителия, ведет к исчезновению пластинок второго порядка и жаберная пластинка превращается в единый отросток, или сращается с соседними пластинками. Для хронического дактилогироза характерно повреждение респираторного эпителия, атрофия многослойного эпителия, некроз пластинок первого и второго порядков. Регенерация наблюдается только локально.

ВЫДЕЛЕНИЕ ФОСФОРА МОЧЕЙ И ЕГО ВЗАИМОСВЯЗЬ СО СНАБЖЕНИЕМ ОРГАНИЗМА КАЛЬЦИЕМ У КРУПНОГО РОГАТОГО СКОТА

ДЬ. ТЁЛДЕШИ

При снабжении коров ежедневно 46 г-ами фосфора, соблюдая соотношение Ca: P=1:4, животные испражнениями, молоком и мочей выделяют 35, 10 и 0,25 г фосфора соответственно (сн. 1). Средняя концентрация фосфора в моче равняется 16 мг%-ам, при стандартной девиации ±100%. У 245 животных крайние показатели равнялись 4 и 67 мг%-ам. В соотношении Ca/P испражнений и содержании фосфора в моче имеется отрицательная корреляция (r = —0,71 и ⁻0,72 соответственно). В сезоне зимнего стойлового кормления эту взаимосвязь выражает линейное регрессивное уравнение y = 35,2—3,12 (сн. 2) при сигнификанции лучше p = 0,1. Между содержанием фосфора в испражнениях и моче нет сигнификантной взаимосвязи (r = 0,48).

Из показателя содержания фосфора в моче можно судить о перебоях в кормлении, обусловленных неблагоприятным соотношением Ca/P.

ИЗУЧЕНИЕ ЦЕРВИКО-ВАГИНАЛЬНОЙ СЛИЗИ ХОЛОСТЫХ КОРОВ

М. А. ЭЛ-НАГГАР и ЕВА БАКШАИ-ХОРВАТ

Собрано 103 образца цервико-вагинальной слизи во время эструса с одной стороны от неоплодотворенных коров, с другой от животных, которые выделяли жидкую слизь. Определяли pH, содержание всех редуцирующих углеводов, фруктозы, хлоридов, кальция, магния и неорганических фосфатов. По сравнению с нормальной слизью при охоте жидкая слизь была более щелочной (pH 7,3), содержание в ней редуцирующих углеводов, фруктозы и фосфатов было сигнификантно меньше, зато она содержала больше хлоридов и магния. Не было заметной разницы в pH, содержании редуцирующих углеводов, фруктозы, хлоридов и фосфатов слизи неоплодотворяющихся (повторно приходящих в охоту) и оплодотворяющихся коров, но имелась заметная разница между ними в содержании кальция и магния.

ОБМЕН КАЛЬЦИЯ И ФОСФОРА ПРИ АТРОФИРУЮЩЕМ РИНИТЕ

I. Изучение роли кальция в возникновении атрофирующего ринита свиней

З. ХОРВАТ и Л. ПАПП

Авторами изучено, можно ли вызвать атрофирующий ринит, если поросята от отбивки будут получать в течение нескольких месяцев корм бедный кальцием. В первой стадии эксперимента изучались поросята от свиноматок, благополучных как клинически, так и патологоанатомически по атрофирующему риниту. Эти свиноматки представляли так называемую резистентную к атрофирующему риниту линию. Среди 29 поросят группа I (16 поросят) получала от отбивки в течение 4 и 5 месяцев с кормом 0,35% металлического кальция и 1,03% элементарного фосфора, группа II — (8 поросят) — 0,22% кальция и 1,16% фосфора, группа III (контрольная, 5 поросят) — 0,96% кальция и 0,98% фосфора. За это время все животные подвергались каждый 10-й день тщательному клиническому осмотру, раз в месяц определяли их вес, щелочную фосфатазу сыворотки, содержание Ca и неорганического P в сыворотке. К концу 4-го месяца половину поросят из каждой группы убили. Определено содержание Ca и P бедренной кости каждого поросенка и проверена носовая полость. На выжившей половине животных наблюдения продолжались еще месяц.

У животных групп I и II наблюдались перебои в движении и патологические изменения, напоминающие рахит; контрольные животные остались бессимптомными. Признаки атрофирующего ринита не наблюдались ни у одного животного. Существенная разница в содержании Ca кормов не сказывалась на содержании Ca сыворотки. Бедренные кости животных групп I и II накопили меньше Ca, чем эти кости контрольных животных. В показателях щелочной фосфатазы сыворотки и привесах существенной разницы не зарегистрировано у животных отдельных групп.

Во второй стадии эксперимента 5 поросятам от больных атрофирующим ринитом свиноматок (группа IV) в течение 5 и 6 месяцев скармливали рацион группы I. Каждый 10-й день все животные подвергались клиническому осмотру и к концу эксперимента проверяли их носовую полость на наличие изменений. Клинических признаков атрофирующего ринита у этой группы тоже не обнаружено. Зато имелись рахитические перебои в движении и изменения костей, подобные таковым у группы I. Только у двух подсвинков этой группы имелись патологоанатомические изменения, вызывающие подозрение на атрофирующий ринит.

На основании своих данных авторы приходят к заключению, согласно которому в этиологии атрофирующего ринита дефицитное снабжение Ca само по себе роли не играет.

ОБМЕН КАЛЬЦИЯ И ФОСФОРА ПРИ АТРОФИРУЮЩЕМ РИНИТЕ СВИНЕЙ

II. Содержание кальция и фосфора костей здоровых и болеющих атрофирующим ринитом свиней

З. ХОРВАТ, Л. ПАПП и Б. ЕЛИАШ

Изучалось содержание кальция и фосфора в позвонках здоровых и больных атрофирующим ринитом свиней разного возраста. В отдельных случаях определено содержание этих элементов и в лобной кости.

В первой группе в 2-х-, 4-хмесячном возрасте и при убое выоперированы для изучения 6-й, 5-й и 4-й хвостовые позвонки соответственно здоровых дочерних потомков 4-х чистопородных корнвальских свиноматок, больных атрофирующим ринитом и 4-х помесных — F_1 (♀ корнвал х ♂ крупная английская мясная) тоже больных свиноматок.

Во второй группе в 7-месячном возрасте при живом весе 103—104 кг анализу подвержены 6-й хвостовой позвонок 15 помесных, здоровых и 15 больных атрофирующим ринитом свиней, дальше, лобные кости 10 здоровых и 9 больных животных.

В третью группу принадлежало потомство — 10 животных со средним живым весом 105 кг — двух свиноматок, матери и собратья которых были всегда благополучными по данной болезни и сами по настоящее время рождали только здоровых поросят. Этих животных авторы считают клинически и «генетически» благополучными по атрофирующему риниту. С данными этих животных сравнены данные изучения 6-го хвостового позвонка потомства трех свиноматок — всего 24 животных со средним живым весом по 105 кг — родители и собратья которых были неблагополучными по атрофирующему риниту и сами рождали больное потомство. Этих животных авторы считали по данной болезни клинически больными и «генетически нагруженными».

На основании анализа костей авторы приходят к заключению, согласно которому больное скороспелое помесное потомство F_1 с прогредирующим возрастом (с 4-хмесячного возраста) накопляет в хвостовых позвонках меньше Ca, чем здоровые свиньи при одинаковых условиях кормления и содержания. Это явление с успехом можно употребить в диагносцировании болезни. Хвостовые позвонки больных животных, независимо от породы, «набухши», пористой структуры.

ИЗУЧЕНИЕ ПЕЧЕНИ СИРИЙСКОГО ХОМЯКА ОБЫЧНЫМ И ЭЛЕКТРОННЫМ МИКРОСКОПАМИ ПРИ ЗАРАЖЕНИИ ВИРУСОМ ЛОШАДИНОГО РИНОПНЕВМОНИТА

П. КАПП

При заражении сирийских хомяков уличным штаммом вируса лошадиного риноппевмонита в возрасте 2—3 дней наиболее характерные изменения наблюдались в печени 48—120 часов после инфекции. Во всех случаях регистрировались изменения единичных паренхиматозных клеток и диффузные таковые. Изменения единичных печеночных клеток представлялись в перерождении клеток, сопровождающемся образованием внутриядерных включений, ацидофильным некрозом и возникновением так называемых светлых клеток. В переродившихся клетках имеется увеличение эндоплазматического ретикула и формирование его в виде пальцевого отпечатка. Наиболее энергический синтез вируса

наблюдается во время формирования внутриядерного включения. Появление вирусных частиц в цитоплазме сопровождается тяжелым поражением эндоплазматического ретикула и митохондриев, образованием цитоплазматических кист, миелино-подобным перерождением и лизисом цитоплазматической оболочки вдоль синусоидной стороны печеночных клеток. Часто наблюдался ацидофильный некроз единичных клеток с последующим появлением эозинофильных телец в синусоидах, что автор считает прямым действием вируса. Вирусные частицы не обнаруживались в светлых клетках. Диффузный некроз паренхиматозных клеток, распространяющийся на часть одной или нескольких долек, был обусловлен васкулярными перебоями. Независимо от поражений паренхиматозных клеток наблюдались и воспалительные процессы в синусоидах и щелях Диссе, междольковые же прослойки были инфильтрированы лимфоцитами и гистиоцитами. Общая картина острого или полуострого гепатита, вызванного вирусом ринопневмонита, морфологически напоминала изменение печени при вирусных гепатитах человека и других животных.

ЭКСПЕРИМЕНТАЛЬНОЕ ИЗУЧЕНИЕ ВИСЦЕРАЛЬНОЙ ЛИЧИНКИ TOXOCARA CANIS В ЛАБОРАТОРНЫХ ЖИВОТНЫХ

И. МОССАЛАМ, И. А. АТАЛЛА и З. ХОСНИ

Мышей, крыс, морских свинок и цыплят экспериментально инвазировали личинками T. canis и через 10, 20, 30 и 40 дней изучались вызванные ими патогистологические изменения в печени, легких, почках и головном мозгу. Личинки при наличии клеточной реакции и без нее обнаруживались во всех перечисленных органах упомянутых животных. Клеточная реакция в печени крыс характеризовалась накоплением фибробластов и эозинофильных клеток, в легких же крыс и морских свинок она представляла собой гранулемы из фибробластов и эозинофильных клеток. Очаговое накопление лимфоцитов было характерным изменением в печени и легких цыплят. В мозгу личинки обычно не вызывают выраженной реакции, но тут-там имеются территории с сателлитозом, нейронофагией и пролиферацией глии с очагами кроветеков.

ПОЛИМОРФИЗМ β-ЛАКТОГЛОБУЛИНА В МОЛОКЕ КОРОВ ПЕСТРОГО ВЕНГЕРСКОГО СКОТА

П. ХОРВАТ

Изучались полиморфизм β-лактоглобулина, распределение фенотипов и их геновая фреквенция. Данные наблюдений сравниваются с таковыми литературы других пород.

Описаны четыре фенотипа β-лактоглобулина: -AA, -BB, -AB и BC. β-лактоглобулин -AB абнаруживается гораздо чаще, чем остальные три. Реже всех встречается β-лактоглобулин-AA. Среди 597 изученных проб молока фенотип BC обнаружен только в одной. Автор констатирует, что вариант C β-лактоглобулина в пестром венгерском скоту имеется, но количество изученных проб молока было недостаточным для выявления отдельных комбинаций этого варианта.

ДАННЫЕ К КИНЕТИКЕ СПОРОЗОИТОВ EIMAERIA STIEDAI (PROTOZOA, SPOROZOA)

У. ДЮРР, Б. МИЛТАЛЕР и Г. Г. ХОЙНЕРТ

Проведено кинематографическое изучение спорозоитов E. stiedai, освобожденных из цист in vitro. Зарегистрированы разные виды движения спорозоитов, как то, поступательное движение вперед и назад, вращательное движение около продольной оси, в комбинации с поступательным движением и без него, изгибание, ощупывание, вращение вокруг одного пункта. Максимальная скорость поступательного движения равнялась 86 μ/сек. При непосредственном наблюдении беспрерывным кажущееся поступательное движение состояло из перерывов, которые можно выявить только путем фильмоскопии. Не обнаружено типичной последовательности в чередовании отдельных типов движения.

К ЦИКЛУ РАЗВИТИЯ EIMERIA STIEDAI

У. ДЮРР

В своем кратком сообщении автор извещает о нахождении спорозоитов в брыжеечных лимфоузлах и костном мозгу бедренной и большой берцовой костей. В лимфоузлах спорозоиты обнаруживались либо свободными клетками, либо включенными в макрофагах; в костном мозгу они были все включенными в макрофагах. Дискутируется путь миграции спорозоитов в печень. На основании предварительных данных можно судить, что спорозоиты попадают в печень не прямо через воротную вену, а сначала попадают в лимфатические сосуды (брыжеечные лимфоузлы) и оттуда потом разносятся кровеносной системой по всему организму. Какова роль в этом макрофагов и механизм поселения мерозоитов в желчных ходах, пока не известны.

ЭКСПЕРИМЕНТАЛЬНОЕ СКЛЕИВАНИЕ ЖЕЛЧНОГО ПРОВОДА

К. ШОМОДЬВАРИ, Ф. СЕНТГАЛИ и Я. КОЛБ

На собаках проводили склеивание желчного хода (d. choledochus) после его поперечного разреза. На основании своих наблюдений авторы приходят к заключению, согласно которому тканевая наклейка при операциях на желчном ходе может во многих случаях заменить или дополнить традиционный шов.

Надежность и тканевая совместимость наклейки доказаны экспериментально на гистологических срезах.

ЭКСПЕРИМЕНТЫ ПО ОБЕЗВРЕЖИВАНИЮ ООЦИСТ КОКЦИДИЙ

А. О. АЙЕНИ, Е. ДИНГЛЕЛДАЙН и У. ДЮРР

На основании собственных экспериментов авторами дискутируются практические и стандартные методы изучения дезинфицирующей эффективности разных препаратов против ооцист кокцидий. Применялись два способа в испробовании препаратов на неспорулированных ооцистах Eimeria stiedai:

I. так наз. способ взвеси, модифицированный по Булден и Ерп (1969),

II. способ фиксации ооцист при использовании
   а) бумажных лент,
   б) керамических плит, применяемых для мощения полов скотных дворов,
   в) способ предметных стекол.

Кроме ряда коммерческих дезинфицирующих средств изучены гидроокись аммония и формалин.

Результаты экспериментов показывают, что один способ взвеси недостаточен для изучения эффекта дезосредств на экзогенные стадии паразитов. Изучение необходимо дополнить другими, более практическими исследованиями, напр. способом фиксации ооцист согласно рекомендации продуцента по практическому использованию дезинфицирующего средства. Есть необходимость в дальнейшей разработке стандартных методов испробования дезосредств, учитывая и физические особенности препаратов

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STUDIES OF Ca AND P METABOLISM IN ATROPHIC RHINITIS OF SWINE

I. STUDIES ON THE PREDISPOSING ROLE OF Ca DEPLETION

By

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Atrophic rhinitis of swine (further on referred to as a.r.) has been known for about one and a half centuries (FRANQUE, 1830, cit. HÁMORI and STIPKOVITS, 1968), but it did not cause severe veterinary medical problems until the recent two decades. It has since spread rapidly among highly productive, fast growing swine breeds all over the world until it now represents one of the main sources of economic loss for the pig breeders. The poor feed utilization and retarded growth of diseased animals, the frequent association of enzootic pneumonia with a.r., mortalities and emergency slaughters, costs of therapy and control, and low reproduction rates now threaten the profitability of intensive swine farming.

The symptoms of a.r. are well known, but opinions are still divergent about its aetiology. There are still experts who attribute the causation to some infectious agent (virus, bacterium, protozoon) (DUME, 1961; HARTWIGH, 1965; KEMÉNY, LITTLEDIKE and CSEVILLE, 1970; SWITZER and L'ECUYER, 1960; VUJOSEVIĆ, DURICHOVIĆ and PETROVIĆ, 1966), while others implicate genetic factors (KOCH, JÖCHLE and KOLB, 1958), poor hygienic conditions (BUSHMAKOV,1966; JANOWSKI, RULSKA, ZULINSKI and SULC, 1966), vitamin depletion or feeding errors (BENDIXEN, 1957; BROWN, KROOK and POND, 1966). Nowadays, however, a.r. is increasingly considered to be a polyaetiologic disease (GWATKIN, 1959).

BROWN, KROOK and POND (1966) have advanced the surprising hypothesis that disturbance of calcium and phosphorus transport due to low calcium supply is the sole causative factor of a.r. They reported that dietary levels of 1.2% Ca and 1.0% P can prevent the establishment of the generalized fibrous ostitis which forms the morphological basis of a.r. (BROWN, KROOK and POUND, 1966) although they recognized that the control of secondary damages is important.

Import of highly-productive swine breeds from Denmark, Sweden and elsewhere has resulted in an endemic occurrence of a.r. in Hungary which has already caused important economic losses (HÁMORI, 1968; ÁLDÁSY and VÁNYI, 1962; HÁMORI and STIPKOVITS, 1968).

This paper is a report of experiments carried out with the aim of obtaining more information on the suggested aetiological role of low dietary calcium and phosphorus levels.

Experimental

In the first series of experiments, piglets from sows either free of, or genetically resistant to, a.r. were fed a low-calcium diet with a relatively high phosphorus content, while in the second series, piglets from sows affected with

a.r. were given a low-calcium diet. Appropriate control groups were set up with each series.

Materials and methods

Litters from three sows were used in the first series. Two of the sows were Hungarian Whites of unknown genetic origin from a herd free of a.r. and had been mated with a Hungarian White boar, also originating from a "clean" herd. They were clinically healthy, as was confirmed by post mortem examination at the close of the experiment. The third sow, descendant of an English Large White boar and a Hungarian White sow, originated from an a.r. infected herd but was judged to be genetically resistant, because neither of its maternal ancestors had become infected with a.r. and its progeny also remained free of the infection, although they were kept with the rest of the herd throughout. The incidence of a.r. in the herd varied between 52.4 and 88.1% on the basis of clinical and pathological inspection.

The experimental piglets, a total of 29 from the three sows, were given a parenteral iron preparation (Chinofer for veterinary use) equivalent to 150 mg iron when 3 days old, to prevent iron deficiency anaemia. They were weaned after 8 weeks and divided into three groups, each given a different diet. Piglets of group I (16 animals) received a diet similar to that fed by BROWN et al., consisting of 74.8% maize meal, 24% soybean meal, 0.9% dicalcium phosphate and 0.3% potassium dihydrogen phosphate. The Ca and P levels of this diet were determined as 0.25% and 1.03%, respectively.

Piglets of group II (8 animals) were given a mixture of 30% pig rearing feed and 70% bran, with Ca and P contents of 0.22 and 1.17%, respectively.

Piglets of group III (5 animals) served as controls and received a commercial pig rearing feed with Ca and P contents of 0.96 and 0.98%, respectively.

Experimental feeding was continued for 4 to 5 months. The animals were kept in a dry, moderately warm, closed pig house with an asphalt floor. They were fed twice daily and fresh drinking water was available ad libitum. They were examined clinically every 10 days throughout the whole period of the experiment. Once every month after weaning they were weighed and blood samples were taken for determination of plasma alkaline phosphatase activity and serum Ca and inorganic phosphorus levels. Half of the animals in each group were killed after four months and their heads were sent to the Pathology Department of the Central Veterinary Institute (Budapest) for evaluation for a.r. The right femoral bone of each carcase was dissected out, de-fatted by extraction with ether and ethanol and examined for dry material, phosphorus and calcium contents. Alkaline phosphatase was determined by Boehringer's Farb-Test, Ca by the permanganate method and phosphorus by the molybdenate method. Results of laboratory tests and the weight data of the animals

are shown in Table I. The remaining animals were kept on the respective experimental diets for one additional month. At the end of the experiment the three sows were also killed and their nasal cavities examined for a.r.

Table I

Effects of dietary Ca depletion. Comparison of serum and bone Ca and P levels and body weights of pigs maintained on a low-calcium diet with data for normally supplied controls

		No. of experimental animals in groups		
		I	II	III (control)
Serum Ca level (mg%)	At weaning	10.5 ±0.7	10.0 ±0.2	10.6 ±0.9
	At 3 months of age	9.6 ±0.5	9.5 ±0.3	10.0 ±0.3
	At 4 months of age	10.2 ±0.9	10.2 ±0.9	10.2 ±0.3
	At 5 months of age	9.7 ±0.6	8.2 ±0.4	8.9 ±0.2
	At 6 months of age	9.7 ±0.9	10.6 ±0.7	9.7 ±0.6
	At 7 months of age	9.1 ±0.2	8.5 ±0.2	—
Serum P level (mg%)	At weaning	9.1 ±2.1	9.1 ±2.1	8.4 ±1.1
	At 3 months of age	7.5 ±1.8	7.9 ±0.9	8.3 ±
	At 4 months of age	10.3 ±2.3	7.7 ±1.8	5.6 ±2.3
	At 5 months of age	10.4 ±1.1	7.2 ±0.6	6.1 ±1.1
	At 6 months of age	9.1 ±0.9	8.2 ±0.9	6.1 ±1.2
	At 7 months of age	9.0 ±0.4	8.1 ±	—
Ca and P levels of de-fatted bone (ash)	Ca-content (mg/g) at 6—7 months of age	379 ±17	361 ±17	410 ±30
	P-content (mg/g) at 6—7 months of age	151.1 ±3	149.9 ±3.2	148.4 ±3
	Ca/P ratio	2.50±0.12	2.40±0.14	2.76±0.23
Body weight (kg)	At birth	1.58±0.1	1.37±0.2	1.41±0.2
	At 3 months of age	10.70±1.5	9.80±1.6	11.10±1.0
	At 4 months of age	18.60±3.5	16.10±2.2	21.00±2.5
	At 5 months of age	30.60±4.1	22.1 ±3.1	28.00±5.1
	At 6 months of age	38.80±3.6	25.5 ±3.2	34.70±4.2
	At 7 months of age	50.20±5.1	34.7 ±5.2	43.10±6.0

The second experiment was performed on 5 piglets (group IV) originating from two sows affected with a.r. The animals received the same diet as group I in the first series. The two dams had clinically manifest a.r. while most of their progeny from previous litters had been affected clinically and all had displayed typical lesions of a.r. when examined post mortem. The five piglets used in this group were therefore regarded as genetically predisposed to a.r. They were examined for symptoms every other week during the experiment. One piglet was killed when 5 months old, the remaining five were killed when 6 months old. The heads of the carcases were sent to the Pathology Department and femoral bones were examined for Ca and P contents as described before.

Results

Piglets of all four groups had a good appetite throughout the experiment, consuming all the feed that was offered. Animals of group II developed diarrhoea of varying severity, lasting for a few days, one month after weaning and on several later occasions, but this apparently did not affect their general condition.

Piglets of groups I, II and IV began to show motoric disorders from 3½ months of age (at the earliest); the best developed individuals became affected first, then gradually the rest as well. They preferably lay on the floor, rose with difficulty and developed a tripping gait. Bone ends in the fetlocks became swollen.

The animals did not fully extend their forelegs even in the standing position (Fig. 1), some indeed were unable to rise on their forelegs and instead assumed a "kneeling" position (Fig. 2). A slight loss of appetite was noticed with the progression of the movement disturbance, but no other clinically detectable changes were observed.

The results of laboratory tests are shown in Table I. Variation of the dietary Ca level apparently had no noticeable influence on the serum Ca content, except that slight falls were recorded for piglets of group II at 5 and 7 months of age. The high dietary P level (group I), however, resulted in a considerable elevation of inorganic P in the serum at 4—5 months of age and thereafter the serum P level persisted throughout at a high level compared to that of controls. Femoral Ca contents fell only in groups I and II but P levels did not change, the femoral bone Ca/P ratio showed a similar tendency. Thus the increased dietary P supply resulted in only a temporary rise of serum inorganic P level, but it did not affect the P content of bones.

Serum alkaline phosphatase levels and body weights did not vary notably between groups.

Pigs fed the Ca-deficient diets (groups I, II and IV) showed symptoms of rickets, whereas the normally fed control pigs (group III) showed no abnormality. No clinical symptoms indicative of a.r. were encountered and post mortem examinations for a.r. also had negative results. This is important, because for pigs of groups I and IV the dietary Ca-levels were kept as low as in the experiments of BROWN et al. (1966). The latter authors reported that with a low-calcium diet only they had been able to produce clinical symptoms of a.r. and gross lesions corresponding to a severe fibrous osteodystrophy. In our hands, only two pigs of the genetically predisposed litter (group IV) showed lesions judged as "probably suspect of a.r.". One of the two suspect animals, killed when 5 months old, had femoral bone ash Ca and P contents of 218 and 94 mg/g, respectively, giving a Ca/P ratio of —2.31; the corresponding mean values for the remaining 4 pigs, killed when 6 months old, were 333 mg/g Ca, 127 mg/g P and —2.36 Ca/P.

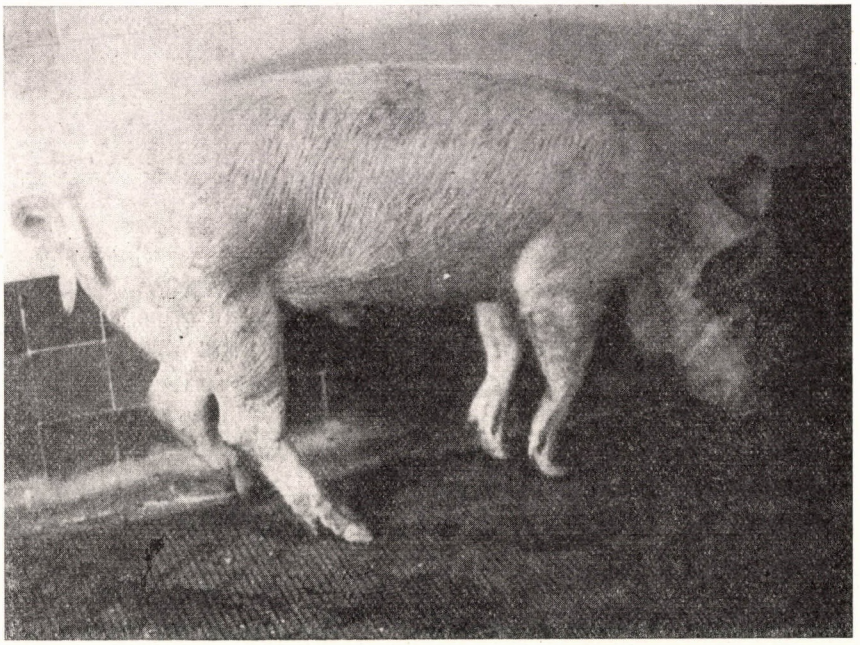

Fig. 1. When the pig is in the standing position its forelegs are slightly bent and its hindlegs drawn under the abdomen

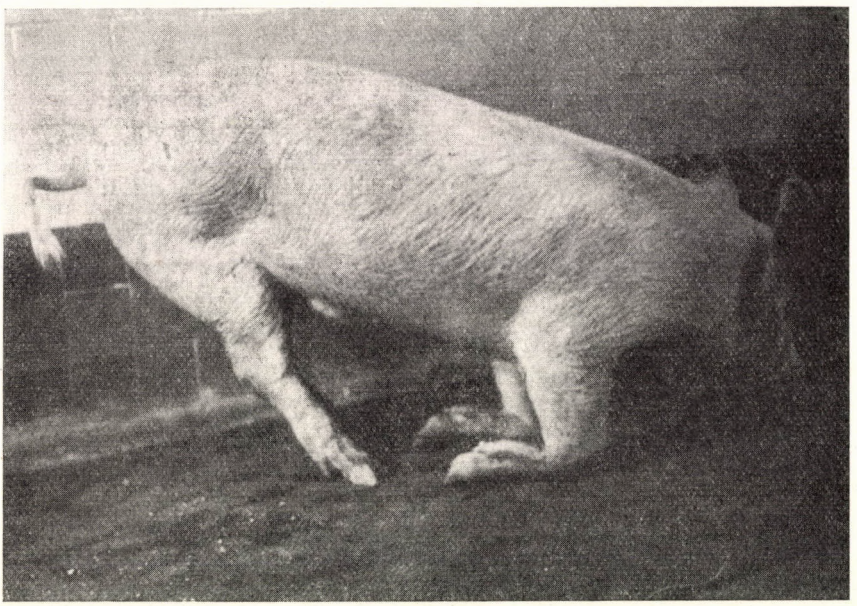

Fig. 2. The pig cannot rise on its forelegs but instead assumes a "kneeling" position

Discussion

It follows from our experimental observations that, contrary to the suggestion of BROWN et al. (1966), disorders resulting from a faulty supply of Ca and P cannot be regarded as the main aetiological factor of swine a.r., on the other hand, reproduction of BROWN et al.'s experiments with a low-calcium diet produced the same rachitic affection of the extremities as reported by these authors. JANOWSKY (1970) has also doubted the causative role of Ca/P imbalance on the grounds that a low-calcium diet was in itself insufficient to elicit any sign of a.r. in a large group of pigs. It seems certain therefore, that the role of dietary Ca depletion is at most secondary. One of the sows used in the present study originated from a herd with a 52.4—83.0% incidence of a.r. Yet neither the sow itself, nor its predecessors and progeny had shown the disease and none of the litter farrowed by in the animal clinic developed a.r. after a long period of gross dietary Ca and P imbalance. It seems likely, therefore, that factors other than a disturbance of Ca and P metabolism might have affected the experimental animals used by BROWN et al. It is very interesting in this content to note MAREK's (1930) earlier observation that one and the same dietary formula elicited rickets in one swine breed and a fibrous osteodystrophy in another.

The experimental results obtained in group IV suggest the importance of management and hygienic conditions in the development of a.r. piglets that could be regarded as genetically predisposed did not develop any sign of a.r. after being fed a low-calcium diet for a prolonged period. This attracts attention to the role of certain environmental ("infectious") factors.

SUMMARY

Induction of swine atrophic rhinitis was attempted by placing weaned pigs on a low-calcium diet for a prolonged period. Piglets used in the first experimental series originated from sows either free of, or genetically resistant to, atrophic rhinitis. Three experimental groups were formed and supplied dietary levels of 0.35% Ca and 1.03% P (group I; 16 animals), 0.22% Ca and 1.16% P (group II; 8 animals) and 0.96% Ca, 0.98% P (group III — control — 5 animals), respectively, up to the age of 4 or 5 months.

Pigs of groups I and II showed motoric disturbances and bone deformation indicative of rickets, whereas the control animals of group III remained free of symptoms throughout. The different dietary Ca supplies caused no notable change in serum Ca levels, but bone Ca concentrations fell slightly in pigs of groups I and II compared to controls. Plasma alkaline phosphatase activities and body weights showed no notable inter-group variation.

In the second experiment 5 pigs (group IV) from two sows affected with atrophic rhinitis were placed on the same diet as group I for 5 or 6 months. No signs of atrophic rhinitis developed, but movement disturbances and bone deformities indicative of rickets did appear. Grossly, only two pigs of group IV showed lesions "probably suspect of atrophic rhinitis", but these were not of a degree that would affect health.

It was concluded that dietary Ca depletion in itself plays no fundamental role in the aetiology of atrophic rhinitis.

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STUDIES OF Ca AND P METABOLISM IN ATROPHIC RHINITIS OF SWINE

II. BONE Ca AND P CONTENTS OF HEALTHY PIGS AND PIGS AFFECTED WITH ATROPHIC RHINITIS

By

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Atrophic rhinitis (further on referred to as a.r.) is a frequent disease of highly productive rapidly growing swine breeds (HÁMORI and STIPKOVITS, 1968). It has been shown with healthy animals that the bone tissue of such breeds already develops at a higher rate than in slow-growing breeds during suckling (ÉLIÁS, 1970) and it may be supposed that for this reason alone their mineral requirements must be higher, especially for calcium and phosphorus.

It seemed therefore worthwhile to compare the bone Ca and P contents of healthy pigs and pigs affected with a.r. and to ascertain whether or not bone Ca and P levels differ between breeds.

Tail vertebrae from healthy and diseased swine were analyzed for Ca and P contents and Ca/P ratio at predetermined time intervals, using the same donors throughout. Specimens of frontal bone were additionally examined in several instances.

Materials and methods

Group I. Female progeny of 4 Cornwall and 4 F_1 (Cornwall X Large White) sows, with manifest a.r., but otherwise in a good condition, were used as donors. The 6th, 5th and 4th tail vertebrae were removed surgically at 2, 4 and 6 months of age, respectively, and their Ca and P contents per 1 g bone ash and per 1 g de-fatted bone dried at 105 °C, and from the former figures the Ca/P ratio were determined. The body weights of the animals were measured at each operation.

The 6th tail vertebra is already palpable at 2 months of age and the 5th and 4th vertebrae develop in succession.

Group II. Thirty female hybrids, 7 months old, weighing on average 103—104 kg (range: 101—109 kg), were used; 15 of these were free of a.r. while the other 15 had a.r. but were in good general condition. The 6th tail vertebra of each animal and frontal bone specimens from 10 healthy and 9 diseased swine were removed and assayed for Ca and P contents.

Group III. A comparison was made of Ca and P contents of the 6th tail vertebra of 10 and 24 swine, weighing on average 105 kg and regarded as genetically resistant and predisposed, respectively. The 10 "resistant" swine originated

from two sows which had no family history of a.r. either in the ancestral or descendant lines, whereas the 24 "predisposed" animals were farrowed by three sows with a history of high a.r. incidence among both predecessors and progeny.

The conditions of management and feeding were kept identical throughout for both experimental and control swine. All received a suitable commercial diet for their age in which the Ca/P ratio was kept at 1 : 1 and the quantity of CaO and P_2O_5 amounted to 12.5 g/kg feed. The hygienic conditions of the farm were checked by workers of the Department of Animal Hygiene of the University of Veterinary Science, Budapest. The microclimate was favourable throughout: average temperature was 14.5 °C (range: 12°—27 °C) and relative air moisture content varied between 65 and 75%. Other microclimatic factors, such as the concentrations of ammonia, carbon dioxide, dust and microbes, remained between normal limits.

At the end of the experiment the animals were slaughtered and during meat inspection nasal cavity examined for gross lesions. All animals judged as diseased during the experiment were found to have atrophic nasal conchae after slaughter, whereas those which had remained healthy showed no gross nasal lesions.

Bone Ca contents were determined by the permanganate, P contents by the molybdenate method.

Results

Ca and P contents of the 6th and 4th tail vertebrae of swine of group I at 2, 4 and 6 months of age, respectively, are shown in Table I.

Results of similar measurements on the 6th tail vertebra of 15 healthy swine and 15 swine displaying a.r. (group II) are shown in Table II.

Data for 10 "genetically resistant" and 24 "genetically predisposed" swine can be seen in Table III.

Discussion

The measurements of Ca and P contents of tail vertebrae of swine affected with a.r. (Table I) showed that under the same conditions the bone Ca content of the fast-growing F_1 cross breeds falls below that of the pure-bred animals from about 4 months of age and the difference becomes quite conspicuous at 6 months. The bone P content, remarkably, is not subject to a similar change. The observation that — irrespective of breed — swine with a.r. incorporate less Ca into tail vertebrae than similarly managed healthy ones, seems to be of a fundamental importance (Tables I and II). The difference between the Ca contents of vertebrae from healthy and diseased swine was statistically signif-

Table I

Ca and P contents of the tail vertebrae of Cornwall and Cornwall X Large White F_1 swine affected with atrophic rhinitis

Serial No.	Swine age (months)	Breed	Weight (kg)	contents (mg/g) of bone ash: Ca	contents (mg/g) of bone ash: P	Ca/P ratio	contents (mg/g) of cooked and dried (105 °C) bone: Ca	contents (mg/g) of cooked and dried (105 °C) bone: P	Remark
1	2	Cornwall	21	460	190	2.42	207	85.5	6th tail vertebra
2	2	Cornwall	20	480	200	2.40	202	84.0	6th tail vertebra
3	2	Cornwall	20	440	174	2.52	198	78.3	6th tail vertebra
4	2	Cornwall	20	461	182	2.53	—	—	6th tail vertebra
		Average:	20	460	186	2.46	202	82.6	
5	2	F_1 (Cornwall)	22	420	173	2.42	193	79.6	6th tail vertebra
6	2	X Large White	29	540	170	3.17	211	66.3	6th tail vertebra
7	2		28	465	168	2.76	—	—	6th tail vertebra
8	2		24	420	170	2.47	244	98.6	6th tail vertebra
		Average:	26	461	170	2.70	216	81.7	
1	4	Cornwall	44	440	175	2.68	224	89.3	5th tail vertebra
2	4	Cornwall	42	460	179	2.57	267	103.8	5th tail vertebra
3	4	Cornwall	50	420	175	2.40	265	110.3	5th tail vertebra
4	4	Cornwall	46	470	170	2.81	268	103.7	5th tail vertebra
		Average:	46	457	174	2.61	265	101.7	
5	4	F_1 (Cornwall)	48	480	160	3.00	230	77.1	5th tail vertebra
6	4	X Large White	50	440	170	2.58	229	88.4	5th tail vertebra
7	4		45	460	176	2.61	253	96.8	5th tail vertebra
8	4		46	400	170	2.35	236	100.3	5th tail vertebra
		Average:	47	445	169	2.63	237	90.6	
1	6	Cornwall	80	404	160	2.52	274	108.0	4th tail vertebra
2	6	Cornwall	100	432	165	2.61	294	112.0	4th tail vertebra
3	6	Cornwall	95	432	167	2.58	295	114.0	4th tail vertebra
4	6	Cornwall	79	420	160	2.62	279	106.0	4th tail vertebra
		Average:	89	422	163	2.58	285	110.0	
5	6	F_1 (Cornwall)	95	396	162	2.44	267	109.0	4th tail vertebra
6	6	X Large White	98	364	156	2.33	244	104.0	4th tail vertebra
7	6		105	384	170	2.25	262	116.0	4th tail vertebra
8	6		98	396	162	2.44	271	111.0	4th tail vertebra
		Average:	99	385	162	2.36	261	110.0	

Table II

Ca and P contents of the 6th tail vertebra and frontal bone of healthy 7-month-old swine and of those diseased in atrophic rhinitis

Serial No.	Swine breed and health state	Body weight (kg)	Frontal bone weight (g)	Ca (tail vertebrae) contents (mg/g) of bone ash	P (tail vertebrae) contents (mg/g) of bone ash	Ca/P ratio	Ca (tail vertebrae) contents (mg/g) of cooked and dried (105 °C) bone	P (tail vertebrae) contents (mg/g) of cooked and dried (105 °C) bone	Ca (frontal bone) contents (mg/g) of frontal bone (ash)	P (frontal bone) contents (mg/g) of frontal bone (ash)	Ca/P ratio
1	F_1 (Cornwall	105	—	520	178	2.92	317	109	—	—	—
2	X Large White),	102	—	412	185	2.22	218	98	—	—	—
3	healthy	104	—	432	178	2.42	337	139	—	—	—
4		106	—	495	178	2.79	189	82	—	—	—
5		105	—	440	182	2.41	194	80	—	—	—
6		101	0.30	394	172	2.29	168	73	416	172	2.41
7		104	0.15	436	175	2.49	186	74	448	166	2.69
8		109	0.29	436	184	2.36	167	70	470	175	2.68
9		107	0.17	434	196	2.21	197	89	374	175	2.13
10		100	0.20	376	200	1.88	161	85	384	172	2.23
11		106	0.10	438	173	2.54	194	76	444	175	2.53
12		104	0.15	496	200	2.48	198	80	504	176	2.86
13		109	0.20	436	195	2.23	145	64	438	175	2.50
14		102	0.30	444	189	2.34	197	83	440	175	2.51
15		101	0.25	484	192	2.52	223	88	488	175	2.78
	Average:	104	0.20	442 ±37	185 ±9.6	2.40	199 ±41.8	82 ±23.1	440 ±35	173 ±3.0	2.53
1	F_1 (Cornwall	100	—	451	187	2.41	194	82	—	—	—
2	X Large White)	106	—	407	178	2.28	114	52	—	—	—
3	diseased	105	—	416	178	2.33	108	46	—	—	—
4		102	—	424	178	2.38	254	107	—	—	—
5		104	—	416	178	2.33	200	85	—	—	—
6		102	0.25	356	160	2.22	148	66	370	162	2.28
7		106	0.45	372	160	2.32	159	68	366	162	2.25
8		100	0.40	364	152	2.39	161	67	368	180	2.04
9		100	0.30	354	155	2.28	163	71	352	165	2.13
10		101	0.20	394	165	2.38	185	77	—	—	—
11		106	0.25	328	132	2.48	182	73	326	162	2.01
12		108	0.50	334	160	2.08	115	55	368	140	2.62
13		99	0.60	354	165	2.14	145	66	356	155	2.29
14		102	0.25	320	147	2.17	133	61	328	157	2.08
15		103	0.25	378	170	2.22	125	56	372	152	2.44
	Average:	103	0.34	377 ±39.8 $P_t>0.1$	164 ±14.5	2.29	158 ±39.6 $P_t>0.1$	68 ±15.2	356 ±14.7 $P_t>0.1$	159 ±10.8	2.23

icant ($Pt > 0.1$) in relation to both bone ash and dried de-fatted bone. Since the analyses of frontal bone specimens gave the same results, it can be concluded that this relative Ca-deficiency exists throughout the skeletal system.

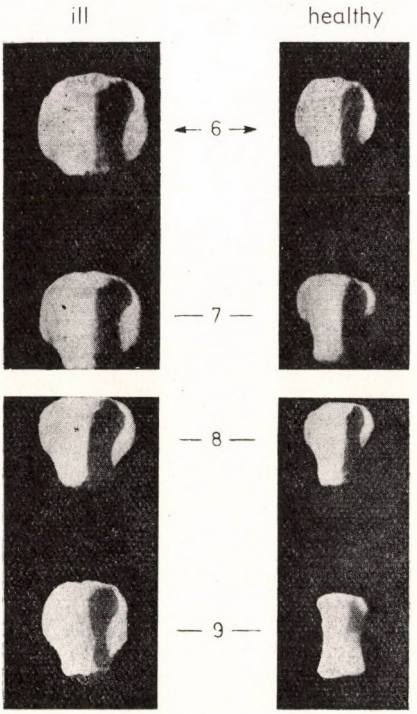

Fig. 1. Tail vertebrae (6th, 7th, 8th, 9th) of 7-month-old healthy swine, and of those diseased in atrophic rhinitis

It follows that bone analysis (of tail vertebra, if the animal is alive) might be of a diagnostic value in the detection of a.r. Simple inspection often reveals that the tail vertebrae of swine with a.r. are enlarged compared to those of healthy swine of similar age (Fig. 1); they are "swollen" and appear to have a spongeous structure. In fact, the average weight of the 6th tail vertebra was higher (0.34 g) in diseased than in healthy animals of the same age (0.2 g), but the Ca contents of the former were lower in relation to 1 g dried bone (Table II).

Ca contents of lower vertebrae were also measured in specimens from "genetically predisposed", diseased animals and compared with those from "genetically resistant", healthy ones (Table III). These observations suggest that a.r. should not be regarded just as a disease localized to the nasal conchae and mucosa, but rather as an irregularity which under as yet unclear circumstances involves the entire skeletal system.

Table III

Ca and P contents of 6th tail vertebra of swine genetically resistant (healthy) and genetically predisposed (diseased) to atrophic rhinitis

Serial No.	Health state	Ca contents (mg/g) of bone ash	P contents (mg/g) of bone ash	Ca/P ratio	Ca contents (mg/g) of dried (105 °C) bone	P contents (mg/g) of dried (105 °C) bone	Ca/P ratio
1	healthy	436	222	1.96	165	84	1.96
2		386	230	1.67	170	101	1.68
3		410	230	1.78	182	102	1.78
4		384	240	1.60	161	101	1.59
5		392	215	1.82	169	92	1.83
6		401	242	1.65	160	80	2.00
7		480	225	2.13	204	95	2.14
8		488	205	2.38	216	91	2.37
9		456	208	2.28	192	87	2.20
10		458	225	2.03	199	97	2.05
	Average:	429 ±39.6	223 ±13.1	1.93	182 ±19.8	93 ±7.5	1.96
1	diseased	384	182	2.10	158	72	2.19
2		381	242	1.57	146	93	1.56
3		388	224	1.70	172	99	1.73
4		384	201	1.92	153	80	1.91
5		381	210	1.81	165	91	1.81
6		379	205	1.84	167	90	1.85
7		375	235	1.59	166	104	1.59
8		379	237	1.59	167	104	1.60
9		401	235	1.70	169	99	1.70
10		375	215	1.74	173	99	1.74
11		384	225	1.70	158	93	1.70
12		388	227	1.70	166	97	1.71
13		379	210	1.80	168	93	1.80
14		375	257	1.45	165	107	1.54
15		370	240	1.54	158	102	1.54
16		366	216	1.70	161	95	1.69
17		370	237	1.56	148	94	1.57
18		355	235	1.59	166	104	1.59
19		396	250	1.58	152	96	1.58
20		392	217	1.80	156	86	1.81
21		388	242	1.60	182	113	1.61
22		379	207	1.83	159	87	1.82
23		379	222	1.70	167	98	1.70
24		392	220	1.78	176	95	1.78
	Average:	381 ±8.5 $P_t > 0.1$	224 ±14.4	1.70	163 ±8.6	95 ±8.7	1.72

SUMMARY

Tail vertebrae of healthy pigs and pigs affected with atrophic rhinitis (a.r.) were examined for Ca and P contents at various ages before slaughter, using as donors the same animals throughout. In several cases, Ca and P were also determined in specimens of the frontal bone.

Group I. Female progeny of 4 Cornwall sows and 4 F_1 crosses (Cornwall X English Large White) all displaying a.r. but otherwise in good condition. The 6th and 5th tail vertebrae were removed surgically at 2 and 4 months of age, respectively, and the 4th vertebra was secured at slaughter, when the animals were 6 months old.

Group II. Thirty female hybrids, 7 months old, weighing on average 103—104 kg; 15 of them were healthy and the other 15 had a.r. The 6th tail vertebra of each animal and specimens of frontal bone from 10 healthy and 9 diseased animals were analyzed.

Group III. The 6th tail vertebrae from 10 and 24 swine, weighing on average 105 kg and classed as "genetically resistant" and "predisposed" to a.r., respectively, were analyzed.

Bone analyses showed that the fast growing F_1 crosses affected with a.r. incorporate less Ca into tail vertebrae than identically managed healthy contemporaries and that this tendency becomes particularly pronounced from about 4 months of age. Examinations of frontal bone specimens had a similar result, indicating that a.r. affects the Ca content of the entire skeleton. Bone P contents, remarkably, are not influenced by a.r.

Thus, Ca determination in tail vertebrae secured from living swine might be of a diagnostic value in the detection of a.r.

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LIGHT AND ELECTRON MICROSCOPIC STUDY OF LIVERS FROM SYRIAN GOLDEN HAMSTERS INFECTED WITH EQUINE RHINOPNEUMONITIS VIRUS

By

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In equine foetuses aborted following maternal rhinopneumonitis virus infection, gross lesions were found almost exclusively in the liver and microscopically proved to consist of focal necroses and a diffuse semi-acute hepatitis (KAPP et al., 1969). In accordance with other opinions (BÜRKI et al., 1965; CORREA and NILSSON, 1966), these hepatic lesions were attributed to direct action of the virus, and it was demonstrated that the disease had certain characteristics in common with viral hepatitis of other animal species.

To obtain more information on liver changes provoked by equine rhinopneumonitis virus, infection experiments with Syrian golden hamsters were carried out. It is known that the virus grows readily in this species and produces inclusions in the parenchymal cells of the liver (ANDERSON and GOODPASTURE, 1942; DOLL et al., 1953). Previous infection experiments of this nature have been aimed primarily at studying viral morphology, so that information on the ultrastructure of the hepatic lesions is scanty. ARHELGER et al. (1963) made electron microscopic examinations on liver specimens from golden hamsters at various times after an intraperitoneal infection with rhinopneumonitis virus at 4 weeks of age. The hamsters died between 18 and 24 hours after infection. Five hours after infection, the distended sinusoids contained many leukocytes and in some parenchymal cells the endoplasmic reticulum aggregated. Clumping and margination of the nuclear chromatin was observed at 5 and 8 hours and viral particles appeared within nuclear inclusions. Between 15 and 18 hours, irregularly arranged filaments were seen in the nuclei of the host cells, the endoplasmic reticulum was distended or aggregated, the mitochondria became swollen, and vacuoles formed in the hyaloplasm. At this time three kinds of viral particles could be distinguished within the nuclear inclusions. Particles lying in the cytoplasm were as a rule larger than those in the nucleus. According to ARHELGER et al., the virions are released through rupture of virus-containing cytoplasmic vacuoles localized near the cytoplasmic membrane. In young golden hamsters treated with propionyl promazine hydrochloride or adrenaline at the same time as they were infected by intraperitoneal injection of rhinopneumonitis virus, the liver cells showed distinct proliferation of the smooth and rough endoplasmic reticulum (BOSTWICK et al., 1968). Mature and immature virus particles lay in evaginations of the nuclear membrane, between its lamellae or in the tubules of the Golgi apparatus. The liver cells of infected controls not treated with drug harboured the virus particles either within the tubular cytoplasmic system, or freely in the cytoplasm.

Experimental

Materials and methods

Golden hamsters were infected intraperitoneally at 2—3 days of age with the non-adapted strain D_p^{-4}, isolated in pig kidney epithelial cell culture from an equine foetus aborted in the course of an outbreak of rhinopneumonitis in a horse stock.* The strain has been maintained ever since in the same type of cell

* The strain was isolated and kindly supplied by Dr. A. BARTHA (Department of Microbiology and Epizootiology, University of Veterinary Science, Budapest).

culture. Hamsters were killed serially at 10, 18, 24, 36, 48, 56, 72, 96 and 120 hours after infection: those exterminated at 96 and 120 hours were already in the pre-lethal stage. Liver specimens were fixed in buffered osmium tetroxide (Pallade), dehydrated in ethanol, and embedded in Durcupan. Ultra-thin sections were cut with a Reichert ultramicrotome and electron micrographs were prepared with a Tesla BS type 613 electron microscope. Semi-thin sections were stained with toluidine blue.

Gross lesions

Hamsters killed 1—2 days after infection had considerably swollen, slightly stubborn livers, usually dark brownish-red in colour. Livers of animals killed after 3—5 days showed additionally greyish-white foci of the size of a pinprick or pinhead. The mucosa of the small intestine exhibited a slight reddening, swelling and deposition of turbid mucus in most animal but in other organs there were no grossly visible changes.

Microscopic lesions

Light and electron micrographs revealed no notable change in the livers of hamsters killed 10 and 18 hours after infection. In places the livers of animals killed after 24 and 36 hours showed a break-up of parenchymal cell rows; most sinusoids were distended and their epithelium swollen and extensively desquamated (Fig. 1a). Granulocytes were aggregated in the lumina of several distended sinusoids. Many of the Disse gaps were distended and filled with a partly homogeneous, partly granular substance (Fig. 1b) while the villi of their parenchymal cell components were swollen to different degrees. Liver parenchymal cells showed no apparent change at this time.

The most characteristic liver lesions appeared from 48 to 120 hours after infection. Their type was essentially similar to that observed during earlier stage of the infection but the damage was more severe due to the longer survival of the animals. In every case, most conspicuous damage was seen in the parenchymal cells of the liver where three forms of lesions could be distinguished.

Liver cell degeneration accompanied by inclusion body formation. Light microscopic sections showed enlargement and cytoplasmic vacuolization of the liver cells with the appearance of Cowdry type A nuclear inclusion bodies. The nuclear membrane was hyperchromatic (Fig. 2). Electron micrographs showed that the nucleus enlarged considerably and became clearer. The nucleolus was fragmented or homogeneous and the chromatin substance aggregated along the nuclear membrane, which was distended in places. At sites of intranuclear inclusions, many viral particles in different stages of development were seen lying among a more or less dense residue of partly finely granular, partly

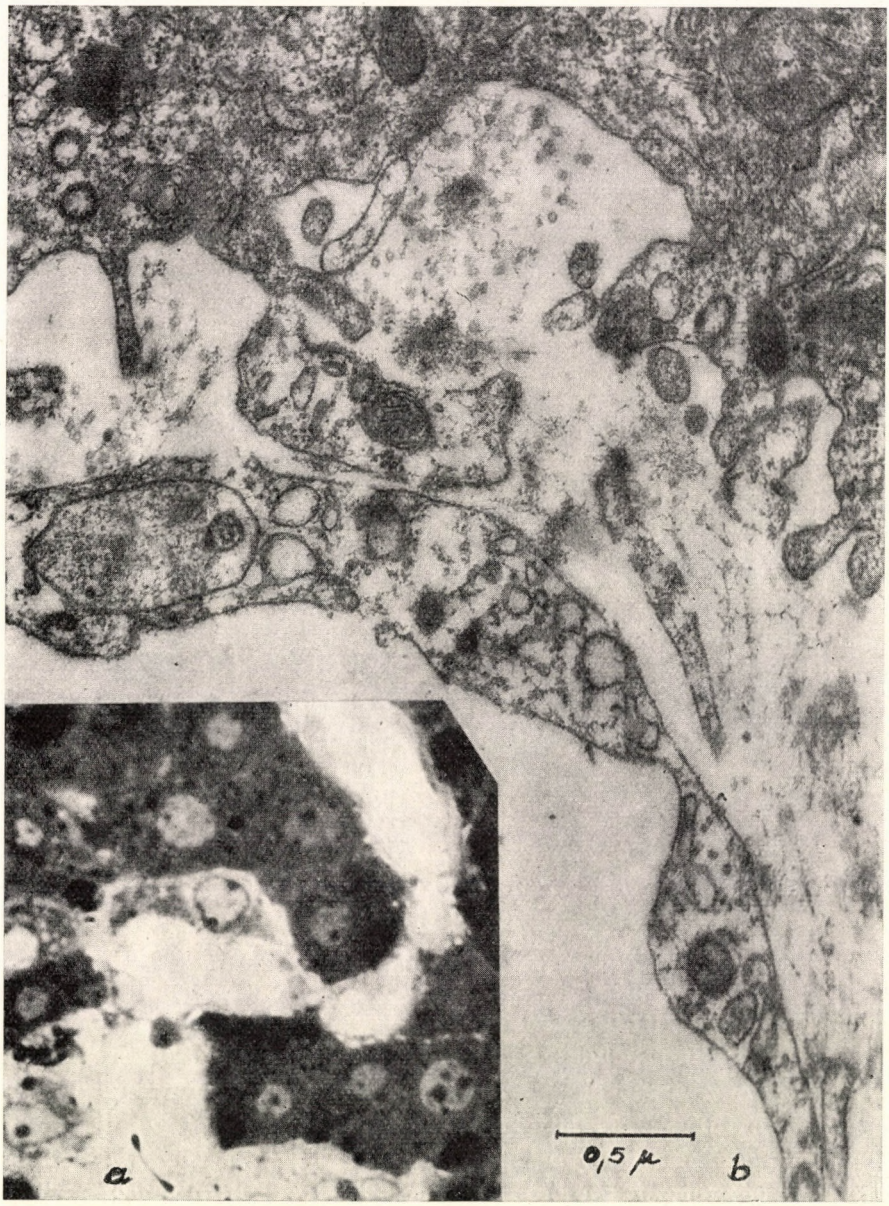


Fig. 1, a, Swollen endothelial cells in the walls of distended sinusoids; semi-thin section, toluidine blue stain. *b,* Markedly distended Disse's gap containing a partly homogeneous, partly granular substance. ×36,400

filamentous chromatin (Figs 3 and 4a, b, c). Although the margins of inclusion body-containing liver cells lost definition in several places, the cytoplasmic organelles were, as a rule, well visible. The mitochondria were distinctly electron-dense and their membranes well preserved, while delicate electron-dense granules appeared in the mitochondrial substance. The membranes of the rough endoplasmic reticulum multiplied, arranging concentrically in fingerprint-like patterns in the cellular cytoplasm. Many free ribosomes were present between the membranes and frequently cysts appeared in the cytoplasm (Fig. 5).

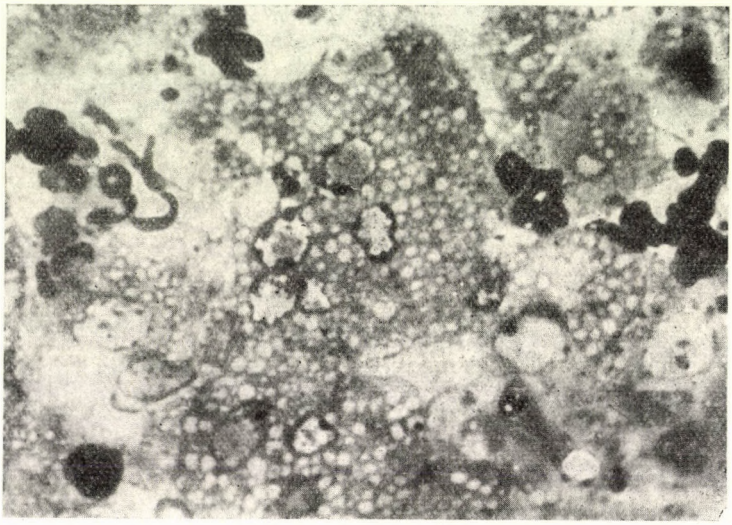


Fig. 2. Cowdry type A nuclear inclusions in liver cells. Semi-thin section, toluidine blue stain. ×900

At later stage of cell degeneration several virions were usually present in the cytoplasm and the organelles showed severe changes. The mitochondria became swollen and rounded, while their cristae either became fragmented or underwent complete lysis, and filamentous, highly electron-dense granules appeared in the mitochondrial substance (Fig. 6). The rough endoplasmic reticulum usually distended considerably and at many places the ribosomes became detached and lay freely in a disorganized manner (Fig. 7). Often cytoplasmic cysts and autophagous vacuoles developed and a myeline-type degeneration appeared (Fig. 8a, b). Cell membranes showed partial lysis, above all at the parts bordering the sinusoids.

Solitary, acidophilic necrosis of liver cells. This process appeared in various parts of the lobules. In semi-thin sections the rows of liver cells appeared to be intact, but the cells were angular and their cytoplasm stained homogeneously with toluidine blue. In electron micrographs the affected cells were initially stellate and could be clearly distinguished from neighbouring normal liver cells.

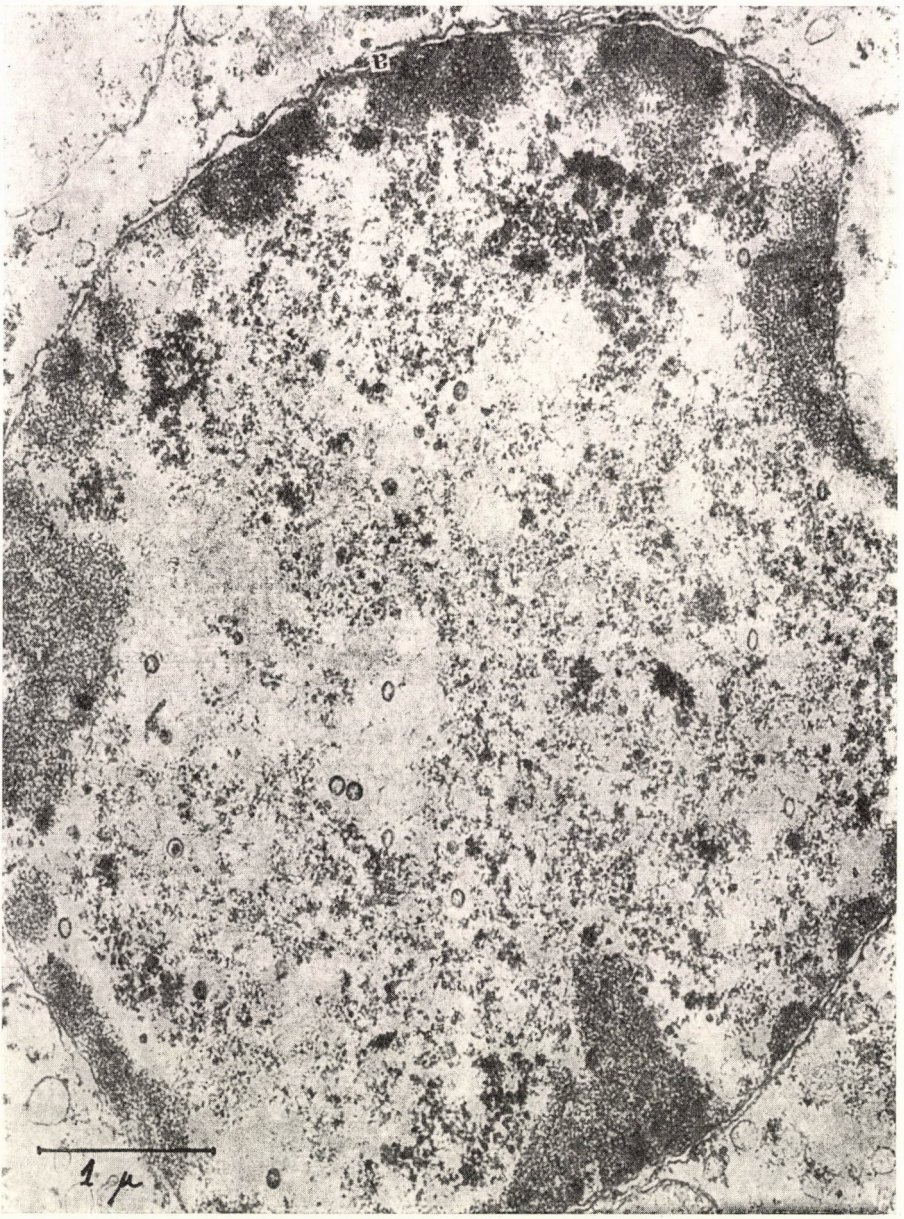


Fig. 3. Nucleus of a damaged liver cell. Note the developing particles of rhinopneumonitis virus between the granular, filamentous residues of the nuclear chromatin. ×23,660

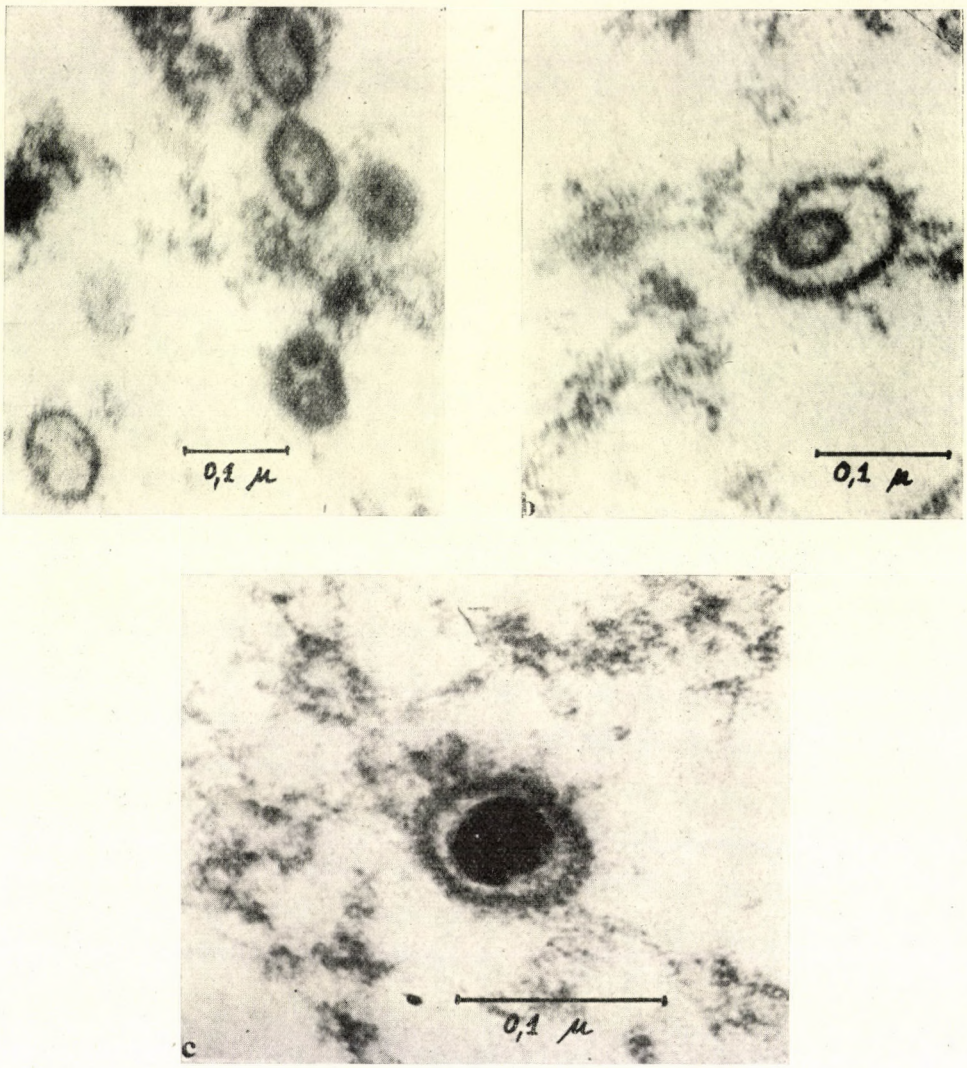


Fig. 4. Developmental stages of the viral particle within the cellular nucleus; *a*, ×136,680; *b*, ×168,000; *c*, ×281,400

The cell membranes were in places sharply delineated, but elsewhere showed signs of lysis. In early stages of acidophilic necrosis, the hyaloplasm was markedly electron dense, sometimes delicately vacuolized, with usually well preserved, clearly visible organelles (Fig. 9). The mitochondria became slightly swollen and showed an increased electron density; the cristae became loose; in places the smooth endoplasmic reticulum distended and multiplied and lipid vacuoles appeared in the cytoplasm. The nuclear subtance cleared slightly with aggre-

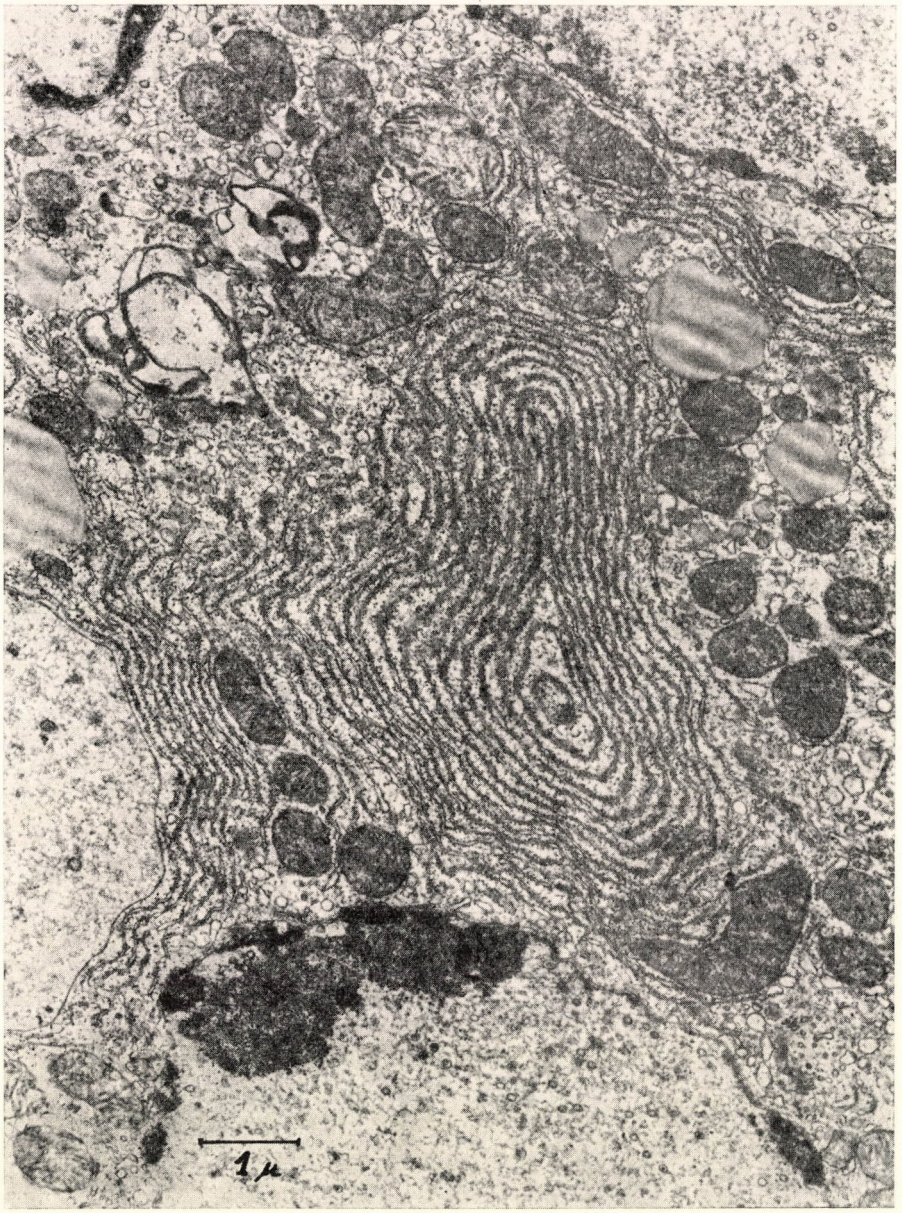


Fig. 5. Fingerprint-like arrangement of rough endoplasmic reticulum and vacuole formation in the cytoplasm of liver cell. ×12,960

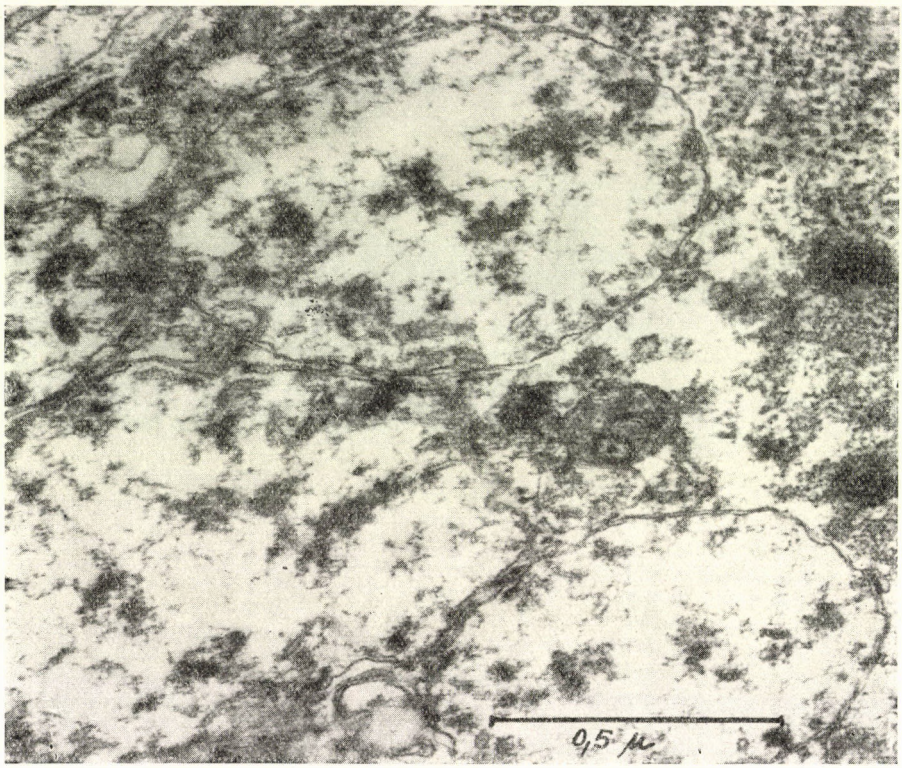


Fig. 6. Swollen, round mitochondria devoid of cristae and enclosing a highly electron-dense substance. ×78,400

gation of the markedly osmiophilic chromatin along the nuclear membrane, and the nucleolus enlarged. A few intranuclear viral particles were usually present.

In later stages, remnants of the affected cells were irregular structures extruding into the sinusoid lumina (Fig. 10). Cell organelles were hardly recognizable in the mostly homogeneous cytoplasm, while vesicles were formed in several places within the ground substance. The clearer parts of the cell residue contained fine granules of glycogen arranged in a rosette-like pattern and the cell membrane was recognizable only in parts. Fully hyalinized residual bodies were not observed.

Liver cell degeneration accompanied by formation of so-called light cells. Many of the liver cells were enlarged and the latter stained pale with toluidine blue in semi-thin sections and became rounded in shape. Electron microscopically the hyaloplasm was less electron dense than that of normal cells. The mitochondria became swollen, their ground substance became clearer and only fragments of the cristae were seen. There was often cystic dilatation of the

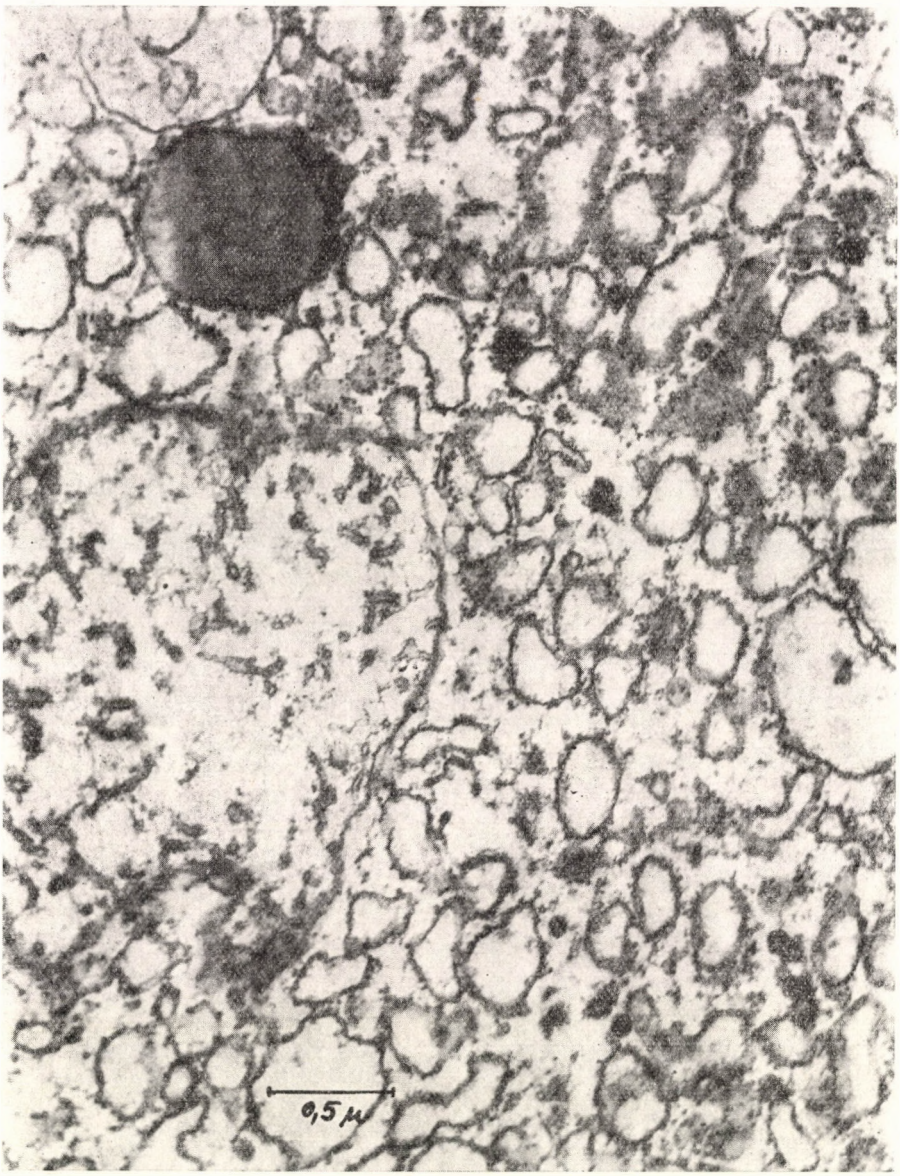
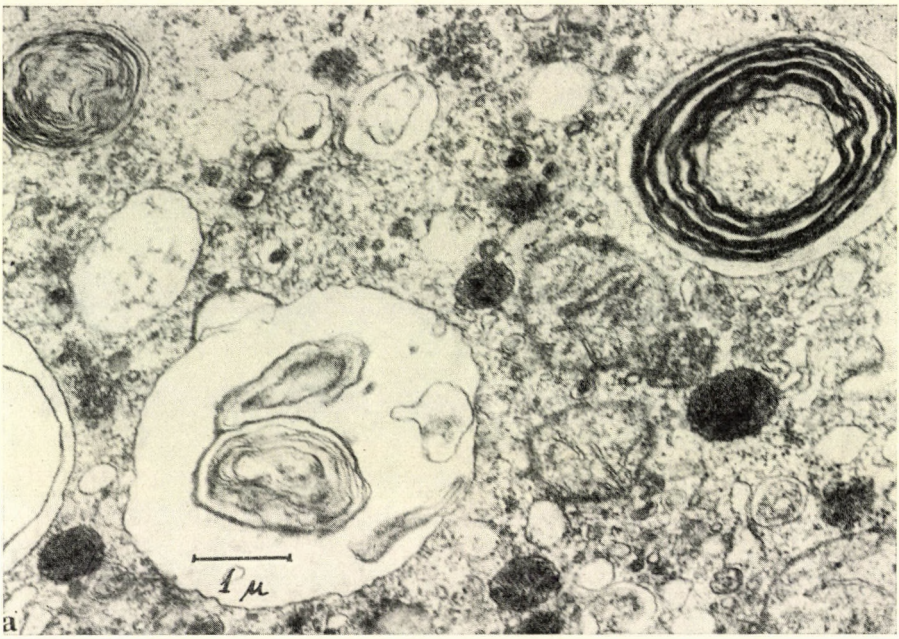


Fig. 7. Part of the cytoplasm of a liver cell. Note the extreme distension and degranulation of the rough endoplasmic reticulum. ×35,600



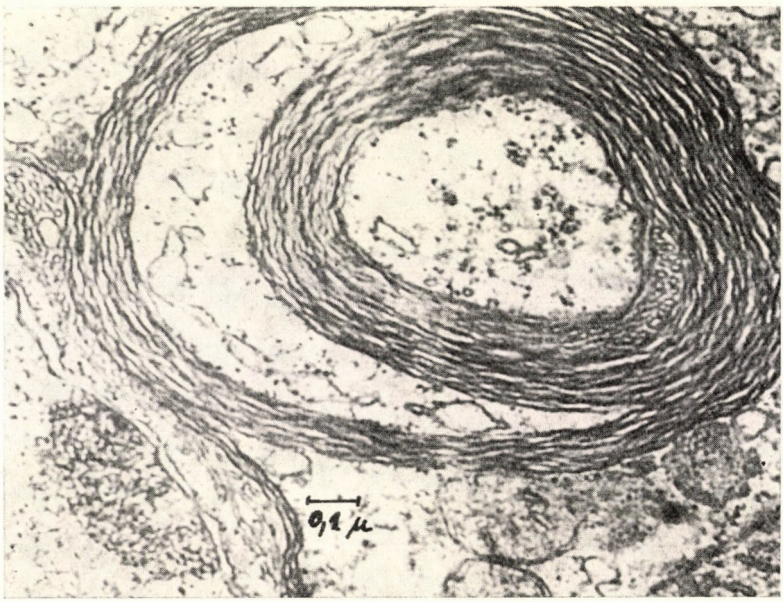


Fig. 8. Part of cytoplasm of a liver cell. Note the autophagous vacuoles, cyst formation and myeline-type degeneration. *a*, ×12,500; *b*, ×67,200

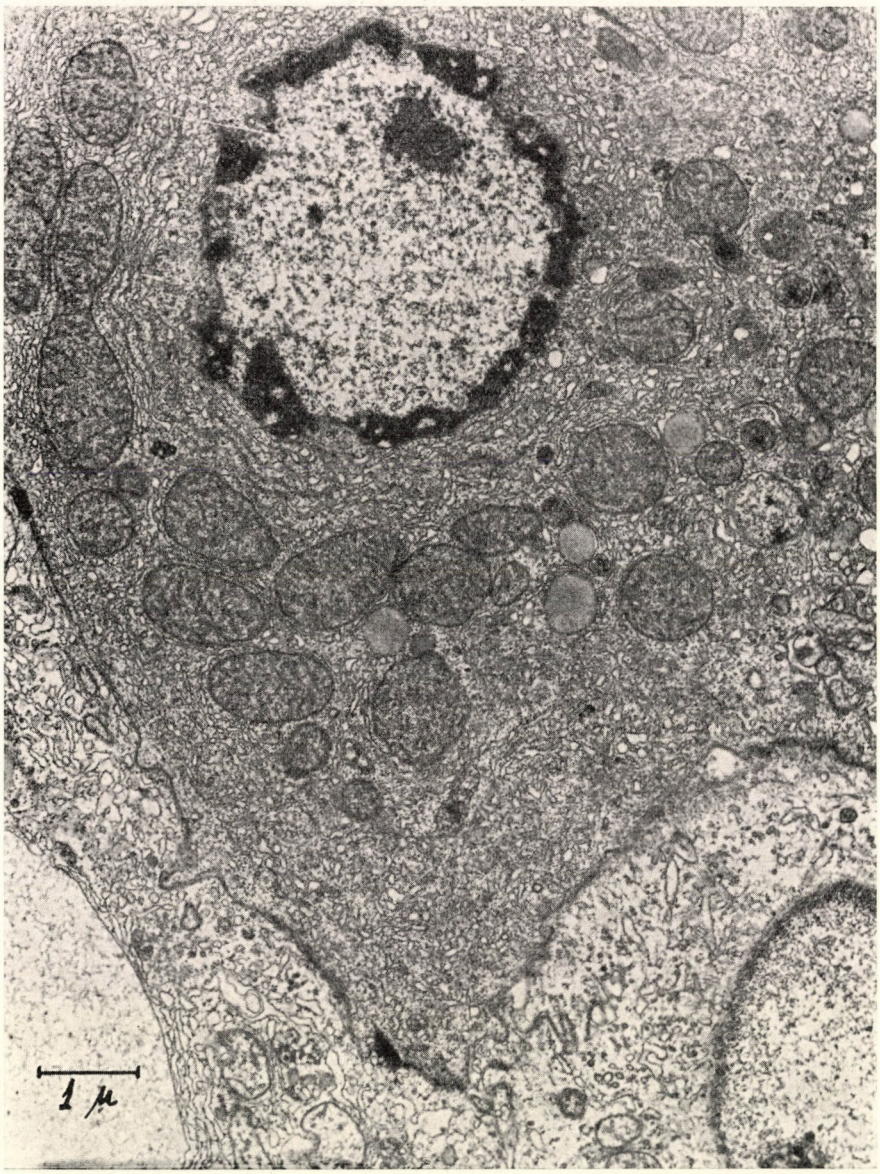


Fig. 9. Early stage of solitary, acidophilic cell necrosis. ×12,960

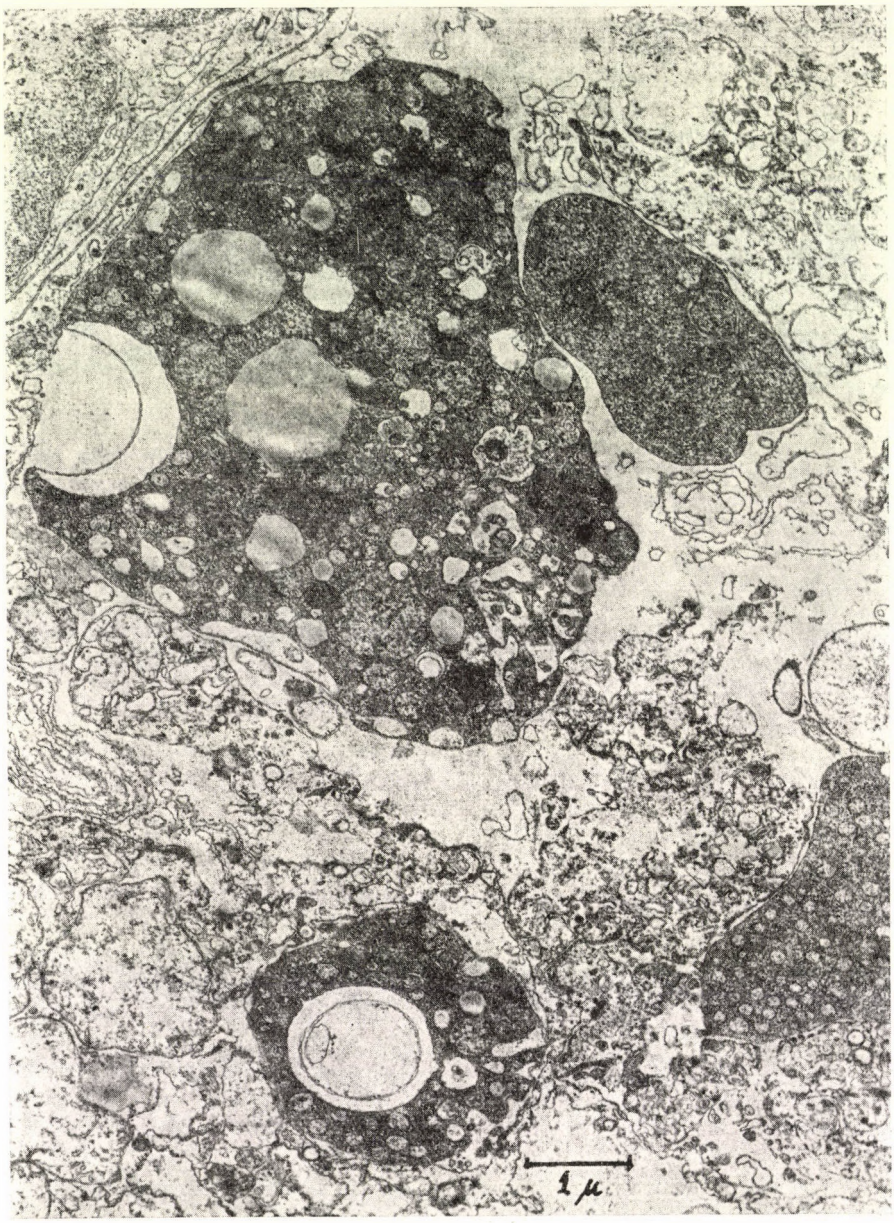


Fig. 10. Acidophilic bodies in a sinusoid. ×12,960

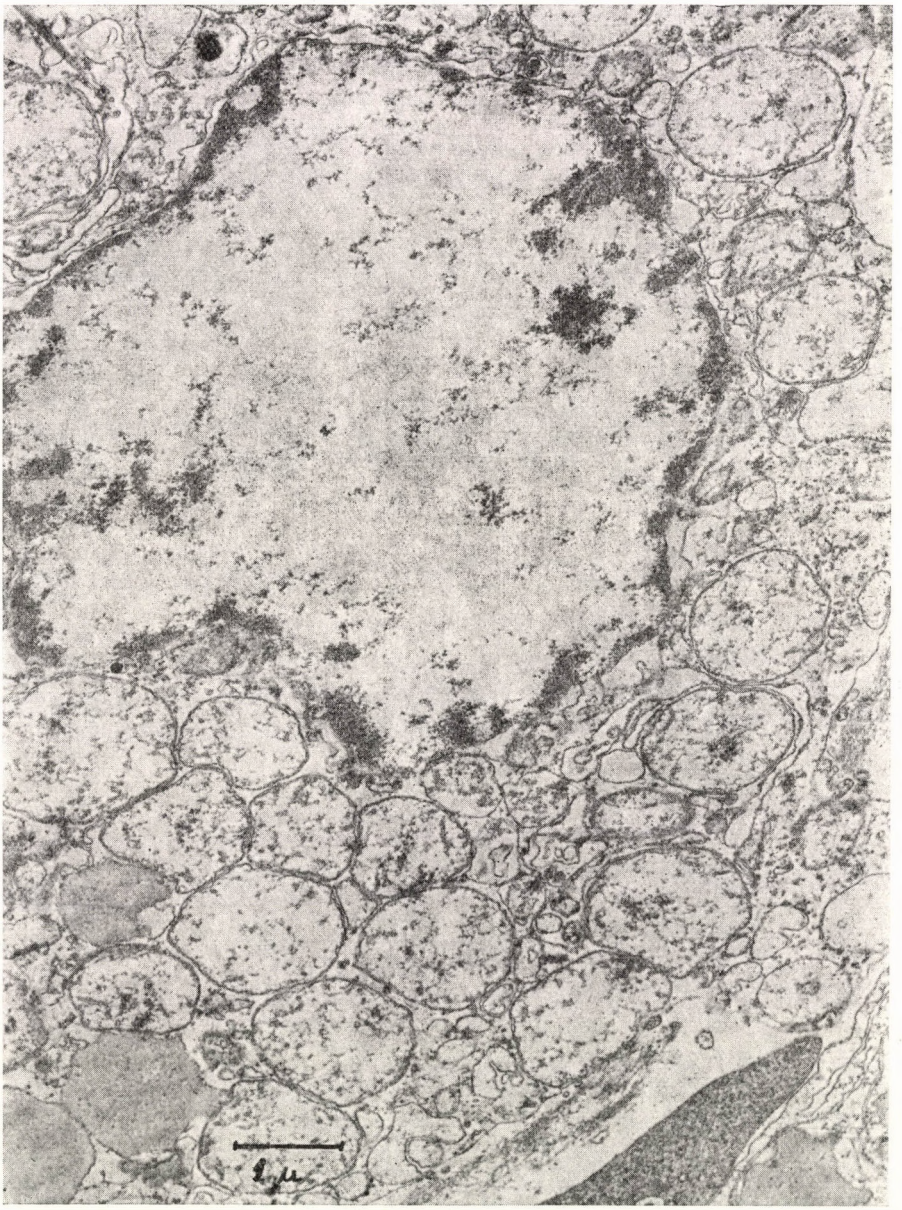


Fig. 11. Pale liver cell of low electron density. ×14,350

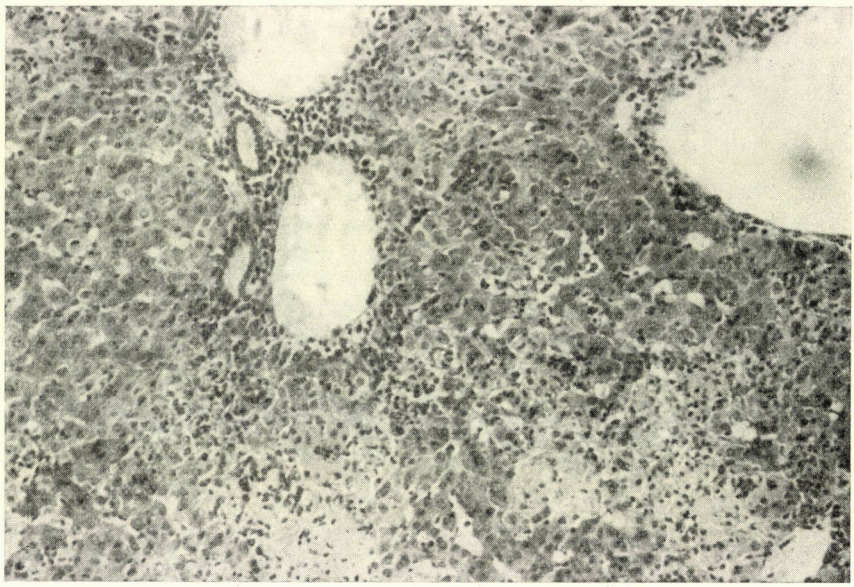


Fig. 12. Extensive focal necrosis in liver lobules. Note the lympho-histiocytic infiltration of interlobular septa. Haematoxylin and eosin stain. ×110

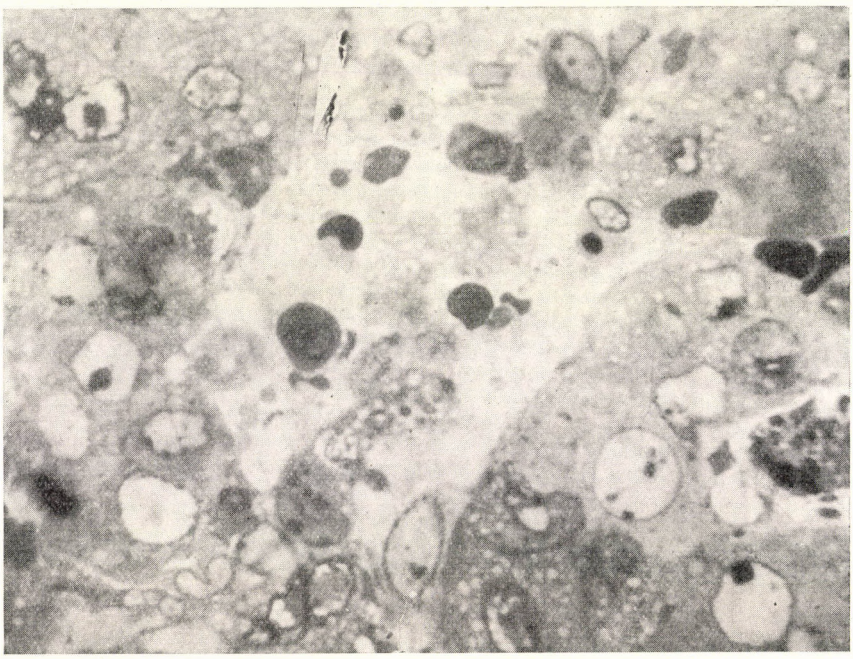


Fig. 13. Sinusoid filled with detached endothelial cells and inflammatory cells. Semi-thin section, toluidine-blue stain. ×1800

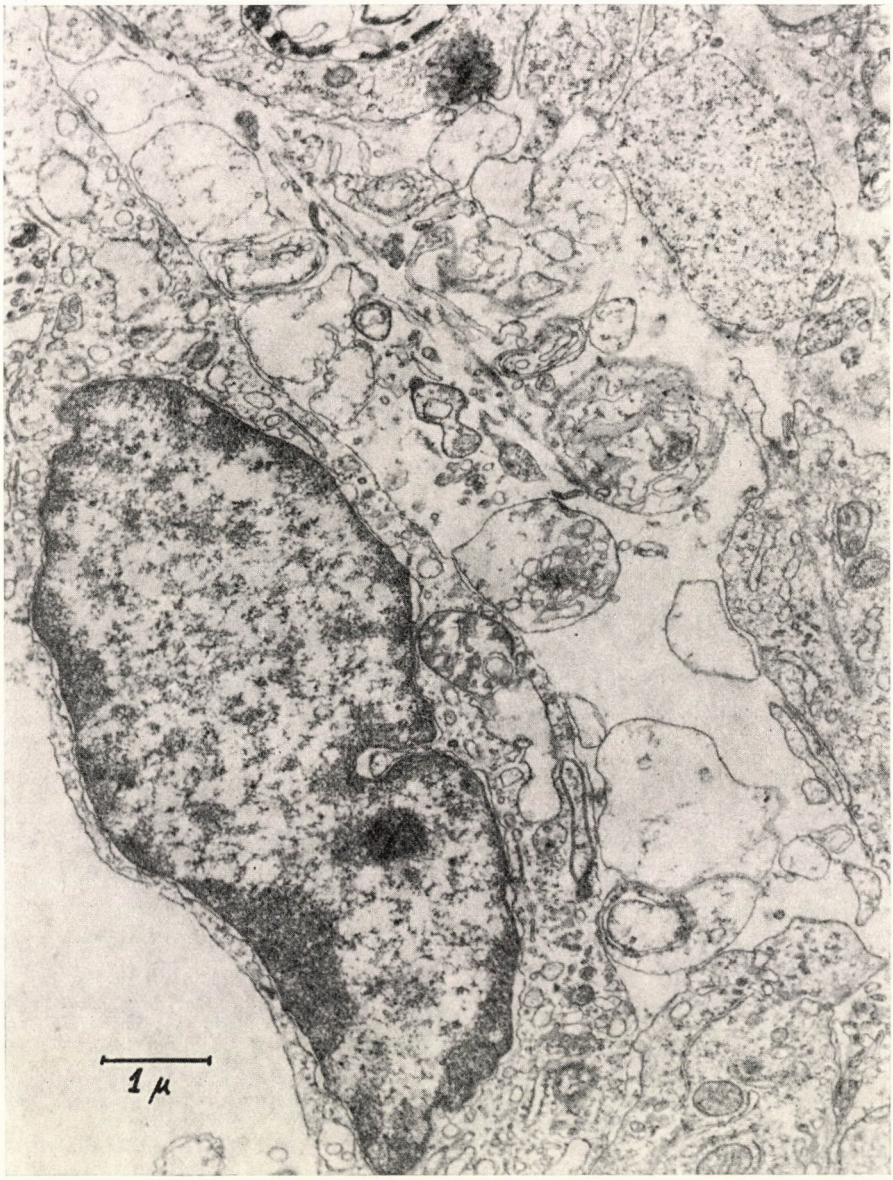


Fig. 14. Distended subendothelial gap filled with swollen microvilli and necrotic cell residues. ×13,420

endoplasmic reticulum. The cell nucleus distended in a vesicular manner, becoming markedly pale, and the little chromatin there was aggregated along the nuclear membrane (Fig. 11). No viral particles were seen in the nuclei of these cells.

In advanced stages of the disease, at 96 and 120 hours, irregular necroses of different sizes were seen in various parts of the liver lobules (Fig. 12). The membranes of cells in affected areas underwent almost complete lysis and the cell organelles were severely damaged. The interlobular septa were infiltrated with lymphocytes and histiocytes (Fig. 12). Biliary ductules were distended and contained coarse or delicate electron dense granules, the microvilli became swollen and detached in places. Most sinusoids appeared distended with endothelial cells often swollen to such an extent that they obliterated the lumen. The endoplasmic reticulum of the endothelial cells usually became distended. Desquamated endothelial cells and leucocytes aggregated in the lumina of the sinusoids (Fig. 13). The subendothelial gaps between the cells were often filled with swollen oedematous microvilli and necrotic cell residues (Fig. 14).

Discussion

Intraperitoneal infection of day-old golden hamsters with a non-adapted rhinopneumonitis virus strain isolated from the livers of aborted equine foetuses induced a diseased condition lasting for 4—5 days. In hamsters killed at various times after infection, severe changes were found only in the liver. Grossly, this was initially enlarged, brownish-red and friable. Later, small greyish-white foci appeared. At 24—36 hours after infection, light and electron micrographs revealed an acute serous hepatitis, but neither the cytoplasm, nor the nucleus of the parenchymal cells contained virus particles at this early stage. The livers of hamsters killed between 48 and 120 hours after infection showed acute or semi-acute hepatitis, involving severe parenchymal cell damage manifested in the form of both sporadic cell necrosis and formation of more extensive necrotic foci in the liver tissue. The cell damage appeared as degeneration accompanied by formation of inclusions, acidophilic necrosis or so-called light cell transformation. Maximum virus synthesis was associated with intranuclear inclusion body formation. In these cells, nuclear changes took place first, accompanied by multiplication and fingerprint-like stratification of the rough endoplasmic reticulum. Later, when the viral particles appeared in the cytoplasm, the endoplasmic reticulum and mitochondria became severely damaged, a myeline-type degeneration developed, and the cell membrane became lysed at the sinusoidal side of the cell. By this time the sinusoids and Disse's gaps were usually involved as well. All these alterations have been observed in viral hepatitis of man (Braunsteiner et al., 1957; Cossel, 1959; Holle, 1960;

YASUZUMI et al., 1963; RUEBNER and SLUSSER, 1968; and others), mice (STARR et al., 1960; SVOBODA et al., 1962; MIYAI et al., 1963; VETTERLEIN and HESSE, 1965) and dogs (LINDBLAD and BJÖRKMAN, 1964).

Although acidophilic necrosis occurs in various other viral and toxic conditions, it has been regarded as characteristic of human viral hepatitis, because the residues of these cells constitute the so-called eosinophilic bodies which become extruded from the parenchymal cell row. This type of necrosis has been observed in viral hepatitis of other animal species too, and according to recent investigations it corresponds with the initial stage of coagulation necrosis. MOPPERT et al. (1967) have suggested on the basis of electron microscopic observations that the predominant early change is the aggregation of the ground substance in consequence of a reduction of the cell volume, which later leads to degeneration of the cytoplasmic organelles through a diminution of intracellular transport. The solitary acidophilic cell necrosis observed in the livers of rhinopneumonitis virus-infected gold hamsters in the present study is essentially the same phenomenon as that observed in other kinds of viral hepatitis. We found that in this animal, aggregation of the cytoplasmic ground substance and nuclear damage are the first steps of the morphogenesis of acidophilic necrosis. The nuclei of the degenerated cells contained a few virus particles, which suggests that the viral agent has a direct role in the acidophilic necrosis. Acidophilic bodies extruded from the parenchymal cells were encountered in practically all stages of the infection.

The third form of solitary liver cell damage is transformation to "light" cells. These swollen, rounded cells had ground substance of diminished electron density, markedly damaged cell organelles, and distended vesicle-like nuclei which did not contain viral particles. Ultrastructurally, these cells were reminiscent the light cells seen in human viral hepatitis. The absence of viral particles nevertheless suggests that in the golden hamster the appearance of such cells is probably unrelated to viral infection.

Apart from the solitary parenchymal cell damages, there were also extensive necroses involving parts of one or more lobules. These changes were in all probability due to vascular disturbance, resulting from the viral infection, in analogy with the similar lesions found in aborted equine foetuses.

The livers of golden hamsters infected with rhinopneumonitis virus showed various additional signs of inflammation. Parenchymal cell damages in the sinusoids and Disse's gaps were accompanied by inflammatory processes, while a lympho-histiocytic infiltration appeared in the interlobular septa.

Light and electron micrographs of liver specimens from golden hamsters infected with rhinopneumonitis virus showed an acute or semi-acute diffuse hepatitis. The gross appearance of the liver corresponded essentially to that observed in aborted equine foetuses (KAPP et al., 1969). Interestingly, neither in this study nor in others was rhinitis or pneumonia observed in the hamsters.

The liver lesions developing in the latter nevertheless bear a striking morphological resemblance to the changes caused by rhinopneumonitis virus in equine foetuses or by hepatitis viruses in man and animals.

Acknowledgements. The author is indebted to Mrs. GY. PETHES for preparing the light microscope and ultra-thin sections and the electron micrographs, and to Miss M. ÖSZY for preparing the photographs.

SUMMARY

In Syrian golden hamsters infected at 2—3 days of age with a field strain of equine rhinopneumonitis virus the most characteristic changes were observed in the liver 48 to 120 hours after infection. Solitary and diffuse lesions of parenchymal cells were prominent in all case. Solitary liver cell damages were manifested in the form of cell degeneration accompanied by intranuclear inclusion body formation, acidophilic necrosis and light cell formation. In the degenerating cells the rough endoplasmic reticulum multiplied and arranged in a fingerprint-like pattern. Maximum viral synthesis took place at the time of intranuclear inclusion body formation. The appearance of viral particles in the cytoplasm was followed by severe damages of endoplasmic reticulum and mitochondria, cytoplasmic cyst formation, myeline-type degeneration and lysis of the cytoplasmic membrane along the sinusoidal side of the liver cells. Solitary acidophilic cell necrosis and the subsequent appearance of eosinophilic bodies in the sinusoids were often observed, and were thought to result from direct action of the virus. No viral particles were seen within light cells. Diffuse parenchymal cell necrosis involving parts of one or more lobules was attributed to vascular disturbances. Apart from the parenchymal cell lesions, inflammatory processes were observed in the sinusoids and Disse's gaps, while the interlobular septa were infiltrated with lymphocytes and histiocytes. The general picture of an acute or semi-acute hepatitis induced by rhinopneumonitis virus morphologically resembled the liver lesions of viral hepatitis in man and animals.

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EXPERIMENTAL STUDIES ON VISCERAL LARVA MIGRANS OF TOXOCARA CANIS IN LABORATORY ANIMALS

By

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The term larva migrans refers in common use to the migration either of canine hookworm larvae in the human skin — cutaneous larva migrans, or of canine ascarides in the viscera — visceral larva migrans (BEAVER et al., 1952). The latter condition occurs mainly among children under four years of age and is usually acquired by the ingestion of soil contaminated with faeces of infected dogs. After being swallowed the infective eggs hatch in the intestine, and the second stage larvae penetrate the intestinal wall to reach the liver. The majority of the larvae remain in the liver, but some pass on to the lungs and other organs.

The histological changes in the liver of mice infected with *T. canis* were first described by HOEPPLI et al. (1949). DONE et al. (1960) found larvae of *T. canis* in the liver, brain, lungs and muscles of experimentally infected pigs, while SCHAEFFLER (1960) recovered them from the liver, muscles and other tissues of sheep. BISSERU (1969) reported on the lesions caused by larvae of *T. canis* in the liver, lungs and brain of mice.

The histopathological changes in the various host species have not been fully described, however. The present investigation was therefore undertaken to get more information about the tissue reaction caused by migrating larvae of *T. canis* in different organs of some laboratory animals and chickens.

Material and methods

Eggs of *T. canis* were obtained from the distal portion of the uterus by dissecting fresh adult female worms. They were cultivated in 0.5% formalin at 28—30 °C for four weeks. Prior to inoculation, the eggs were washed several times with tap water, and their number was estimated. Eight individuals each of 30-day-old mice, rats, guinea-pigs and 11-day-old chickens were experimentally infected with about 2000 embryonated eggs except the mice which received about 500 eggs. The eggs were introduced into the esophagus with the aid of a dropper. Two animals from each group were killed by cutting their throats 10, 20, 30 and 40 days after infection. Fresh samples taken from the lungs, kidneys, liver and brain were fixed in 10% formalin, embedded in paraffin, serially cut, and the sections stained with hematoxylin and eosin.

Results

Liver

Mouse: Liver examined 30 days after the infection showed larvae surrounded by fibroblasts and eosinophils (Fig. 1). Kupffer cells and endothelial cells lining the hepatic sinusoids were swollen.

Rat: The livers examined on the 10th day of the infection were congested and showed focal hepatic necrosis and interstitial aggregations of eosinophils.

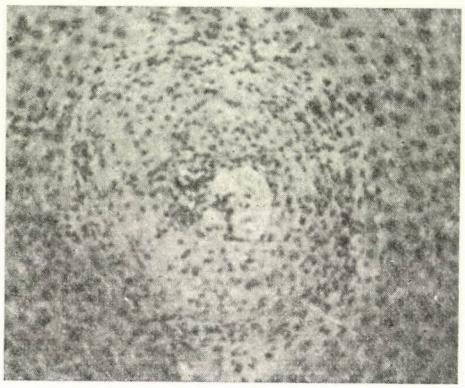

Fig. 1. Larva of *Toxocara canis* surrounded by fibroblasts and eosinophils in the liver of a mouse 30 days after infection. ×150

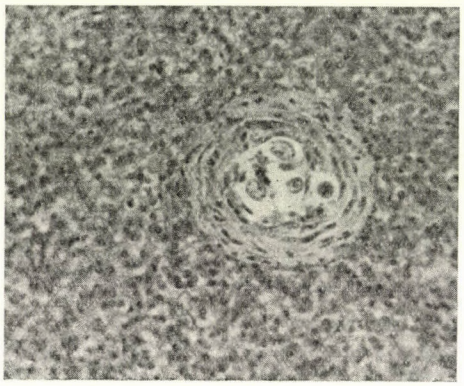

Fig. 2. Larvae surrounded with a thick fibrous capsule in the liver of a guinea-pig 40 days after infection. ×200

Guinea-pig: Larvae were found among hepatic cells 10, 20, 30 and 40 days after the infection. Lymphocytic infiltration with fibroblastic proliferation was present in the portal tracts of the liver, which was congested and exhibited numerous necrotic foci after 10, 20 and 30 days of the infection. Some larvae failed to induce inflammation, but others were surrounded by necrotic debris, fibroblasts and eosinophils from 10—20 days after the infection. Yet other larvae were enclosed in a thick fibrous tissue capsule (Fig. 2) infiltrated with eosinophils. From day 30 after the infection, the cytoplasm of most hepatic cells was vacuolar or hydropic and showed focal necrosis, but there were also signs of repair and regeneration. Reticulo-endothelial cells were swollen.

Chicken: Larvae were mostly seen subcapsularly among the hepatic cells and inside the sinusoids, both with and without surrounding inflammation, after 10, 20 and 30 days of the infection (Fig. 3). The reaction around the larvae on the 10th day consisted of necrotic debris, eosinophils and lymphocytes (Fig. 4). Numerous nodular interstitial lymphocytic infiltrations together with a few eosinophils were seen among the hepatic cells. The Kupffer cells and endothelial cell lining of hepatic sinusoids were swollen. Some bile ducts revealed a

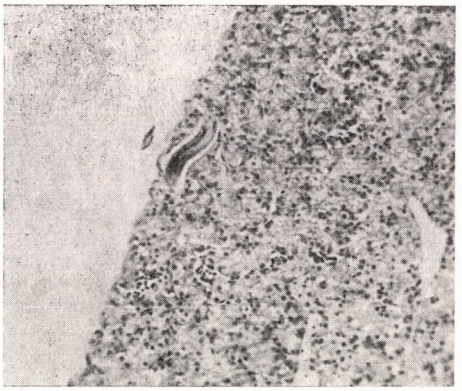

Fig. 3. Larva under the capsule without inflammation in the surrounding tissue of the liver of a chicken 10 days after infection. ×550

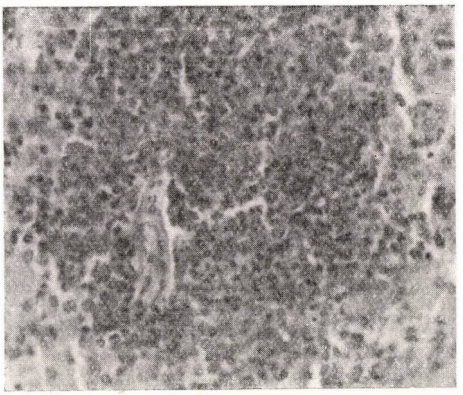

Fig. 4. Larva surrounded by necrotic debris, eosinophils and lymphocytes in the liver of a chicken 10 days after infection. ×330

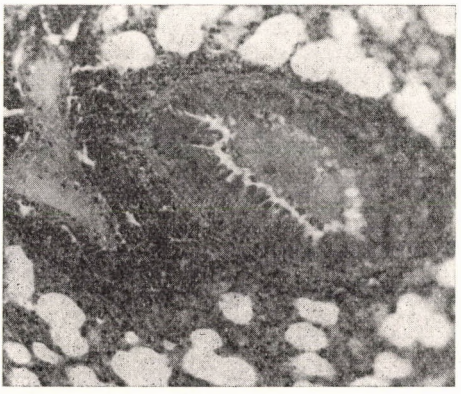

Fig. 5. Peribronchial and perivascular infiltration with eosinophils and lymphocytes in the lung of a mouse 30 days after infection. ×150

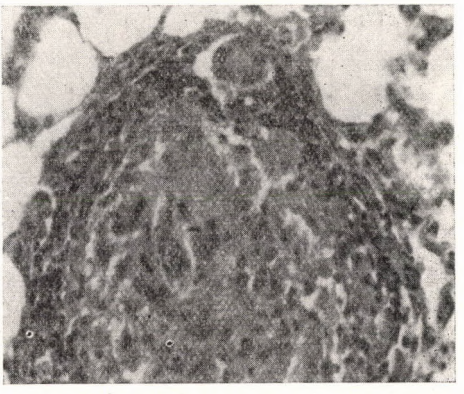

Fig. 6. Larvae surrounded by necrotic debris, macrophages, giant cells, fibroblasts and eosinophils in a rat lung 30 days after infection. ×330

hyperplastic epithelial lining with increased fibrous tissue in the wall 10 days after infection.

Lungs

In general, a picture of interstitial pneumonia was present in all experimental animals.

Mouse: On the 10th day lungs were congested and showed haemorrhagic patches together with proliferated and desquamated bronchial epithelium. Larvae surrounded by a layer of fibroblasts and eosinophils were present in the interstitial tissue on days 10 and 30. Bronchioles and blood vessels were surrounded with eosinophils by the 10th day and with lymphocytes and eosinophils by the 30th day of the infection (Fig. 5).

Rat: Larvae were present in all pulmonary tissues examined. On days 10, 20 and 30 of the infection they were surrounded by necrotic debris, macrophages, giant cells, fibroblasts and some eosinophils (Fig. 6). Hyalinized larvae covered by a thick fibrous tissue capsule surrounded by macrophages, giant cells of foreign-body type and eosinophils were seen on days 30 and 40. Eosinophils appeared around bronchioles and more extensively around blood vessels in all cases (Fig. 7). Hyperplastic peribronchial lymph follicles with lymphocytic aggregation were established in the lamina propria of bronchioles on the 30th day of the infection.

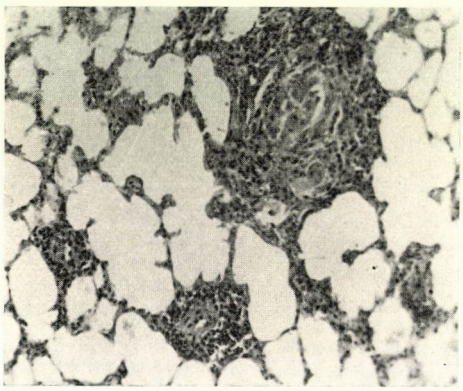

Fig. 7. Arterioles showing perivascular eosinophilic infiltration and thickened wall in the lung of a rat 30 days after infection. ×150

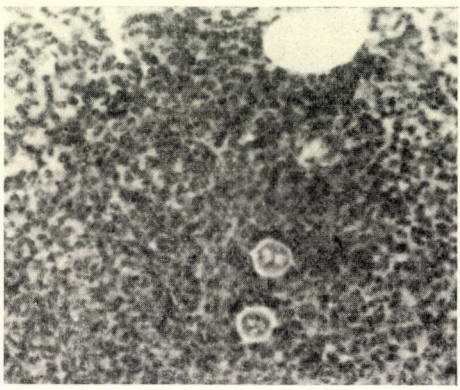

Fig. 8. Thickened pleura covering a parasitic nodule in the lung of a guinea-pig 20 days after infection. ×125

Guinea-pig: Larvae were observed in the interstitial pulmonary tissue on days 10, 20, 30 and 40 after infection. On the 20th day some were surrounded by granulation tissue infiltrated with eosinophils, sometimes followed by a zone of macrophages and giant cells of the foreign-body type. At the same time the endothelial lining of the blood vessels was swollen, bronchial epithelium hyperplastic and desquamated. Portions of the pleura covering the parasitic nodules were disrupted or thickened by eosinophilic infiltration and fibroblastic proliferation (Fig. 8). Larvae in the lungs examined on the 30th and 40th days were surrounded by necrotic debris, eosinophils and fibroblasts (Fig. 9). Peribronchial and perivascular eosinophilic infiltration and fibroblastic proliferation were also visible. The wall of some arterioles in the pulmonary tissue was thick and showed obliterated lumen on day 40 (Fig. 10).

Chicken: Lungs contained larvae surrounded by lymphocytes, eosinophils and a few fibroblasts on the 10th day of the infection (Fig. 11), but by the 20th day only granulation tissue infiltrated with eosinophils and surrounded by lymphocytes was seen. Some of the latter nodules had a necrotic centre surrounded by macrophages and giant cells. A few subpleural nodules examined on

the 30th day consisted of fibroblasts, lymphocytes and blood capillaries. Numerous nodular lymphocytic infiltrations were observed in the pulmonary tissue and bronchial lamina propria on days 20 and 30 (Fig. 12). There was focal eosinophilic infiltration under the pleura or in the interstitial tissue on the 30th day.

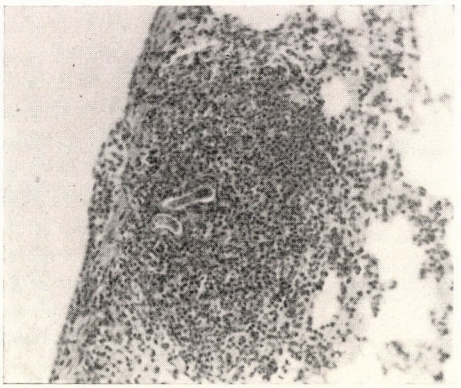

Fig. 9. Larvae surrounded by necrotic debris, eosinophils and fibroblasts in the lung of a guinea-pig 40 days after infection. ×330

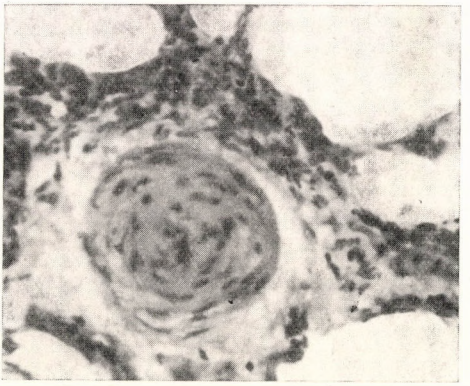

Fig. 10. Arteriole with a thickened wall and obliterated lumen in the lung of a guinea-pig 40 days after infection. ×330

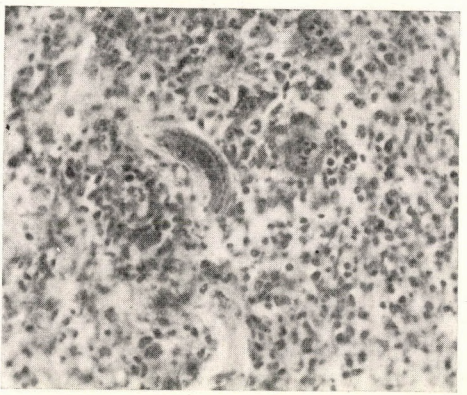

Fig. 11. Larva surrounded by lymphocytes, eosinophils and a few fibroblasts in a chicken lung 10 days after infection. ×220

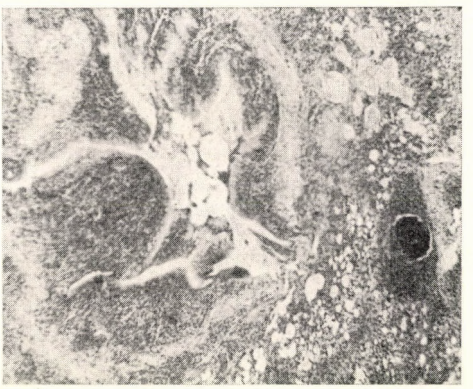

Fig. 12. Nodular lymphocytic infiltration in the pulmonary tissue and bronchial lamina propria of a chicken 20 days after infection. ×75

Kidneys

Mouse: Kidneys examined on the 30th day of the infection contained larvae among the renal tubules (Fig. 13), without any reaction in the nearby tissue. The cortex showed numerous aggregations of macrophages and fibroblasts (Fig. 14). The renal epithelium in the affected areas was dissociated or

formed syncytial giant cells. Renal tubules inside and adjacent to the affected areas were reduced in diameter and had a thick basement membrane. Some of the tubule cells displayed a minimum cytoplasm and large vesicular nuclei which almost obliterated the lumen. Bowman's capsules beside the inflammatory nodules were thickened and the endothelial cell lining of blood vessels was swollen.

Rat: The kidneys examined 10 and 30 days after infection presented larvae surrounded by fibrous tissue capsules. On the 30th day granulomatous

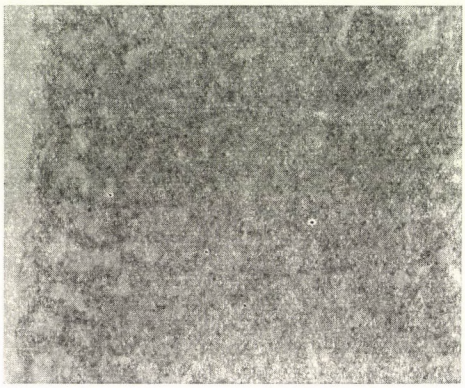

Fig. 13. Larvae among renal tubules without inflammation of the surrounding tissue, in the kidney from a mouse 30 days after the infection. ×100

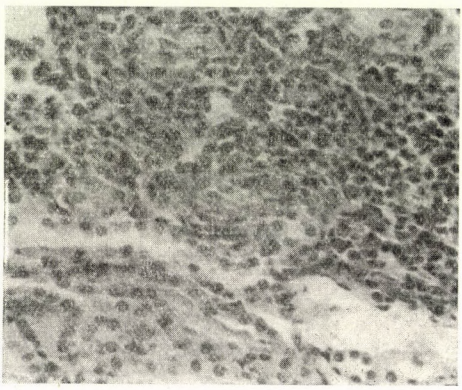

Fig. 14. Aggregation of macrophages and fibroblasts in the renal cortex of a mouse 30 days after infection. ×330

tissue and eosinophils were prominent around the larvae (Fig. 15). Renal tubules and Bowman's capsules in the affected areas revealed changes similar to those described for mice. The endothelial cell lining of the blood vessels was swollen.

Guinea-pig: Kidneys examined after 10 and 20 days were congested and revealed larvae surrounded by fibroblasts. Some larvae were found inside the medullary capillaries on the 20th day (Fig. 16). Dissociated cortical renal epithelium forming syncytial giant cells was seen on the 20th, 30th and 40th days (Fig. 17). Cloudy swelling and hydropic degeneration were present after 20, 30 and 40 days of the infection, together with focal interstitial eosinophilic and lymphocytic infiltrations. Bowman's capsules close to affected areas were thickened.

Brain

Mouse: Larvae inside small vacuoles but no tissue reaction were observed on days 10, 20 and 30 of the infection. Areas of satellitosis, neuronophagia and glial proliferation were apparent in all specimens, especially in those examined

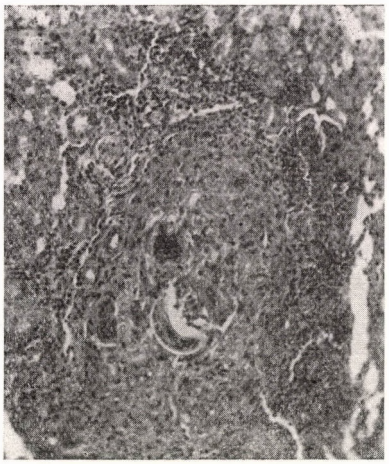

Fig. 15. Larvae surrounded by necrotic debris, fibroblasts, macrophages, giant cells and eosinophils in the kidney of a rat 30 days after infection. ×150

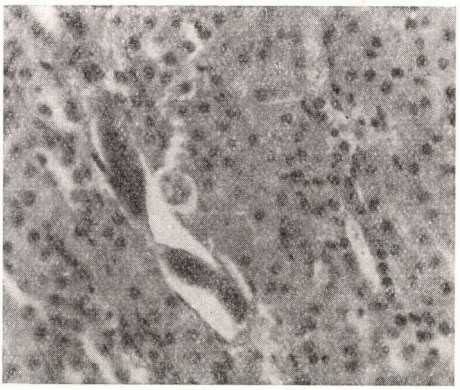

Fig. 16. Larva inside a medullary capillary of kidney from a guinea-pig 20 days after infection. ×330

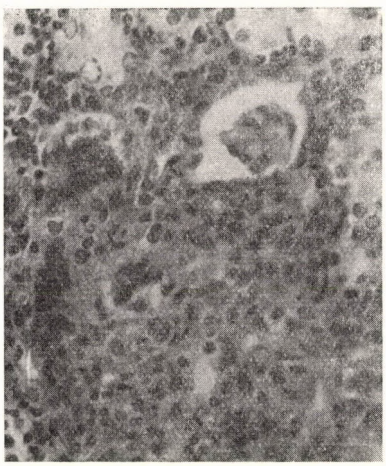

Fig. 17. Syncytial giant cells from dissociated renal epithelium of a guinea-pig 20 days after infection. ×330

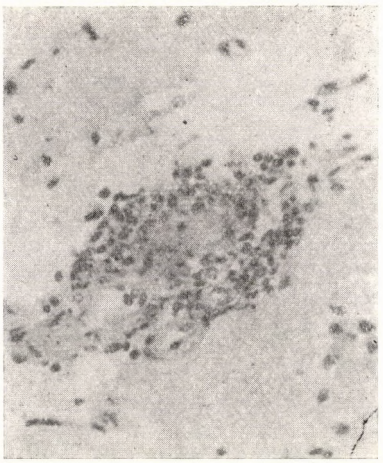

Fig. 18. Perivascular lymphocytic infiltration in the pia mater of a mouse brain 30 days after infection. ×330

after 20 and 30 days of the infection. In the pia mater focal haemorrhages were seen on the 10th day and perivascular lymphocytic infiltration and fibroblastic proliferation on the 30th day (Fig. 18).

Rat: Larvae inside small vacuoles without inflammation were found in the brain after 10 and 40 days. Haemorrhagic foci were present on the 10th

day, malacia, satellitosis, neuronophagia and focal gliosis (Fig. 19) on the 40th day.

Guinea-pig: Larvae were present in the brain tissue without any sign of inflammation (Fig. 20). Focal congestion and malacia were observed on the 40th day of the infection.

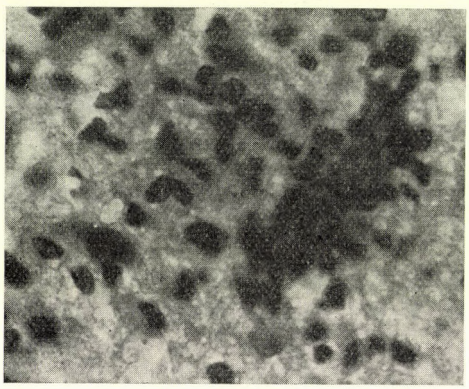

Fig. 19. Glial nodule, satellitosis and neuronophagia in the brain of a rat 40 days after infection. ×1380

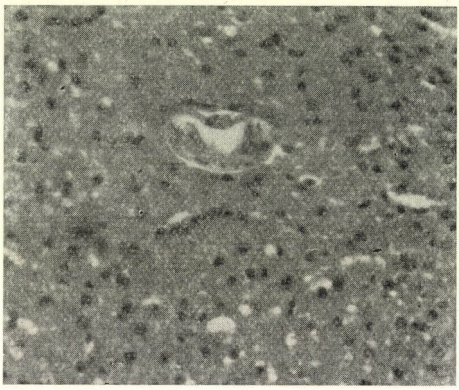

Fig. 20. Larva inside vacuole without inflammation of the surrounding tissue, in the brain from a guinea-pig 40 days after infection. ×330

Discussion

It seems clear from the above study that larvae of *Toxocara canis* migrated through various organs of all the experimentally infected animals. This is in agreement with the statement made by BISSERU (1969) that except man and the dog all animals are paratenic hosts for *T. canis*.

The livers, lungs and kidneys of all animals showed inflammatory reactions with and without the presence of larvae and frequently surrounded by fibrous capsule. This is in accordance with WEBSTER's report (1958) of *T. canis* larvae invading lymph vessels, lymph nodes and venous capillaries up to the portal circulation in the course of migration to the liver, heart, lungs and systemic circulation.

The reaction in the liver of mice 20—30 days after infection consisted of foci of eosinophils and fibroblasts around the larvae. HOEPPLI (1949) reported the occurrence of foci of eosinophils, neutrophils and giant cells around larvae surrounded by a fibrous tissue capsule, while BISSERU (1969) saw infiltrations with eosinophils, lymphocytes and neutrophils without the larvae. In the liver of guinea-pigs, CHAUDHURI and SAHA (1959) observed numerous granulomatous areas, often with remnants of larvae in the centre and profuse peripheral eosinophilic infiltration. Their results are in agreement with the picture noted

in this study on days 30 and 40 after infection. GALVIN (1964) investigated the lesions produced by *T. canis* in the liver of chickens and found small foci of lymphocytic infiltration with necrotic centres surrounded by a few giant cells of the foreign-body type and an outer fibrous tissue capsule with a partial or complete boundary of lymphoid cells, a few eosinophils and neutrophils. In the birds examined in the present study, however, the larvae were surrounded by necrotic debris, eosinophils and lymphocytes without giant cells, while the nodular interstitial lymphocytic infiltration did not surround the parasitic nodules but was haphazardly distributed.

The picture of interstitial pneumonia found in the lungs of all examined animals is the result of injury caused by the migrating larvae (JUBB and KENNEDY, 1963). BISSERU (1969) found free larvae in haemorrhagic areas without inflammatory cells and separate inflammatory foci of lymphocytes with occasional neutrophils and a few eosinophils in the lungs of mice infected with *T. canis*. In the present study the larvae were surrounded by fibroblasts and eosinophils. Eosinophils with lymphocytes were only found around bronchioles and blood vessels. The lungs showed haemorrhagic areas which is in agreement with BISSERU's description (1969). In the lungs of guinea-pigs infected with *T. canis* CHAUDHURI and SAHA (1959) found diffuse interstitial eosinophilic infiltration. This infiltration was conspicuous around the bronchioles and eosinophilic exudate filled the bronchioles and alveoli. Similar observations were made in the present study, but in addition larvae surrounded by necrotic debris, eosinophils and fibroblasts, or macrophages and giant cells, or even by a thick fibrous tissue capsule, were noted. The cellular reaction of pulmonary tissue to migrating larvae of *T. canis* was essentially granulomatous in the rat and guinea-pig, with some fibroblasts and eosinophils. The latter were especially prominent in the guinea-pig lungs. In chickens, interstitial foci of lymphocytic infiltration and the persistence of lymphocytes in the parasitic nodules were the outstanding features.

In his study of the lesions in the kidneys of dogs infected with *T. canis* BOTTI (1957) noted subcapsular nodules showing a tendency to necrosis and composed of fibroblasts, macrophages and plasma cells. The available literature did not refer to similar lesions in the kidney of laboratory animals. In the present material, parasitic nodules occurred mostly in the renal cortex of the experimental animals. The nodules consisted of larvae, eosinophils, macrophages, giant cells and fibroblasts with degenerated adjacent renal tubules. Some larvae were surrounded by a fibrous capsule or found inside the capillaries. Generally, the kidneys are the most common location for the small granulomas caused by *T. canis* larvae (JUBB and KENNEDY, 1963).

BISSERU (1969) found numerous larvae in the brain of mice infected with *T. canis*. This invasion was accompanied by little or no cellular reaction in the parenchyma except for some migration tracts infiltrated with lymphocytes.

Mice, rats and guinea-pigs used in the present work exhibited very similar lesions, but in addition there were areas showing satellitosis, neuronophagia and glial proliferation with focal haemorrhage. These lesions were probably caused by constant movement of the parasites. The failure of a fibrous capsule to be formed around larvae in the brain is due mainly to the lack of fibrous tissue in the central nervous system. JUBB and KENNEDY (1963), in contrast, detected no lesions in the central nervous system. This point needs further investigations, however, in view of the brain lesions described in the present paper.

SUMMARY

Mice, rats, guinea-pigs and chickens were experimentally infected with *T. canis*, and the histopathological lesions in the liver, lungs, kidneys and brain examined 10, 20, 30 and 40 days after the infection were described. In general, larvae with or without cellular reaction were demonstrated in most organs of all animals. The cellular reaction consisted of fibroblasts and some eosinophils in the liver, and was mainly granulomatous with some fibroblasts and eosinophils in the lungs of rats and guinea-pigs. Nodular lymphocytic infiltration was the characteristic lesion in the liver and lungs of chickens. Larvae in the brain generally failed to produce explicit reaction, although some areas of satellitosis, neuronophagia and glial proliferation with focal haemorrhage could be detected.

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β-LACTOGLOBULIN POLYMORPHISM IN THE MILK OF HUNGARIAN SPOTTED COWS

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Polymorphism of β-lactoglobulin in cows' milk was first reported by ASCHAFFENBURG and DREWY (1955), who by starch gel electrophoresis found two variants: a faster one, A, and a slow one, B. This variation was explained on the basis of family investigations as being under the control of a single locus with two co-dominant alleles. In Australian cows BELL (1962) found a third variant, C, which migrated considerably slower than the former two. LARSEN and THYMAN (1966) also reported in Danish Jersey breed a new β-lactoglobulin allele, which they termed D; this migrated yet slower than C. They found —CC homozygous and —BD heterozygous phenotypes, but no —CD and —DD animals. The gene frequency values of the different alleles in the Danish Jersey breed were as follows: $Lg^A = 0.39$; $Lg^B = 0.55$; $Lg^C = 0.04$ and $Lg^D = 0.02$. Thus at present four β-lactoglobulin variants are known in cattle, controlled by four co-dominant alleles. The purpose of this paper is to report findings on the β-lactoglobulin polymorphism of the Hungarian Spotted breed and to compare the data with those for other breeds examined so far and presented in the literature.

Material and method

Milk samples were collected from cows of six herds and stored at +5 °C until use. The starch gel electrophoretic procedure described by MEYER (1966) was employed, with slight modifications. The gel slides were inserted with small pieces of filter paper submerged in skimmed milk. The electrophoretic runs were performed at 120 V voltage, with a running time of about 3.5—4 hours.

Results and discussion

All β-lactoglobulin phenotypes found to occur in the sampled cattle are presented in Fig. 1. The single cow in which the —BC phenotype was found was registered in the herd-book, but none of its ancestors or offspring could be traced, so that it was not possible to carry out an inheritance study. It would seem that the reason why no further combinations of the very rare Lg^C allele were detected must have been the relatively small size of the sampled population. From the distribution of the other β-lactoglobulin phenotypes, given in Table I, it is clear that, except for the nearly constant proportion of —BB, these showed considerable variation in their incidence in different herds. The —AA and —AB phenotypes therefore fluctuate in opposite directions, —AB being the most common, —AA less common than both this and —BB.

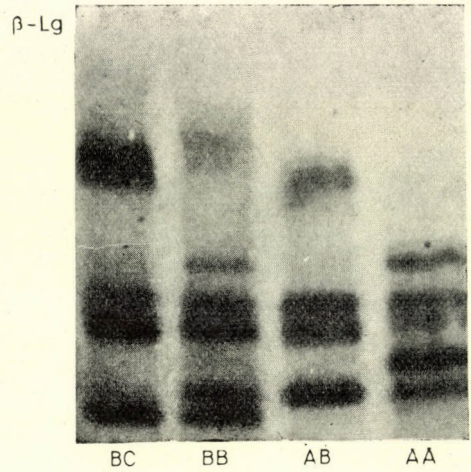

Fig. 1. β-lactoglobulin phenotypes in Hungarian Spotted cattle

Table I

The distribution of β-lactoglobulin phenotypes and the

Herd No.	No.		β-lactoglobulin —AA	%	—BB	%	—CC	%
		obs:	11	9	35	27	—	—
I	123	exp:	19.94		43.91		—	—
			−8.94		−8.91		—	—
		obs:	16	20	22	26	—	—
II	81	exp:	17.35		23.36		—	—
			−1.35		−1.36		—	—
		obs:	32	18.4	58	34.5	—	—
III	171	exp:	30.30		56.15		—	—
			+1.70		+1.85		—	—
		obs:	34	27	32	26	—	—
IV	124	exp:	31.99		30.00		—	—
			+2.01		+2.00		—	—
		obs:	13	26	10	20	—	—
V	50	exp:	14.04		11.04		—	—
			−1.04		−1.04		—	—
		obs:	4	8	14	29	—	—
VI	48	exp:	7.51		17.52		—	—
			−3.51		−3.52		—	—
		obs:	110	18	172	28	—	—
Total:	497	exp:	119.81		181.36		—	—
			−8.81		−10.36		—	—

A comparison of the gene frequency values of the different β-lactoglobulin alleles and the distribution of their phenotypes in Hungarian Spotted and other breeds of cattle is presented in Table II. Marked variations in the phenotype distributions are apparent. While breeds placed at the head of the table show very low frequencies of —AA coupled with high frequencies of —BB, this disproportion equalizes as we pass down the table, and at the same time the heterozygous —AB phenotype becomes predominant. In the case of the Hungarian Spotted breed, —BB is the more frequent of the two homozygous phenotypes, but —AB shows the highest frequency. Similar substantial differences can be seen in the observed gene frequencies for other breeds. It may be concluded from this that the ratios of the different phenotypes vary between breeds, the distribution of phenotypes as well as the gene frequency values being characteristic of each individual breed.

gene frequency values in Hungarian Spotted cattle

phenotypes						Gene frequencies			χ^2	P
—AB		—AC		—BC		Lg^A	Lg^B	Lg^C		
	%		%		%					
77	63	—	—	—	—					
59.13		—	—	—	—	0.4025	0.5075	—	11.1	0.001
+17.87		—	—	—	—					
43	53	—	—	—	—					
40.27		—	—	—	—	0.4629	0.5371	—	0.3	0.50
+2.73		—	—	—	—					
80	46.5	—	—	1	0.5					
82.49		—	—	1.97	—	0.4210	0.5731	0.0059	0.67	0.30
−2.49		—	—	−0.97	—					
58	47	—	—	—	—					
61.97		—	—	—	—	0.5080	0.4920	—	1.03	0.30
−6.97		—	—	—	—					
27	54	—	—	—	—					
24.91		—	—	—	—	0.5300	0.4700	—	0.33	0.50
+2.09		—	—	—	—					
30	63	—	—	—	—					
28.71		—	—	—	—	0.3958	0.6042	—	2.39	0.10
+1.29		—	—	—	—					
315	52	—	—	1	0.4					
294.79		—	—	0.47	—	0.4480	0.5512	0.0008	4.02	0.02
+20.21		—	—	+0.53	—					

Table II

Comparison of β-lactoglobulin polymorphisms of various cattle breeds

Breeds	No. of animals	Distribution of phenotypes						Gene frequencies		References
		—AA		—BB		—AB		Lg^A	Lg^B	
			%		%		%			
Zebu	137	2	1.4	113	82.6	22	16	0.09	0.91	BHATTACHARYA et al., 1963
Red Danish	282	2	1	230	82	50	17	0.10	0.90	LARSEN et al., 1966
Ayrshire	178	7	5	108	60	63	35	0.22	0.78	MÁCHA et al.,1968
Swiss Brown	24	2	10	13	54	9	36	0.27	0.73	KIDDY et al.,1965
Pinzgau	148	22	15	76	31	50	34	0.32	0.68	MÁCHA et al., 1968
Holstein—Friesian	963	173	18	300	31	490	51	0.43	0.57	COMBERG et al., 1964
Hungarian Spotted	598	110	19	173	29	315	52	0.44	0.56	HORVÁTH, present paper
Slovakian Spotted	170	39	23	47	28	84	49	0.48	0.52	MÁCHA et al., 1968
Black and White Danish	118	30	25	29	25	59	50	0.50	0.50	LARSEN et al., 1966
Danish Jersey	142	37	25	31	25	74	50	0.42	0.58	LARSEN et al., 1966
Bohemian Spotted	611	169	28	164	27	278	45	0.50	0.50	MÁCHA et al., 1968

SUMMARY

The distribution of different β-lactoglobulin phenotypes and the frequencies of alleles were examined in milk samples collected from 597 individuals in six herds of Hungarian Spotted cattle. Four phenotypes were found: —BC, —AA, —BB and —AB, in order of increasing frequency. The —BC phenotype was detected in the milk of only one cow, so that while it is clear that the Lg^C allele occurs in this breed, the population examined was probably too small for other combinations to be found. A comparison is made of these results with data on this polymorphism reported in the literature for other breeds.

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ZUR BEWEGUNG DER SPOROZOITEN VON EIMERIA STIEDAI (PROTOZOA, SPOROZOA)*

Von

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(Eingegangen am 16. November 1971)

Ausführliche Bewegungsstudien an den im allgemeinen nur wenig Motilität zeigenden Sporozoiten der Kokzidien sind trotz zahlreicher, in Tabelle I zusammengestellter Einzelbefunde selten.

DORAN, JAHN und RINALDI (1962) führten an *E. acervulina*-Sporozoiten Filmaufnahmen durch und folgerten, daß als Bewegungsursache schraubenförmig über den Sporozoitenkörper hinweggleitende wellenförmige Kontraktionen (»travelling helical waves«) in Frage kämen. Diese Hypothese, die nicht ganz mit der ihr zugrundeliegenden mathematischen Theorie übereinstimmte, konnte durch eine spätere Änderung der in Frage kommenden Gleichungen derart bestätigt werden, »daß die hydrodynamischen Theorien es diesen Organismen jetzt gestatten, so zu schwimmen, wie sie es tun« (JAHN und BOVEE, 1970). Die bisher ausführlichsten Berichte über Sporozoitenbewegungen bei meist bovinen Kokzidien wurden im Rahmen zytologischer Studien von HAMMOND, CHOBOTAR und ERNST (1968) sowie ROBERTS und HAMMOND (1970) gegeben.

Im allgemeinen werden sowohl bei Sporozoiten als auch bei Merozoiten vier Bewegungstypen unterschieden, von denen drei in der Regel zu keiner Ortsveränderung des Organismus führen (s. auch Tab. I). Die Fortbewegung (»gliding«) wird als in unregelmäßigen Zeitabständen stattfindendes ruck- oder stoßartig beginnendes und langsam auslaufendes Gleiten beschrieben, dessen Ursache die bereits erwähnten »travelling helical waves« sein sollen. Als Bewegungen am Ort gelten:

1. »probing«, Seitwärtsbewegungen und/oder periodische Kontraktionen der vordersten Spitze des Sporozoiten bei sonst nicht bewegtem Körper (Tastbewegungen);

2. »flexing«, Beugebewegungen, bei denen das Vorderende, meist das vordere Drittel des Sporozoiten langsam seitlich bis zu 90° und mehr abgebeugt wird und schneller in die Normallage zurückkehrt;

3. »pivoting«, Pendelbewegungen des ganzen Sporozoiten um einen Fixpunkt an seinem Hinterende.

Ziel der vorliegenden Arbeit war es, an Hand umfangreicher Filmaufnahmen und Direktbeobachtungen von *Eimeria stiedai*-Sporozoiten eine ausführliche Analyse der Bewegungstypen dieser Art zu geben.

* Mit der Unterstützung der Deutschen Forschungsgemeinschaft.

Tabelle I

Zusammenstellung der in der Literatur angegebenen Bewegungstypen von Sporozoiten

Kokzidienart	Bewegungstyp	Autor
Cocc. cuniculi	Krümmungen und Streckungen, ruckweise Vorwärtsbewegung, Tastbewegungen des Vorderendes	METZNER (1903)
E. cuniculi	Krümmungen und Streckungen, Vorwärtsbewegung	v. WASIELEWSKI (1904)
E. stiedai und *E. falciformis*	Krümmen und Sichstrecken, ruckweise Fortbewegung, Drehen um die Längsachse und Vorwärtsbewegung	REICH (1913)
E. necatrix	bending (=flexing), gliding	THYZZER, THEILER und JONES (1932)
E. stiedae	contracting and twisting the body	SMETANA (1933)
E. bovis	moving in a spiral path; flexing	HAMMOND, BOWMAN, DAVIS und SIMMS (1946)
E. adenoides	probing, pivoting, gliding, curling and uncurling (=flexing?)	CLARKSON (1958)
E. meleagrimitis	probing, pivoting, gliding, curling and uncurling (=flexing?)	CLARKSON (1959)
E. stiedae	undulating movement, gliding, bending	ROSE (1959)
E. acervulina	movement is helical and resembles a corkscrew boring its own path	DORAN, JAHN und RINALDI (1962)
E. bovis, ellipsoidalis, auburnensis	flexing, gliding	NYBERG und HAMMOND (1964)
E. debliecki	probing, flexing, gliding	VETTERLING (1966)
E. nieschulzi	forward movement	MARQUARDT (1966)
E. labbeana	flexing, elongating and contracting one end	SRIVASTAVA (1967)
E. bovis	probing, flexing, pivoting, gliding	FAYER und HAMMOND (1967)
E. bilamellata	flexing, gliding	TODD, HAMMOND und ANDERSON (1968)
E. bovis *E. auburnensis*	flexing, gliding, probing (?) probing, flexing, pivoting, gliding, rotating	HAMMOND, CHOBOTAR und ERNST (1968)
E. spec., ninakohlyakimovae	probing, flexing, gliding, pivoting	
E. bovis, ellipsoidalis, auburnensis	probing, flexing, gliding	ROBERTS und HAMMOND (1970)

Material und Methodik

E. stiedai-Sporozoiten aus bis zu 8 Wochen alten Oocysten wurden nach der von KRIEG (1971) beschriebenen Methode gewonnen. Die Direktbeobachtung erfolgte auf einem Wärmetisch bei etwa 39 °C im hängenden Tropfen und

im Deckglaspräparat bei Hellfeldbeleuchtung. Die Filmaufnahmen wurden am Deckglaspräparat im Mikroskop-Heizschrank nach HEUNERT (1962) bei etwa 36 °C durchgeführt. Die Aufnahmen erfolgten mit einer Askania-Z- Kamera unter Verwendung eines Zeiss-WL-Mikroskops auf 35 mm Normalfilm. Es wurden Phasenkontrast- und Differential-Interferenzkontrastbeleuchtung nach Normarski verwendet (Objektive: Neofluar und Planachromat 100 und 40×; Fotookulare 4 und 3,6×). Die Aufnahmegeschwindigkeit betrug 24 und 60 Bilder pro Sekunde. Die Auswertung der Filmaufnahmen erfolgte bei bis zu 3000 facher Vergrößerung.

Ergebnisse

Die freien Sporozoiten waren ca. 17 μ (16,6$\pm$1,5 μ; $n = 11$) lange und etwa 5 μ (4,5$\pm$0,7 μ; $n = 11$) dicke, bananenähnlich erscheinende und auch so gebogene Gebilde mit einem sehr formvariablen Vorderende und einem konstant leicht abgerundeten hinteren Ende.

Bei Interferenzkontrastbeleuchtung schien das vorderste Ende des Sporozoiten aus einem mehr oder weniger homogenen Zytoplasma zu bestehen. Es folgte eine Zone, die stärkere Granulationen enthielt und in deren Mitte sich eine nahezu kreisförmige Vertiefung befand, deren Durchmesser etwa der halben Dicke des Sporozoiten entsprach. Nahezu die ganze hintere Hälfte wurde von dem hyalinartig erscheinenden refraktilen (Paranuclear-, Hyalin-) Körper eingenommen, auf den bei einigen Sporozoiten noch einmal eine halbkreisförmig erscheinende homogen aussehende Zytoplasmamasse mit einem Durchmesser von 2—3 μ folgte (Abb. 1a).

In Phasenkontrastbeleuchtung erschien das Vorderende stets dunkel. Einzelheiten waren in der Regel nicht zu erkennen. Nur in seltenen Fällen zogen streifenförmige, meist jedoch nicht deutlich geformte Gebilde nach hinten. Der folgende Zellteil zeigte eine oder zwei größere, aufeinanderfolgende, oft nicht ganz scharf begrenzte Aufhellungen und mehrere wahllos verteilte, etwa 1/4 bis maximal 1 μ große, sehr dunkle Punkte, die auch gelegentlich in dem auf den refraktilen Körper folgenden Zytoplasma vorhanden waren. Der refraktile Körper stellte sich als einheitlich grau getönte, scharf begrenzte Masse dar (Abb. 1b).

Auf der Körperoberfläche der Sporozoiten wurden bei Hellfeldbeleuchtung vereinzelt dunklere Granula gesehen, die ihre Lage verändern konnten oder sich ab und zu ganz von dem Sporozoiten lösten.

Alle im folgenden geschilderten Bewegungen erfolgten spontan und unvorhersehbar, ohne daß ein bestimmtes Bewegungsmuster eingehalten worden wäre. Neben Sporozoiten, die längere Zeit unbeweglich lagen und dazwischen nur vereinzelt Beugebewegungen ausführten, lagen Sporozoiten, die mit kurzen Pausen fast ununterbrochen in Bewegung waren. Sporozoiten, die aus

frisch sporulierten Oocysten stammten, zeigten oft eine höhere Bewegungsintensität als Sporozoiten aus älteren Oocystenkulturen bzw. aus Sporozysten, die nach dem Zermörsern längere Zeit im Kühlschrank gelegen hatten. Das gleiche war der Fall, wenn die freien Sporozoiten einige Zeit gelagert worden waren.

Da die für die einzelnen Bewegungsabläufe gemessenen Wege und Zeitspannen sehr unterschiedlich waren, ist eine Angabe von Mittelwerten nur in wenigen Fällen oder aber für Teilstrecken möglich. Erschwerend auf die

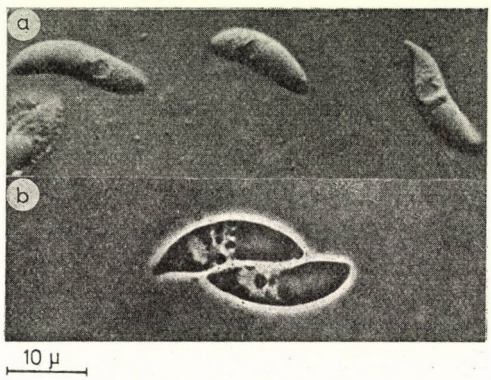

Abb. 1. *a*, Sporozoiten in Differential-Interferenzkontrastbeleuchtung nach Normarski; b, Sporozoiten in Phasenkontrastbeleuchtung

Beobachtungen und Auswertung der Filmaufnahmen wirkte sich die Tatsache aus, daß alle Bewegungsabläufe fast stets dreidimensional verfolgt werden mußten.

Die am häufigsten auftretenden und am meisten ins Auge fallenden Bewegungstypen waren Gleit- und Beugebewegungen.

1. Gleitbewegungen (Abb. 2 und 3)

Ortsveränderungen der Parasiten wurden durch bei direkter Beobachtung scheinbar schnell beginnende und sich allmählich verlangsamende gleitende, stoßartige Bewegungen vollzogen, bei denen unterschiedliche Wegstrecken zurückgelegt wurden. Die einzelnen Bewegungsstöße, die in unregelmäßigen Zeitabständen erfolgten, waren durch mehr oder weniger lange Liegepausen, in denen meistens Beugebewegungen durchgeführt wurden, unterbrochen.

Im Verlauf einer Gleitbewegung änderte sich die Form des Sporozoitenvorderendes, das sowohl zugespitzt als auch abgestumpft sein konnte, nur selten; ein einziges Mal konnte während eines ruhigen Gleitens ein gleichzeitiges »probing« beobachtet werden.

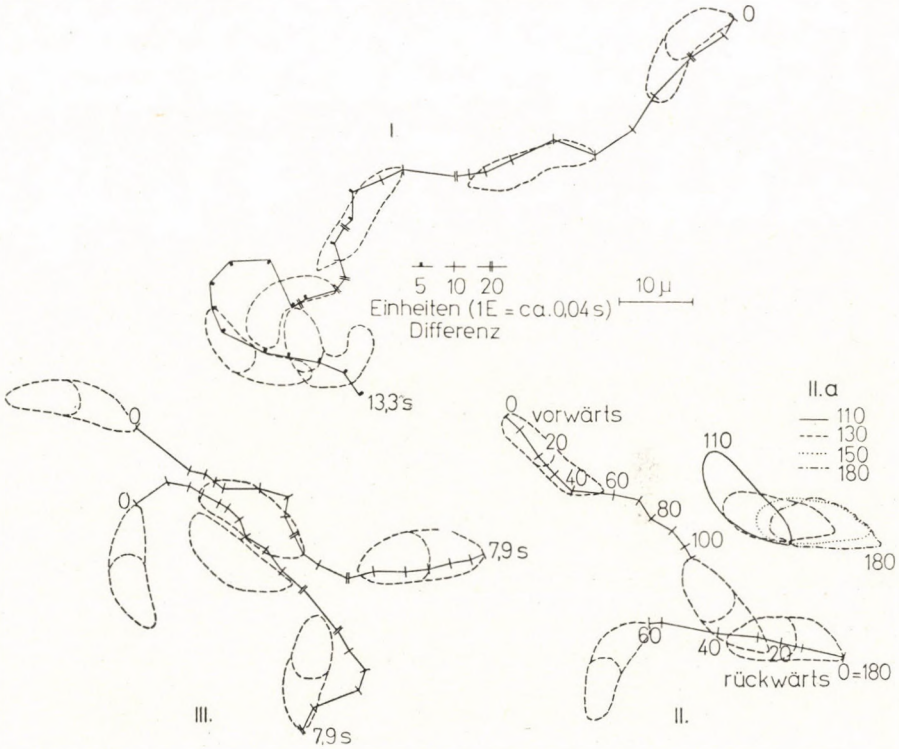

Abb. 2. Form und Länge der Wegstrecken sowie Teilgeschwindigkeiten vor- und rückwärtsgleitender Sporozoiten

Bei der Analyse der Filmaufnahmen erwies sich der visuelle Eindruck einer schnell beginnenden und langsam auslaufenden Bewegung als Täuschung. Wie aus Tab. II ersichtlich, begannen die Sporozoiten entweder schnell oder langsam sich zu bewegen, steigerten u. U. ihre Geschwindigkeit oder verlangsamten sie streckenweise und hörten, ohne auf ein Hindernis zu stoßen, abrupt oder allmählich mit dem Gleiten auf. Die höchste gemessene Teilgeschwindigkeit betrug auf einer Weglänge von 1,4 μ 86 μ/Sekunde. Als durchschnittliche, »normale« Geschwindigkeit, die auch über längere Wegstrecken erzielt wurde, können 9—15 μ/Sekunde angenommen werden. Eine untere Geschwindigkeitsgrenze schien nicht zu existieren. Neben einem kontinuierlichen Gleiten über kürzere oder längere Strecken kam es auch vor, daß größere Strecken in einzelnen Bewegungsstößen zurückgelegt wurden, zwischen denen minimale Pausen von $1-5 \times 0{,}042$ Sekunden (=1—5 Einzelbilder) eingelegt wurden.

Tabelle II

Beispiele für Wegstrecken und Teilgeschwindigkeiten einzelner Sporozoiten

Prot. Nr. 9a				9b				10a				15a			
Bild Nr. von—bis	Zeit in Sek. Σt	Weg in μ Δs	Teil-geschw. μ/Sek. Δv	Bild Nr. von—bis	Zeit in Sek. Σt	Weg in μ Δs	Teil-geschw. μ/Sek. Δv	Bild Nr. von—bis	Zeit in Sek. Σt	Weg in Δs	Teil-geschw. μ/Sek. Δv	Bild Nr. von—bis	Zeit in Sek. Σt	Weg in μ Δs	Teil-geschw. μ/Sek. Δv
vorwärts				vorwärts				vorwärts				vorwärts			
1— 10	0,4	2,3	5,6	610—620	0,4	8,4	20,1	290—295	0,2	1,8	9,0	0— 10	0,4	2,5	6,1
20	0,8	4,0	10,0	30	0,8	1,9	4,7	300	0,4	3,3	16,6	30	1,3	4,7	5,7
30	1,3	3,0	6,6	40	1,3	1,7	4,0	5	0,6	1,8	9,0	40	1,7	6,5	15,5
40	1,7	3,3	8,0	50	1,7	1,2	2,9	8	0,75	3,6	24,0	50	2,1	4,7	11,5
50	2,1	2,2	5,3	60	2,1	4,5	11,0	10	0,8	3,4	68,0	60	2,5	6,1	14,4
60	2,5	2,9	7,0	70	2,5	3,5	8,4	12	0,9	3,1	31,0	70	2,9	5,7	13,5
70	2,9	3,3	8,0	80	2,9	2,2	5,3	14	1,0	3,4	34,0	80	3,3	5,7	14,0
80	3,3	2,7	6,5	700	3,8	2,5	3,9	20	1,3	1,3	5,2	90	3,8	3,5	8,6
90	3,8	2,8	6,9	10	4,2	2,6	6,2					100	4,2	2,2	5,3
100	4,2	2,6	6,1	20	4,6	2,5	6,0					20	5,0	1,3	1,6
110	4,6	1,5	3,5	40	5,4	3,8	4,6	rückwärts				25	5,2	6,8	32,4
				50	5,8	3,4	8,2	365—380	0,6	2,5	4,2	35	5,6	2,5	6,2
rückwärts				60	6,3	3,9	9,3	90	1,1	1,9	3,8	40	5,8	4,0	18,3
250—260	0,4	5,3	12,7	70	6,7	2,8	6,7	400	1,5	1,7	4,3	45	6,0	3,4	17,2
70	0,8	2,8	6,8	80	7,1	2,5	6,1	10	1,9	1,3	3,3	64	6,8	1,5	1,8
80	1,3	2,8	6,7	90	7,5	2,7	6,9	30	2,7	3,4	4,3	70	7,1	2,5	10,2
90	1,7	5,0	11,9	800	7,9	1,6	4,0	40	3,1	2,6	6,5	80	7,5	2,0	4,7
300	2,1	6,7	16,4					55	3,8	1,9	2,7	200	8,3	2,4	2,8
10	2,5	1,5	3,6									20	9,2	1,9	2,3
												25	9,4	3,9	17,6
2. Sporozoit: rückwärts				Alle Werte wurden auf 2 Dezimalen berechnet								50	10,4	1,0	1,0
280—290	0,4	2,6	6,2	und auf 1 Dezimale auf- bzw. abgerundet								70	11,3	1,1	1,2
300	0,8	1,1	2,7									75	11,5	6,2	30,8
10	1,3	2,1	4,9									80	11,7	4,8	24,1
20	1,7	3,3	8,0									85	11,9	3,5	17,2
25	1,9	3,8	18,1									90	12,1	3,2	16,1
30	2,1	3,2	15,2									95	12,3	2,9	14,7
40	2,5	6,5	15,5									300	12,5	5,8	29,0
50	2,9	6,7	15,9									5	12,7	3,2	15,9
55	3,1	3,3	16,7									10	12,9	3,8	19,0
60	3,3	3,0	13,5									15	13,1	3,3	16,6
												20	13,3	3,4	17,2

Die ohne eine Pause zurückgelegten Wegstrecken schwankten von 1,4 bis mindestens 62,8 μ (der Sporozoit verschwand hier aus dem Gesichtsfeld), in der Regel waren sie jedoch nicht länger als die Körperlänge der Sporozoiten.

Die Form der Bewegungsbahnen variierte stark. Nahezu gestreckt liegende Sporozoiten glitten meist geradlinig vorwärts, während leicht gebogene Sporozoiten eher entsprechend der Beugung ihrer Körperachse kreis-, und S-förmigen oder elliptischen Bahnen folgten. Durch zusätzliche, sich mehr oder weniger schnell drehende (»schlängelnde«) Bewegungen des Vorderendes

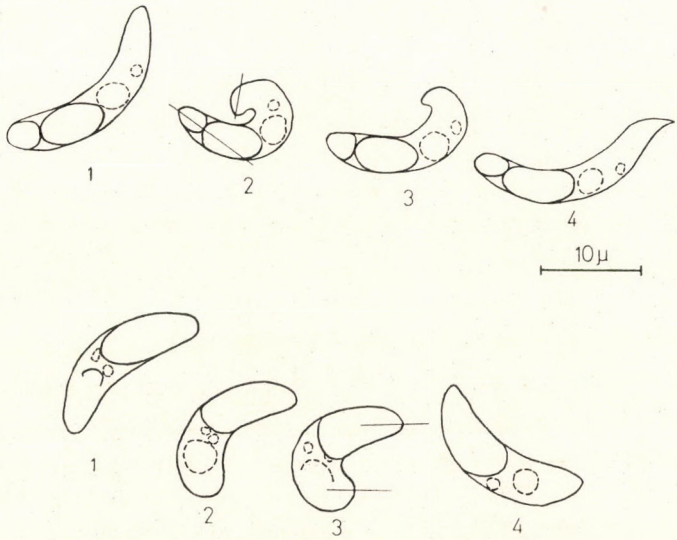

Abb. 3. Vorwärtsgleiten von Sporozoiten kombiniert mit Rotationsbewegungen

konnten diese Bahnen korkenzieherähnliche Formen annehmen. Insbesondere bei letzteren Bewegungslinien, teilweise jedoch auch bei der geradlinigen Fortbewegung, ergab die Filmanalyse eine zusätzliche Drehbewegung des Sporozoiten um seine Längsachse (»Rollen«) (Abb. 3). Es war nicht immer eindeutig zu entscheiden, in welcher Richtung dieses Rollen ausgeführt wurde, es schien jedoch so, als ob keine Richtung bevorzugt würde. Bei ungestörtem, langsamem Gleiten wurde des öfteren im Verlauf einer Wegstrecke, die etwa der Körperlänge des Sporozoiten entsprach, eine derartige Drehbewegung um fast 180° ausgeführt. Vereinzelt war eine Rotation der Sporozoiten um ihre Längsachse, meist unmittelbar nach der Excystation, zu beobachten, ohne daß es gleichzeitig zu einer Ortsveränderung kam.

Gelegentlich wurde die eingeschlagene Richtung während des Gleitens durch schnellere, peitschenschlagähnliche Bewegungen (eine Wendung um ca. 90° in knapp 0,2 Sekunden), die nur von der vorderen Hälfte des Sporozoiten

ausgeführt wurden, spontan geändert. In einigen Fällen führten Sporozoiten derartige »peitschende« Bewegungen ohne Vorwärtsbewegung oder unmittelbar im Anschluß an eine Gleitbewegung durch, wobei sie sich gleichzeitig mit ihrem Hinterende an einer Unterlage festzuheften schienen.

Neben Vorwärtsbewegungen wurden von verschiedenen Sporozoiten auch Rückwärtsbewegungen durchgeführt, die fast stets geradlinig erfolgten. Sie begannen entweder bei frei liegenden Sporozoiten spontan im Anschluß an eine Ruhepause nach einem Vorwärtsgleiten oder entwickelten sich aus einer Vorwärtsbewegung, die durch ein Auftreffen auf ein Hindernis abgebremst wurde. In letzteren Fällen konnte gelegentlich ein mehrfaches Zurückweichen und Vorwärts-»schießen« beobachtet werden, als ob der Sporozoit versuche, durch wiederholtes (bis zu sechsmal beobachtetes) »Anlaufnehmen« das Hindernis zu überwinden. Vereinzelt führte der Sporozoit dabei nach dem Abbremsen und vor dem Rückwärtsgleiten noch einmal einen kurzen aber kräftigen Nachstoß aus. Die Rückwärtsbewegung erfolgte sowohl langsamer als auch schneller als die Vorwärtsbewegung und wurde vereinzelt auch mit Rollbewegungen kombiniert.

2. Beugebewegungen

Im Verlaufe dieser Bewegung traten deutliche Formveränderungen des Vorderendes der Sporozoiten auf. Das im Ruhezustand leicht abgerundete Vorderteil stumpfte weiter ab und begann sich in Richtung der kleinen Kurvatur des Sporozoiten einzurollen. Die Bewegung griff anschließend auf immer weitere Teile der vorderen Hälfte des Parasiten über, bis diese schließlich eine deutliche Abweichung gegenüber der Ruhelage aufwies. Das Abknicken erfolgte in unterschiedlichem Maße und an verschiedenen Stellen des Vorderendes, jedoch fast immer vor der Stelle, an der der refraktile Körper begann. In einem einzigen Falle konnte bei einem Sporozoiten, bei dem der refraktile Körper durch 30-minütiges Zentrifugieren bei mehr als 200 000 *g* nach vorne verlagert war, beobachtet werden, wie bei einer Beugebewegung auch dieser abgeknickt wurde.

Der maximale Beugungswinkel betrug mehr als 180°, doch wurde diese Stellung nur bei wenigen Sporozoiten beobachtet. Auch das Einrollen des Vorderendes erfolgte, wie bereits erwähnt, in unterschiedlichem Maße, so daß neben plumpen, auf den ersten Blick nur stark kontrahiert wirkenden Formen auch Sporozoiten beobachtet wurden, die eine wirklich U-förmige Gestalt mit zwei deutlich ausgebildeten Schenkeln eingenommen hatten (Abb. 4).

Die erreichte Beugestellung wurde in der Regel einen kurzen Moment (1—2 Sekunden) lang einbehalten, wobei, insbesondere bei längerem Verharren in diesem Zustand (maximal 5 Sekunden beobachtet), der Eindruck entstand, als ob sich der Sporozoit vergeblich bemühe, eine noch stärkere Abbeugung zu

erreichen. Das anschließende Strecken erfolgte schneller, allmählich oder deutlich ruckartig beginnend.

Die für eine Beugebewegung benötigten Zeiten variierten stark. Für das Abbeugen wurden 3 bis maximal 17 Sekunden gemessen, während das Entspannen in 1,5 bis etwa 7 Sekunden erfolgte. Die von einem Sporozoiten für Beugen und Strecken benötigten Zeitspannen verhielten sich dabei in der Regel etwa wie 3 : 1 bis 2 : 1.

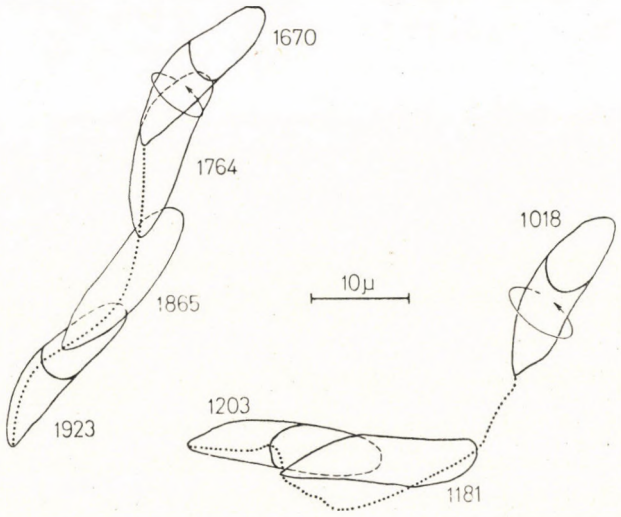

Abb. 4. Beugebewegungen zweier Sporozoiten. Obere Reihe: Geringfügige Lageveränderung während des Abbeugens durch Strömungsbewegungen; untere Reihe: Durch eine Streckbewegung des Hinterendes deutlicher Wechsel der Bewegungsrichtung des vorherigen und nachfolgenden Gleitens

Im Anschluß an eine Beugebewegung folgte entweder eine Ruhepause oder eine Gleit-, Tast- oder erneute Beugebewegung. Unmittelbar aufeinander folgendes Beugen wurde entweder ohne ein vollständiges Strecken aus einer noch leicht abgebeugten Haltung oder aus einem schon fast als Tastbewegung (s. unten) zu deutenden Strecken der vordersten Sporozoitenspitze entwickelt. Einmal konnte ein Sporozoit beobachtet werden, der innerhalb von knapp 4 Minuten 14 Beugebewegungen hintereinander ohne sichtbare Pause durchführte.

Normalerweise wurden die Beugebewegungen von frei schwimmenden oder mit dem Hinterende festsitzenden Sporozoiten durchgeführt, wobei dieses seine Lage kaum veränderte. Ganz selten konnte jedoch auch beobachtet werden, wie ein Sporozoit, mit offensichtlich fixiertem Vorderende sein Hinterende mit dem refraktilen Körper nach vorne um ca. 90° abbeugte. In einem Einzelfall wurde gesehen, wie ein Sporozoit im Anschluß an eine Gleitbewegung

das Vorderende ganz normal beugte, dann jedoch plötzlich nicht dieses, sondern sein Hinterende streckte und in der auf diese Weise um etwa 90° verschobenen Richtung davonglitt (Abb. 4, unterer Sporozoit). Eine längere Gleitbewegung in gebeugter Haltung konnte nur ganz ausnahmsweise und dann nur auf Kreisbahnen festgestellt werden.

3. Tastbewegungen

Bei in der Regel ruhig liegenden Sporozoiten waren diese Bewegungen lediglich auf das Vorderende beschränkt. Die normalerweise leicht abgerundete vorderste Spitze der Sporozoiten verlängerte sich deutlich um etwa 2—3 μ und nahm im Extremfall eine leicht rüsselförmige Gestalt an. Entweder wurde diese Spitze daraufhin wieder zurückgebildet, so daß der Eindruck eines Streckens mit darauffolgender Kontraktion entstand, oder sie führte seitliche, manchmal sogar kreisförmige »tastende« Bewegungen mit einer Amplitude von etwa 3 μ aus. Die typischsten Bilder waren dabei zu sehen, wenn sich diese Spitze entgegen der normalerweise vorhandenen Beugerichtung des Sporozoiten krümmte, so daß dieser eine leicht S-förmige Gestalt annahm (Abb. 1a).

Die Dauer einer solchen Tastbewegung schwankte je nach der Intensität der Suchbewegungen der ausgestreckten Spitze von reichlich 1 Sekunde bis zu knapp 5 Sekunden, wobei jedoch die Abgrenzung zwischen dem Ruhezustand und dem Beginn bzw. Ende der Bewegung nicht immer einwandfrei zu ziehen war.

Tastbewegungen wurden in der Regel nur ein- bis zweimal zwischen anderen Bewegungsarten durchgeführt. Ein dreimaliges hintereinander durchgeführtes Strecken und Kontrahieren der Spitze war bereits eine Seltenheit. Am häufigsten schloß sich das Tasten an Beugebewegungen an bzw. ging kontinuierlich aus diesen hervor und in sie über, so daß auch hier eine Abgrenzung nicht leicht war. Ganz vereinzelt konnten Tastbewegungen während langsamer Gleitbewegungen gesehen werden, etwas mehr wurden sie von ruhig daliegenden oder eventuell mit ihrem Hinterende festgehefteten Sporozoiten ausgeführt. Wiederholt wurde beobachtet, wie zwei nebeneinander liegende Sporozoiten wechselseitig aneinander vorwärts und rückwärts glitten und, wenn sie sich »vorne« befanden, eine Tastbewegung ausführten (Abb. 1b).

4. Pendelbewegungen

Dieser Bewegungstyp, der die meisten Variationsmöglichkeiten aufwies, wurde in seiner ausgeprägtesten Form bei frisch excystierten Sporozoiten nur selten gesehen, war jedoch bei Sporozoiten, die nach der Excystation 24—48 Stunden in physiologischer Kochsalzlösung im Kühlschrank gelagert worden waren, häufiger. Da die Bewegungen in der überwiegenden Mehrzahl der Fälle

senkrecht zur Beobachtungsebene verliefen, lagen infolge der damit verbundenen Tiefenschärfeschwankungen keine auswertbaren Filmaufnahmen vor. Alle berichteten Daten beruhen nur auf Direktbeobachtung.

Unbedingte Voraussetzung für die Pendelbewegungen war stets das Anheften der Sporozoiten mit ihrem Hinterende auf einer Unterlage. In den typischsten Fällen führte der Sporozoit dann mit einem stets gestreckten Vorderende, das dem gestreckten Vorderende bei den Tastbewegungen ähnelte, langsam kreisende Bewegungen aus. Der Durchmesser des von seiner äußersten Spitze beschriebenen Kreises schwankte von wenigen μ, die etwa der Dicke des Sporozoiten entsprachen, bis zu einem Radius, der der Länge des Sporozoiten entsprach, wenn dieser die Bewegung in der Beobachtungsebene (= Anheftungsebene) ausführte. In diesen Fällen konnten jedoch nie volle Kreise, sondern höchstens angenäherte Halbkreise der Sporozoitenspitze beobachtet werden, während bei kleineren Radien öfters 2—3 volle Kreisbewegungen ausgeführt wurden. In weniger ausgeprägten Fällen schienen die von festgehefteten Sporozoiten ausgeführten Bewegungen reine Pendelbewegungen mit unterschiedlicher Amplitude in einer Ebene zu sein oder den bereits beim Gleiten beschriebenen peitschenschlagähnlichen Bewegungen zu gleichen, bzw. sich aus den Gleit- und gleichzeitig schlängelnden Bewegungen des Sporozoitenvorderendes durch plötzliches Festsetzen mit dem Hinterende zu entwickeln.

Unterbrochen wurden die Pendelbewegungen durch mehr oder weniger lange Ruhepausen, in denen gelegentlich Beuge- oder Tastbewegungen ausgeführt wurden.

Etwa 80% der in typischer Weise ausgeführten Kreisbewegungen erfolgten in einer Richtung entgegen dem Uhrzeigersinn, vom Hinterende des Sporozoiten aus nach vorne gesehen.

Für die Zeitdauer des Anheftens können so gut wie keine Angaben gemacht werden. Wurde es als Unterbrechung einer Gleitbewegung ausgeführt, so betrug die Mindestzeit etwa ½ Sekunde, bei länger ruhig liegenden Sporozoiten konnte nicht immer einwandfrei entschieden werden, ob sie noch angeheftet waren oder nicht.

Wie bereits oben angedeutet, war in keinem Fall ein typisches Bewegungsmuster der Sporozoiten zu bemerken. Gleitbewegungen wechselten mit Beugen ab, gingen vereinzelt in Pendelbewegungen über oder entwickelten sich, z.T. stoßartig, aus Beugebewegungen. Mit ziemlicher Zuverlässigkeit zeigten sich häufiger beugende Sporozoiten zwischen den einzelnen Beugebewegungen auch Tastbewegungen, während sich Gleit- und gleichzeitige Tastbewegungen fast immer ausschlossen. Pendelbewegungen konnten mit Beuge- und Tastbewegungen kombiniert werden, Rollbewegungen wurden im »Ruhezustand« oder während des Gleitens durchgeführt. Zwischen allen Einzelbewegungen konnten jederzeit beliebig lange bzw. auch nur äußerst kurze Ruhepausen eingelegt werden.

Bei einer Sporozoitensuspension, deren Bewegungsintensität als mittelmäßig bezeichnet werden konnte, zeigten von 139 frisch excystierten Individuen, die jeweils 3 Minuten lang beobachtet wurden, 36 Sporozoiten nur Beugebewegungen, 12 nur Gleitbewegungen und 34 sowohl Beuge- als auch Gleitbewegungen. Typische Pendelbewegungen wurden nicht ausgeführt. Die nur mit Ölimmersion und bei intensiver Beobachtung gut wahrnehmbaren Tastbewegungen wurden nicht registriert.

Diskussion

Die lichtmikroskopisch wahrnehmbaren Einzelheiten der Sporozoiten gestatten nur wenig Rückschlüsse auf ihre Organisation. Vermutlich befindet sich an der Stelle der bei Interferenzkontrastbeleuchtung wahrnehmbaren Vertiefung der Kern, während alle übrigen, von elektronenmikroskopischen Untersuchungen an anderen Arten her bekannten Strukturen insbesondere bei Phasenkontrastbeleuchtung nur undeutlich sichtbar waren. Die im Anschluß an den refraktilen Körper bei einigen Sporozoiten festgestellte Zytoplasmamasse dürfte durch eine Verschiebung desselben während der Excystation nach vorne an diese Stelle gelangt sein.

Bereits in dem allerersten über Sporozoitenbewegungen vorliegenden Bericht, der auf Beobachtungen an *E. stiedai*-Sporozoiten beruht (METZNER, 1903), werden Tast-, Beuge- und Gleitbewegungen erwähnt. Trotz zahlreicher weiterer Beobachtungen an den verschiedensten Kokzidienarten werden jedoch erst von CLARKSON (1958) bei *E. adenoides* alle 4 Bewegunstypen der Sporozoiten kurz beschrieben. Auch in den folgenden Jahren wird die Pendelbewegung nur zweimal (FAYER und HAMMOND, 1967; HAMMOND, CHOBOTAR und ERNST, 1968) erwähnt, ohne daß dabei näher auf sie eingegangen wird. Über Rotationsbewegungen wird nur von REICH (1913) für *E. stiedai* und *E. falciformis* sowie von HAMMOND, CHOBOTAR und ERNST (1968) für *E. auburnensis* berichtet, beide Male sind sie mit einer Gleitbewegung kombiniert.

Bei *E. stiedai*-Sporozoiten konnten jetzt alle in der Literatur beschriebenen Bewegungstypen gefunden werden: Gleit-, Beuge-, Tast-, Rotations- und Pendelbewegungen, wobei das Gleiten sowohl vor- als auch rückwärts erfolgte.

Bei der Filmanalyse der Gleitbewegungen konnten selbst bei den Aufnahmegeschwindigkeiten von 60 Bildern/Sekunde keine morphologischen Hinweise auf die von DORAN, JAHN und RINALDI (1962) sowie JAHN und BOVEE (1970) postulierten schraubenförmigen Wellen gefunden werden. Das Rückwärtsgleiten könnte in einigen Fällen als mechanisches Anstoßen an ein Hindernis gedeutet werden. Doch läßt sich aus der Tatsache, daß nicht jedes mechanische Abbremsen der Sporozoiten zu einer Rückwärtsbewegung

führt sowie daß Rückwärtsbewegungen sowohl aus der Ruhelage heraus erfolgten als auch teilweise höhere Geschwindigkeiten als die Vorwärtsbewegung aufwiesen, schließen, daß es sich in jedem Fall um ein aktives Gleiten handeln muß. Inwieweit die Bewegungsbahnen, insbesondere das häufig auch in der Literatur erwähnte korkenzieherartige Gleiten durch eine Kombination der Vorwärts- und Rotationsbewegungen zustandekommen, muß vorläufig offengelassen werden, da bei den gleichen Bewegungsbahnen unterschiedlicher Sporozoiten eine Rotation sowohl vorhanden war als auch nicht. Des weiteren kann vermutlich erst aufgrund weiterer Untersuchungen an anderen Kokzidienarten gesagt werden, ob die beobachteten peitschenschlagähnlichen, drehend-wendenden Bewegungen des Sporozoitenvorderendes als ein eigener Bewegungstyp aufgefaßt werden müssen, oder ob es sich hier lediglich um eine richtungsändernde Modifikation der Gleitbewegung handelt.

Über absolute Geschwindigkeiten von Sporozoiten wurde bisher noch nicht berichtet. Die durchgeführten Untersuchungen ergaben, daß die Sporozoiten keine, auch nicht innerhalb einer Gleitbewegung, einheitliche Geschwindigkeit besitzen, sondern daß diese sehr stark variieren kann. Eine bei direkter Beobachtung kontinuierlich aussehende Bewegung kann sich u. U. aus mehreren durch sehr kurze Pausen unterbrochenen Teilbewegungen zusammensetzen, die infolge der Trägheit der Netzhaut für den Beobachter zu einem einheitlichen Bewegungsablauf verschmelzen.

CLARKSON (1958) berichtet bei *E. adenoides* über Wegstrecken von 5- bis 6-facher Körperlänge, die beim Gleiten zurückgelegt werden. ROBERTS und HAMMOND (1970) maßen bei *E. ellipsoidalis* und *E. ninakohlyakimovae* Entfernungen von einigen bis über 100 μ. *E. stiedai*-Sporozoiten legten in der Mehrzahl der Fälle kaum mehr als 10 bis 20 μ zurück, konnten aber auch mehr als 60 μ weit ohne Unterbrechung gleiten.

Nach den Untersuchungen von HAMMOND, CHOBOTAR und ERNST (1968) bzw. ROBERTS und HAMMOND (1970) scheint die Formvariabilität des Sporozoitenvorderendes je nach Art verschieden zu sein. *E. auburnensis* bzw. *E. ellipsoidalis* erwiesen sich dabei als wesentlich variabler als *E. bovis* bzw. *E. ninakohlyakimovae*. *E. stiedai*-Sporozoiten scheinen in dieser Beziehung mehr *E. auburnensis* und *E. ellipsoidalis* zu ähneln, es wurden etwa die gleichen, bei HAMMOND, CHOBOTAR und ERNST (1968) bereits abgebildeten Formen gefunden.

Im Gegensatz zu den bei diesen Autoren berichteten Befunden glitten jedoch *E. stiedai*-Sporozoiten nicht nur in der schlanken Gestalt vorwärts, sondern auch mit leicht abgerundetem Vorderende. Die extrem schlanke Form konnte nur während der Tast- und Pendelbewegungen gefunden werden. Eine von SRIVASTAVA (1967) für *E. labbeana*-Sporozoiten berichtete Gestaltveränderung des Hinterendes konnte weder in weiteren Literaturangaben noch in den eigenen Untersuchungen gefunden werden.

Aufgrund der großen, bei den Beugebewegungen gefundenen Variationsbreite erscheint es fraglich, ob hier von Speziesunterschieden gesprochen werden kann, obwohl HAMMOND, CHOBOTAR und ERNST (1968) auf einige diesbezügliche Unterschiede in der Intensität des Beugens zwischen *E. bovis* und *E. auburnensis* hinweisen. Die von diesen Autoren bei *E. auburnensis* beschriebene Faltenbildung konnte bei *E. stiedai* in diesen lichtmikroskopischen Untersuchungen nicht beobachtet werden. Ein auffallender Unterschied bestand zu dem Beugeverhalten noch nicht excystierter Sporozoiten in den Sporozysten (DÜRR, HEUNERT, MILTHALER und GALLE, 1971), bei dem das Abknicken wesentlich deutlicher, stärker und ohne das vorherige Einrollen des Vorderendes erfolgte und zu einer Wendung in der Sporozyste führte.

Fast immer wurde das Vorderende der Sporozoiten abgebeugt. In Übereinstimmung mit den Beobachtungen von SRIVASTAVA (1967) an *E. labbeana* wurde jedoch in Einzelfällen auch ein Beugen des den refraktilen Körper enthaltenden Hinterendes gesehen. Allerdings wurde dabei das Vorderende vorher nicht eingerollt, sondern erweckte den Eindruck, als ob es sich versteife und festzuhalten suche. Eine dritte, ebenfalls äußerst selten festgestellte und in der vorliegenden Literatur bisher nicht erwähnte Variation erfolgte derart, daß sich zwar das Vorderende normal abbeugte, dann aber die durch das Beugen erreichte Lage beibehielt und das Hinterende gestreckt wurde, was gleichzeitig zu einer Richtungsänderung in der Längsachse des Sporozoiten führte.

Die relativ seltenen Berichte über Pendelbewegungen hängen möglicherweise damit zusammen, daß dieser Bewegungstyp, zumindest bei *E. stiedai*, erst an Sporozoiten, die längere Zeit excystiert waren, vermehrt auftrat. Inwieweit die bei den typischen Pendelbewegungen gefundene Bevorzugung der einen Bewegungsrichtung mit durch Silberimprägnation darstellbaren, leicht spiralig verlaufenden Oberflächenstrukturen (DÜRR, 1970/71) korrelierbar ist, denen bisher keine elektronenmikroskopisch nachweisbaren Strukturen zugeordnet werden können, muß offenbleiben. Ähnliche, allerdings quer oder nur vereinzelt schräg verlaufende Streifungen konnten HAMMOND, CHOBOTAR und ERNST (1968) an lebenden Sporozoiten einer nicht näher bezeichneten *Eimeria*-Art aus *Dipodomys ordii* im Hellfeld sowie LUDVIK (1959) durch Silberimprägnation an *Toxoplasma*-Organismen nachweisen.

Über die den Bewegungen möglicherweise zugrunde liegenden elektronenmikroskopisch darstellbaren Feinstrukturen der Sporozoiten soll ausführlicher an anderer Stelle berichtet werden.

Funktionell ließen sich sowohl die Pendel- als auch die Tastbewegungen als Suchbewegungen der Sporozoiten deuten, die ihnen das Auffinden der Wirtszellen ermöglichen sollen. Gleitbewegungen bewerkstelligen die dazu notwendigen Ortsveränderungen bzw. können u. U. dazu dienen, durch das wiederholt beobachtete Anlaufnehmen kleinere Hindernisse zu überwinden.

Lediglich den Beugebewegungen kann vorläufig keine klar ersichtliche Funktion zugeordnet werden.

ZUSAMMENFASSUNG

An in vitro-excystierten Sporozoiten von *E. stiedai* wurden Direkt- und kinematographische Beobachtungen durchgeführt. Es konnten Gleitbewegungen vor- und rückwärts, Rollbewegungen, mit und ohne Gleitbewegungen kombiniert, Beuge-, Tast- und Pendelbewegungen festgestellt werden. Bei den Gleitbewegungen erreichten die Sporozoiten eine Geschwindigkeit von maximal 86 μ/Sekunde. Längere, bei Direktbeobachtung kontinuierlich erscheinende Gleitbewegungen konnten durch wiederholte, nur durch die Filmanalyse zu ermittelnde kürzeste Pausen unterbrochen sein. Eine typische Reihenfolge im Ablauf der einzelnen Bewegungsformen war nicht zu bemerken.

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LIFE CYCLE OF EIMERIA STIEDAI*

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Using the same title, OWEN (1970) reported last year having detected sporozoites of *E. stiedai* 24 hrs. p.i. in the bone marrow and 48 hrs. p.i. in the liver and blood buffy layer of infected rabbits using a mode of xenodiagnosis by infecting SPF-rabbits with the biological materials in question. DÜRR (1971) reported the presence of radioactivity in all organs of mice and rabbits being fed labelled sporozoites. However up till now, to my knowledge, there has been no report of the direct evidence of *E. stiedai* sporozoites outside the shortest passage between the intestines and the liver, including the mesenteric lymph nodes (HORTON, 1967; FITZGERALD, 1970; PELLÉRDY and DÜRR, 1970).

Now I have been able to demonstrate *E. stiedai* sporozoites histologically also in the bone marrow of rabbits.

Rabbits 8—10 weeks old, already naturally slightly infected with liver coccidiosis as demonstrated in one control were again infected orally for 3 to 8 consecutive days with several (up to 100) millions of *E. stiedai* oocysts per day. Two to three days after the last infection the rabbits were killed and their organs fixed with formalin for histological techniques. Sporozoites (in the liver also schizonts and merozoites) could be demonstrated in this manner in the liver, the mesenteric lymph nodes and the bone marrow of femur and tibia. Also in fresh smear preparations of the bone marrow sporozoites had been seen. In the mesenteric lymph nodes the sporozoites were lying either free between the cells or enclosed within macrophages (Fig. 1). (Further detailed studies on this localization are in progress.) In the fresh smear preparations of the bone marrow the sporozoites were seen only engulfed in macrophages, whereas in the histological preparations they were also very scarcely demonstrable lying free between the cells.

The most characteristic feature of the sporozoites is their refractile body, the pictures resembling those given by HORTON (1967) in his drawings. The differentiation between sporozoites and possibly engulfed erythrocytes seems to be very difficult and needs a lot of experience, especially in cross sections of sporozoites through the region of their refractile bodies. Here a very small cytoplasmic margin, not visible around red blood cells, and a more or less distinct parasitophorous vacuole may serve as criterium.

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Concluding from these findings it may be assumed that *E. stiedai* sporozoites, after excysting in the small bowel, penetrate the intestinal wall and are distributed through the body mainly along with the intestinal lymph passing the mesenteric lymph nodes and the ductus thoracicus. The liver may serve as filtration organ (see also YAKIMOFF, 1926). A direct transportation of the sporozoites with the portal blood to the liver (SMETANA, 1933; FITZGERALD, 1970; PELLÉRDY and DÜRR, 1970) may occur, but certainly plays a minor role.

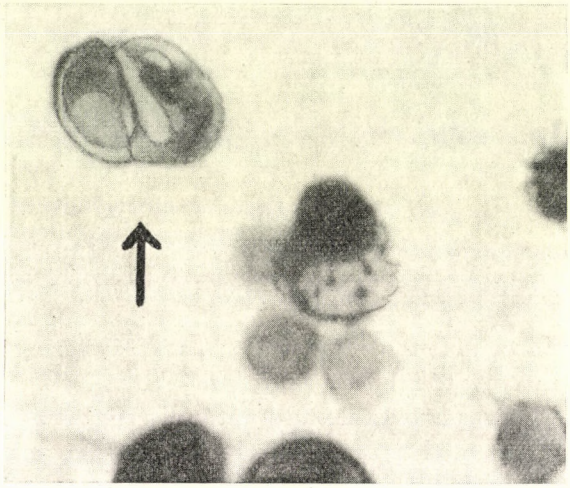

Fig. 1. Histological section of femur bone marrow. Arrow indicates a sporozoite-bearing macrophage. H. E.-staining. About 2000×

The way through efferent blood vessels from the mesenteric lymph nodes to the liver (HORTON, 1967) seems to be dubious in the light of newer findings. Firstly there are no references available demonstrating a direct connection between these two organs through blood vessels, nor to the contrary. Secondly, according to HEITMANN (1970) and HEITMANN et al. (1970) there is no other transfer of immunological competent committed cells (i.e. sporozoite-bearing macrophages) from the lymph nodes to the blood than through the ductus thoracicus. Furthermore, considerable numbers of sporozoites in the liver are demonstrable only from the 48th hr. p.i. (OWEN, 1970; PELLÉRDY and DÜRR, 1970). This seems to be in good congruence with the findings of HEITMANN (1970) and HEITMANN et al. (1970) that there is practically no output of lymphocytes from a lymph node during the first 12 hours after contact with a strong antigen (i.e. the sporozoites, probably within the macrophages) and that the output of large lymphocytes (i.e. the macrophages, containing the sporozoites) with the lymph commences after this time and reaches a peak during the following two to four days depending upon the force of the antigen.

Up till now it is uncertain where the sporozoites enter the macrophages and if they are capable of leaving them actively in the mesenteric lymph nodes, supposing they entered these in the intestinal wall. Further it is questionable if there are at all sporozoites reaching the liver outside of macrophages, whether directly from the intestines with the portal blood or by hypothetical blood vessel connections between the mesenteric lymph nodes and the liver, actively penetrating them in the lymph node, or transported with the lymph stream passing the ductus thoracicus. Perhaps lymph vessel canaliculation will answer this question one day.

Histological demonstration of sporozoites, however, in other parts of the body than the liver seems to be nearly impossible considering their relatively low number being present in each of these parts at one given moment. Hitherto unknown is the mechanism inducing the sporozoites to settle in the liver when passing it within the blood vessels. Possibly cell culture or other in vitro techniques may be suitable in detecting the reason of this tropism.

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EXPERIMENTELLES KLEBEN AM DUCTUS CHOLEDOCHUS

Von

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Die Gewebeklebemittel finden in allen Gebieten der Chirurgie eine stets häufigere Anwendung.

Über die gewebeklebende und blutungstillende Wirkung konnten wir selbst gute Erfahrungen sammeln. Wir verwenden sie routinemäßig bei Hauttransplantation und Gefäßoperationen, in erster Linie bei der Embolektomie und bei Rekanalisationsoperationen (SZENTGÁLI und Mitarb., 1969; MÁTÉ und Mitarb., 1971; SZENTGÁLI und MÁTÉ, 1970; OKOS und Mitarb., 1969).

OTA und Mitarb. (1965) sowie GOETZ und WEISSBERG (1964) untersuchten die Toxizitäts- und Absorptionsverhältnisse der Klebemittel. GOTTLOB und BLÜMEL (1965, 1968) haben die Klebemethode der Gefäßanastomosen ausgearbeitet. In Ungarn berichteten BORNEMISZA (1968, 1969) und NEMES (1969a, b) in mehreren Veröffentlichungen über die mit Gewebeklebemitteln gesammelten Erfahrungen.

All dies gab einen Anlaß für unsere Experimente, um zu prüfen, mit welchem Ergebnis die Nähte an Gallenwegen mit Gewebeklebemitteln ersetzt oder ergänzt werden können. Unter den zur Verfügung stehenden zahlreichen Literaturangaben fanden wir über das Choledochuskleben nur die Arbeit von MESTER und Mitarb. (1970).

Eigene Untersuchungen

Mit den Klebemitteln Histoacryl-N-Blau und Vulnocoll wurde am Ductus choledochus von 20 Hunden das Kleben durchgeführt.

Das Gewicht der Tiere betrug 18—25 kg. Sie wurden mit Hibernal und Depridol narkotisiert. Nach Eröffnung der Bauchhöhle wurde der Ductus choledochus freigelegt, dann eine der folgenden chirurgischen Lösungen gewählt:

I. An der Wand des Hauptgallenganges wurde eine 15—20 mm lange Inzisionsöffnung verfertigt. Für die Schließung der Choledochotomieöffnung verwendeten wir folgende drei Varianten:

(a) Bei 5 Tieren wurden die Wundränder ohne Naht, einschichtig geklebt.

(b) Bei 5 Tieren klebten wir einen Faszienstreifen als zweite Schicht auf die einschichtig geklebte Inzisionsöffnung.

(c) Bei 5 Tieren wurde die Inzisionsöffnung mit zwei Haltenähten vereinigt und geklebt.

II. Die »end-to-end« Anastomose erfolgte auf zweierlei Weise:

(a) An 2 Hunden wurde am Hauptgallengang, 2 cm von der Duodenaleinmündung entfernt, ein 6—8 mm langer Längsschnitt verfertigt. Über die Öffnung wurde ein Katheter in den Ductus choledochus eingeführt, dann in Richtung der Leberpforte vorgeschoben und die Choledochuswand oberhalb des Katheters in Längsrichtung zirkulär durchtrennt. Die sich elastisch trennenden Enden wurden mit einer Pinzette aneinandergefügt und über dem Katheter zirkulär geklebt. Nach Anhaften der Ränder wurde der Katheter durch die Choledochotomieöffnung entfernt, die Öffnung — mit Anheben der Ränder und ohne Haltenaht — durch Kleben geschlossen.

(b) Bei 3 Hunden haben wir die Choledochuswand über den durch die Inzisionsöffnung eingeführten Katheter ebenfalls zirkulär durchtrennt, dann mit zwei Haltenähten vereinigt und zirkulär geklebt.

Nach Beendigung des Klebens prüften wir durch Zusammendrücken der Gallenblase, ob an der Verbindungsstelle keine Gallenentleerung erfolgt. Nach intraabdomineller Injektion von kristalliger Penicillinlösung wurde die Bauchwand geschlossen.

Postoperativ wurden die Tiere fortlaufend kontrolliert, dann zwecks histologischer Aufarbeitung am 10., 20., 35., 50., 75. Tag getötet. Während der Beobachtungszeit hatten die Tiere guten Appetit, der Ernährungszustand war befriedigend, Ikterus trat nicht auf. Die am 20., 35. und 60. postoperativen Tag verfertigten Röntgenaufnahmen zeigten einen normal weiten Ductus choledochus. Zwei Tiere, bei denen wir die »end-to-end« Anastomose ohne Haltenaht, nur mit Kleben angelegt hatten, verendeten am 3.—4. postoperativen Tag infolge Anastomoseinsuffizienz.

Der Sektionsbefund ergab das Bild einer günstigen Wundheilung. Die mit Klebestoff vereinigten Inzisionswunden sowie die mit Haltenaht versorgten Stumpfe hielten fest, in der Bauchhöhle waren Spuren einer Gallenentleerung weder während der frühen, noch späten postoperativen Phase bemerkbar. Als Folge des Klebens kamen geringe, bzw. in Fällen der mit Faszienstreifen erfolgten Kleben etwas reichlichere Bindegewebsadhäsionen zustande. In keinem Fall zeigten die Bindegewebsnarben akute Entzündungszeichen zwischen den 10. und 75. postoperativen Tagen. An den mit unterschiedlicher Technik bereiteten Inzisions- und »end-to-end« Anastomosestellen wies der Ductus choledochus weder eine Einengung, noch eine Erweiterung auf. Im übrigen heilten die Inzisionen mit einer so minimalen Narbe, daß am 50.—75. Tag die genaue Klebestelle in mehreren Fällen nur im histologischen Schnitt nachweisbar war. Die *histologischen Untersuchungen* (Formalinfixierung, Paraffineinbettung, Färbung mit Hämatoxylin-Eosin und nach van Gieson) bewiesen eine rasche Reparation im Operationsgebiet des Choledochus.

Am 10. postoperativen Tag war die Operationslinie der Inzisionen und »end-to-end« Anastomosen durch ein zellenreiches Angiofibroblastgewebe angedeutet. An der Schleimhaut des Ductus choledochus war eine Propriadrüsengranulation bemerkbar und aus dem Wundrand wuchs neu gebildetes Zylinder-

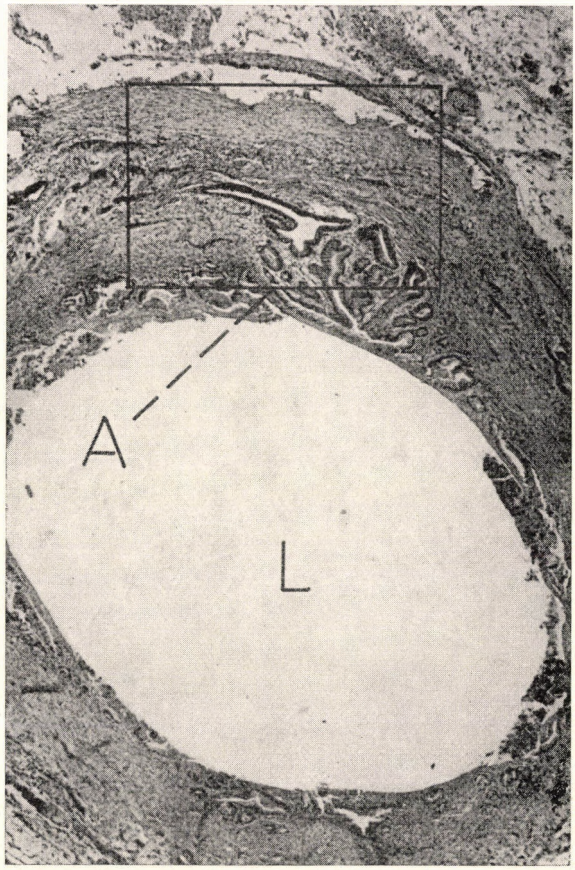

Abb. 1. Ohne Haltenähte, einschichtig geklebte Inzision am 20. postoperativen Tag. Am oberen Rand ist das Klebemittel sichtbar. Der eingerahmte Teil (A) ist in Abb. 2 vergrößert dargestellt. Häm.-Eos.-Färbung. Vergr. 10 ×

epithel auf die Propriaoberfläche. Klebestoffreste waren nur in der Muskelschicht und Adventitia sichtbar. Die Umgebung der Klebemittelreste sowie die benachbarten Wandteile waren mit geringeren neutrophilen Granulozyten- und Lymphozytenmengen und in größerer Zahl mit Histiozyten und Fibroblasten infiltriert. Das Ausmaß der Infiltration war von der Größe der Klebemittelreste abhängig. In der Umgebung von Haltenähten war überall eine bedeutende Gewebereaktion bemerkbar, welche in erster Linie durch die Anhäufung akut entzündlicher Zellen gekennzeichnet war.

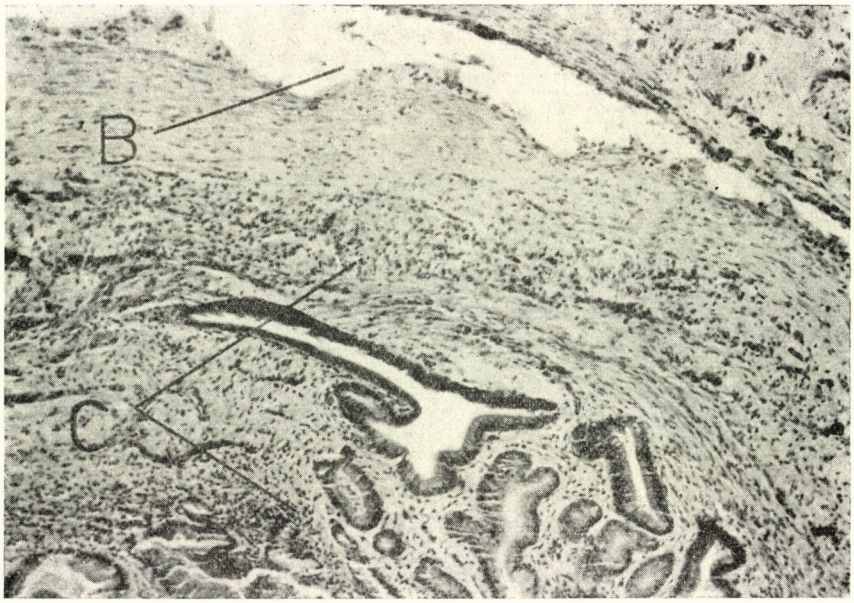

Abb. 2. Vergrößertes Bild der vorigen Klebestelle. B, Während der Einbettung hat sich die Klebemittelsubstanz gelöst; C, An der Inzisionsstelle bindegewebige Reparation. Häm.-Eos.-Färbung. Vergr. 1 : 40

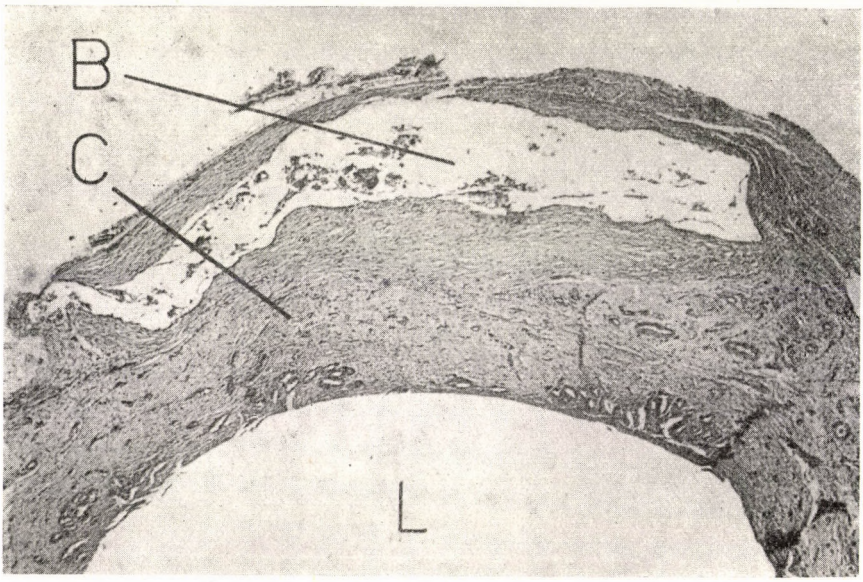

Abb. 3. End-to-end vereinigte Ductus choledochus-Wand am 50. postoperativen Tag. In die Klebemittelsubstanz dringen Zellelemente. L, Choledochuslumen; B, Klebestelle; C, Narbengewebe. Häm.-Eos.-Färbung. Vergr. 1 : 25

Bei den am 20. und 35. postoperativen Tag getöteten Tieren war die Schleimhaut des Ductus choledochus überall organisiert. In der Propria waren die muzinösen Endkammern regeneriert, die Propriaoberfläche war überall mit Zylinderepithelzellen bedeckt. An der Ductus choledochus-Wand war die Operatonslinie mit aufgesplittertem Granulationsgewebe begrenzt, wo stellenweise Fremdkörperriesenzellen sichtbar waren (Abb. 1, 2, 3).

Bei den am 50.—75. postoperativen Tag getöteten Tieren wurde das Operationsgebiet durch aufgesplittertes Bindegewebe angedeutet. In die Klebestoffteile drangen Histiozyten und Fibroblasten ein, und eine neugebildete Bindegewebsfaserschicht entstand. Die Nähte waren zwar mit Bindegewebe eingekapselt, aber zwischen den Fasern war eine auf lebhafte Gewebereaktion hinweisende Zellansammlung bemerkbar. Der Charakter und die Größe der Gewebereaktion der mit Histoacryl, bzw. Vulnocoll geklebten Gallengängen zeigten im histologischen Schnitt keinen Unterschied.

Besprechung

Unsere experimentellen Operationen zeigten, daß Klebemittel am extrahepatischen Gallengang mit gutem Erfolg verwendet werden können.

Die Schließung von Längsinzisionen durch Haltenähte und Kleben sowie Anlegen von Fasziastreifen kann gut versorgt werden. Aufgrund unserer Versuche sind wir der Meinung, daß es gefährlich ist eine »end-to-end« Anastomose ohne Haltenähte, ausschließlich mit Kleben anzulegen, denn der Widerstand der frisch geklebten Wand allein genügt nicht, um die Anastomose zu erhalten.

Die Stümpfe können mit 2—3 Haltenähten und zirkulärem Kleben vereinigt werden, ohne daß es später zu einer Choledochuseinengung kommt — wie dies die am 35.—75. Tag verfertigten histologischen Schnitte bewiesen. Außerdem zeigten die histologischen Schnitte, daß das Ergebnis um so vollkommener ist, je weniger Haltenähte an die Gallengangwand angelegt werden.

Histoacryl und Vulnocoll organisierten sich unter ähnlichen Gewebereaktionen, aber die Resorption ist auch nach mehr als 2 Monaten nicht beendet.

Unter Berücksichtigung der oben angeführten Gesichtspunkte haben wir die Gewebeklebemittel bei Gallengangoperationen eingeführt. Wir beabsichtigen, die Bewertung des klinischen Materials in einer anderen Arbeit zu veröffentlichen.

ZUSAMMENFASSUNG

Bei Hunden wurden am Ductus choledochus querlaufende Kleben unternommen. Anhand der Erfahrungen kann das Gewebeklebemittel bei Choledochusoperationen die traditionelle Naht in vielen Fällen ersetzen oder ergänzen.

Die Verläßlichkeit und gewebefreundliche Eigenschaft des Klebemittels wurde durch die Sektions- und histologischen Befunde bestätigt.

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STUDIES ON THE INACTIVATION OF COCCIDIAN OOCYSTS

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Modern animal keeping in which large numbers of animals are kept in a small space facilitates parasitic infections. Regular and careful disinfection of the animal rooms is the most important countermeasure. Eggs of Ascarids and Coccidian oocysts, however, are resistant to nearly all disinfectants.

In West Germany bactericidal and fungicidal disinfectants are tested according to specific regulations. A list of all effective disinfectants is published periodically.

There are no regulations up till now, however, for parasiticidal agents. This is particularly unfortunate because parasitic diseases of humans are gaining increasing importance (e.g. visceral larva migrans and toxoplasmosis).

Many producers of parasiticidal disinfectants already have their agents tested. As there are no standard regulations, the trials are conducted under non-uniform conditions. This has been responsible for a number of contradictory results obtained recently.

Since ENIGK's investigations (ENIGK, 1939), three procedures are commonly used for testing disinfectants against parasites in the laboratory:

1. In the suspension test eggs of helminths or coccidian oocysts are suspended in the chemical agent for a definite time and examined quantitatively for their viability. This method is often used because of its simplicity. Here most investigators failed to provide information on the volume of active substance used per unit surface area tested (ENIGK, 1939, 1947; YAKIMOFF, 1932 and 1934; SECK and SCHUMACHER, 1941; REX, 1943; GEITMANN, 1961; JUNGMANN, 1965; BRAUER, 1966; ENIGK and HILBRICH, 1968). In their experiments with a disinfectant containing carbon disulfide, v.d. GULDEN and v. ERP (1969) showed that, especially in the case of volatile substances the results obtained in a suspension test depend on the volume of the agent applied per unit surface area.

2. The wooden plate method (ENIGK, 1939) or its variation with filter paper strips (BRAUER, 1966) is a carrier technique.

3. Faeces or soil mixed with oocysts are used in the practical experiment.

Two sorts of oocysts may be used: unsporulated ones, whose exogenous development (sporulation) is observed, and sporulated oocysts, whose reproductivity is examined. If unsporulated oocysts are tested one has to bear in mind that they have a reduced resistance to chemicals as compared with sporulated ones (PELLÉRDY, 1965; SCHNEIDER, AYENI and DÜRR, 1971b).

In the first case the sporulation rate of in vitro treated unsporulated oocysts is determined. The reproductivity is examined in the infection test. In this case also the mortality and sometimes the morbidity of the natural host after inoculation with the treated oocysts are determined. LONG (1970) has made an attempt to infect chicken embryos and tissue cultures with excysted sporozoites, but this method has not yet been sufficiently refined for practical investigations.

Table I

Anticoccidial agents used and the method of application as prescribed by the suppliers

Agent	Active substance	Manufacturer	Treatment before disinfection	Treatment after disinfection	Method	Disinfection		
						Application		Calculated volume ml/cm²
						conc. in %	Minimum contact time	
Ammonia	no commercial recommendation available					3.7		0.025
Formalin	no commercial recommendation available					3.6+10		0.025
Dekaseptol®	carbon disulphide and others, not specified	Chem. Fabrik, Marienfelde, Hamburg, BRD	thorough cleaning	ventilation, leave to dry	spray or scrub	6	2 h	0.01—0.02
DesL20®	halogenated condensation products of alkylated and arylated hydroxytoluenes and cresol sulphonic acids	TAD Cuxhaven, BRD	thorough cleaning	leave to dry	spray or scrub	4	15—30 min	0.02
Eimeran-Neu®	not given	G. Röhner, Froschhausen, BRD	not given	leave to dry	spray	5	30 min	0.01—0.02
Helasept®	chlorinated hydrocarbons and carbon disulphide	Dr. Hesse & Co., Hohenlockstedt, BRD	thorough cleaning	not given	spray or scrub	5	15 min	0.01—0.02
Izal®	mixture of phenols, monocyclic and polycyclic aromatic hydroxy compounds	Izal Ltd., Thorncliffe, Sheffield, England	no recommendations given for parasitic disinfection			5		0.025

Lomasept®	same composition as DesL20	TAD, Cuxhaven, BRD	thorough cleaning	leave to dry	spray or scrub	5	15—30 min	0.014
Lysococc®	organic carbon disulphide depots combined with phenol mixture and proton donor	Schülke & Mayr GmbH, Glashütte bei Hamburg, BRD	thorough cleaning	leave to dry	spray	5	not given	0.01
Salernil "spezial"®	chlorinated hydrocarbons and carbon disulphide in special soap solution	Pfizer GmbH, Karlsruhe, BRD	thorough cleaning	leave to dry wash away on metals after some hours	wash	6	not given	0.02—0.025
Triseptol®	carbon disulphide, cresols, chlorinated cresols, chlorinated hydrocarbons	Manosit-Chemie, Wistedt, BRD	thorough cleaning	not indicated	spray	5—6	15 min	0.01—0.02

* As soon as the results have been obtained, a new formulation of Eimeran-Neu is said to be available, which is reported to be capable of destroying 100% of sporulated *E. tenella* oocysts in 60 min using the suspension test with undefined levels.

To test the reliability of the cited methods and the effectiveness of some commercial disinfectants we have examined their activity by means of different methods of investigation. The instructions of the suppliers have been considered as far as possible.

Material and methods

Unsporulated oocysts of *Eimeria stiedai* were obtained from the biliary ducts of experimentally infected rabbits using the method described by AYENI (1969). The oocysts were suspended in distilled water to give a suspension

Table II

Results of the suspension test according to V. D. GULDEN and v. ERP (1969)

Agent	Reaction time						Control	
	5 min			15 min				
	n	% spor.	% path.	*n*	% spor.	% path.	% spor.	% path.
Ammonia	2	3.0	2.3	2	0.0	0.0	90.0	0.0
Formalin (10%)	2	89.0	0.1	2	90.3	0.1	90.0	0.0
Dekaseptol	2	2.3	0.1	2	0.3	0.0	89.0	0.3
	2	37.5	1.0	—	—	—	91.0	0.3
	—	—	—	2	0.5	0.1	89.7	0.5
DesL20	2	89.9	0.0	2	65.3	0.5	89.7	0.0
	—	—	—	2	86.7	0.7	89.5	0.3
Eimeran-Neu	2	89.9	0.0	2	89.7	0.0	89.7	0.0
	—	—	—	2	88.3	1.3	89.7	0.5
Helasept	2	43.0	0.8	2	0.5	0.3	89.7	0.0
	—	—	—	2	5.5	8.3	89.7	0.5
	—	—	—	2	0.1	9.5	77.3	0.0
Izal	2	90.0	0.1	2	90.8	0.0	89.7	0.0
	—	—	—	2	86,9	0.7	89.5	0.3
Lomasept	2	89.9	0.0	2	91.3	0.0	89.7	0.0
	—	—	—	2	89.1	1.3	89.7	0.5
Lysococc	2	89.5	0.0	2	81.5	0.0	89.7	0.0
	—	—	—	2	45.9	0.5	89.5	0.3
	—	—	—	2	84.7	0.0	77.3	0.0
Salernil "spezial"	2	43.7	1.8	2	0.5	1.0	89.7	0.0
	—	—	—	2	1.3	3.2	89.5	0.3
Triseptol	2	0.3	0.0	2	0.0	0.0	89.7	0.0
	—	—	—	2	0.7	0.1	89.7	0.0

n, number of runs per trial; spor., sporulated oocysts (mean value of the double runs); path., pathologically sporulated oocysts (mean value of the double runs)

containing about 14.4×10^6 oocysts/ml. This suspension was kept in the refrigerator at a temperature of about 4 °C until it was used.

Suspension test (V.D. GULDEN and V. ERP, 1969)

1.75 ml of an oocyst suspension was pipetted into a shallow glass Petr dish 7 cm in diameter. To this occyst suspension 1.75 ml of a doubly concentrated dilution of disinfectant (see Table II) was added under constant shaking to ensure satisfactory mixing, and then left to stand open for periods of 5 or 15 minutes.

The final oocyst suspension reached a level of 0.9 mm. After the reaction time the suspension was diluted with water to give a total volume of about 70 ml which was immediately divided into 6 parts and centrifuged. The supernatant fluid was decanted and kept to determine the sporulation rate of the oocysts remaining in it. The washing process was repeated twice. The sediment was then collected into a single centrifuge tube and washed again twice.

The resulting oocyst sediment was suspended in about 10 ml of a 2.5 per cent potassium dichromate solution, poured into a Petri dish of about 9 cm diameter and left covered to sporulate within three days. On the third day, 300 randomly distributed oocysts were examined according to their sporulation stages using the method of KOTLÁN and PELLÉRDY (1934). A total count of the oocysts was taken before and after treatment to determine the loss resulting from the treatment procedures.

Carrier method (tissue paper)

One drop of the oocyst suspension was pipetted on round pieces of tissue paper (Kleenex tissue) 4 cm in diameter. After a short part-drying period the tissue paper was transferred to Petri dishes and wetted with the prescribed amount and concentration of disinfectant (see Table I). Subsequently, one Petri dish each was immediately turned into a moisture chamber, while the other one was left standing uncovered for 15 minutes to allow volatile components to evaporate. After a storage at room temperature for three days the oocysts were scratched from the tissue paper and the sporulation rate was determined.

In these experiments it did not matter which side of the contaminated tissue paper was in contact with the Petri dish.

Moreover the effect of formalin and ammonia, which are frequently used for disinfection, was examined by placing larger pieces of tissue paper besides small ones bearing oocysts and wetting them with one of the above solutions. The Petri dishes were then covered immediately and turned into a moisture chamber for three days.

Carrier method (ceramic tiles and slides)

One drop of the oocyst suspension was pipetted on uncleaned ceramic tiles and tiles cleaned with detergents. To this suspension we added the amount of disinfectant calculated from the suppliers specifications (see Table I). With a glass spatula this mixture was distributed over the surface of the tile. After a time of six hours during which the tiles were left uncovered all tiles were brought into moisture chambers; the sporulation rate was determined after three days.

Slides degreased with ethanol were treated in the same way. In addition, slides were contaminated with a mixture of oocysts and faeces. The disinfectant was added subsequently. All experiments included controls, in which tap water was substituted for the disinfectants.

In all of the above cited experiments the moisture chambers had not been closed hermetically. SCHNEIDER (1971), however, demonstrated that testing volatile agents in hermetically closed chambers may lead to different results. To ascertain this aspect we repeated the carrier technique with tissue papers with hermetically closed moisture chambers.

Results

The results of the suspension experiments are shown in Table II. Ammonium hydroxide (3.7%) destroyed 97 per cent of the oocysts within five minutes. Triseptol had a nearly hundred per cent effect in five minutes, while during the same period of time the effect of Dekaseptol ranged between 97.7 and 62.5 per cent. Helasept and Salernil "spezial" inhibited the sporulation of about 57 per cent of the oocysts within five minutes. All the other tested agents showed nearly no activity.

After a time of 15 minutes ammonium hydroxide, Dekaseptol, Helasept, Salernil "spezial", and Triseptol reduced the rate of sporogony from 90 to 100 per cent. The other agents (except Lysococc in one trial) showed no effect.

The loss of oocysts due to centrifugation as calculated from the controls ranged between 4 to 18 per cent, while the loss of treated oocysts varied between 1 and 85 per cent but remained fairly constant for the same agent. A check of the sporulation of these "lost oocysts" in the supernatant fluids gave results corresponding to the values described above for the different agents.

Table III, first position shows the results of the oocyst-carrier methods using tissue paper. Solutions of Izal and Triseptol as well as 10 and 3.6 per cent formalin had an almost 100 per cent effect after an evaporation time of 0 and 15 minutes. The effectiveness of 3.6 per cent formalin and Triseptol, however, was distinctly lower in one further experiment. Dekaseptol showed a high

effectiveness only in one out of 13 experiments, when a plastic Petri dish instead of a glass dish was used.

The carrier experiments with ceramic tiles (Table III, 2nd position) produced different results with regard to the degree of sporulation using the cleaned and uncleaned tiles. With the exception of Lysococc, DesL20, Helasept and Izal the sporulation rate on the uncleaned surfaces was lower than on the cleaned one.

On the unwashed plates ammonium hydroxide, 10 per cent formalin, Dekaseptol, Eimeran-Neu, Lomasept, Salernil "spezial" and Triseptol reduced

Table III

Results of the oocyst-carrier tests

Oocyst carrier	Moisture chamber not hermetically closed									Chamber hermetically closed (tissue paper)	
	tissue paper				ceramic tiles ($n = 1$)			glass slides ($n=1$)			
					uncleaned	cleaned		pure oocyst susp.	oocyst faecal mixture		
						I	II				
Evaporation time	0 min		15 min		6 hours					0 min	
	n	% sp.*	n	% sp.*	% sp.	% sp.	% sp.	% sp.	% sp.*	n	% sp.*
Ammonia	4	0.0	3	88.3	2.0	84.3	72.7	88.0	87.7	2	0.0
Formalin 3.6%	3	0.0	3	0.0	59.7	85.3	2.0	83.7	85.3	2	0.0
10%	1	25.7									
	4	0.0	3	0.0	0.0	16.3	1.0	17.0	2.3	—	—
Dekaseptol	9	88.7	5	86.9	9.0	80.7	56.3	85.3	85.0	2	0.0
	3	61.8	1	48.0							
	1	10.0**									
DesL20	4	86.9	3	88.6	82.3	85.0	54.7	80.0	84.3	2	0.8
										1	15.6
Eimeran-Neu	4	81.8	3	91.2	3.1	80.7	75.7	84.0	87.0	3	50.6
										1	4.3***
Helasept	4	86.9	3	89.0	88.7	82.3	32.3	82.3	82.3	3	56.0
										4	0.3***
Izal	7	0.0	6	0.0	30.3	0.0	46.7	0.0	35.0	2	0.0
Lomasept	6	84.6	9	84.2	0.6	81.7	57.0	82.0	84.7	2	1.8
	1	61.0									
Lysococc	4	89.6	3	89.9	85.3	86.7	80.7	85.0	78.7	2	67.6
Salernil "spezial"	3	87.2	3	88.0	0.3	85.0	65.0	74.3	82.0	2	0.0
	1	64.0									
Triseptol	3	0.0	3	0.6	0.0	50.0	4.0	67.7	74.7	2	0.0
	1	9.7									
Control	6	87.0	—	93.0	90.7	86.7	81.7	85.7	87.0	2	80.3

* mean value, if the results did not differ for more than 10 per cent.
** using a plastic Petri dish.
*** using 40 per cent more of the agent than prescribed.

Table IV

Disinfecting effect of the vapours of ammonia and formalin on oocysts (tissue paper carrier-method)

Agent	Volume applied	*n*	% sporulated oocysts	% pathologically sporulated oocysts
Formalin 10%	32×10^{-2} ml/85 cm^3	1	3.3	5.7
		2	0.0	0.0
Formalin 3.6%	32×10^{-2} ml/85 cm^3	2	2.7	0.0
Ammonia	32×10^{-2} ml/85 cm^3	1	0.0	5.7

the sporulation of the oocysts to less than 10 per cent. Izal prevented 70 per cent, and 3.6 per cent formalin only 40 per cent of the sporogony. On the washed tiles 10 per cent formalin destroyed 84 to 99 per cent, Izal 54 to 100 per cent, and Triseptol 50 to 96 per cent of the oocysts. 3.6 per cent formalin and Helasept inhibited the sporogony of 15 to 98 per cent and 18 to 69 per cent respectively. All other agents failed in both series to prevent the sporulation of more than 50 per cent of the oocysts.

Table III, 3rd position shows the effect of the agents under investigation on oocysts and oocyst-faecal mixture spread on glass slides. Apart from 10 per cent formalin and Izal reducing the sporulation of the unmixed oocysts to 17 and 0 per cent, respectively, and those of the oocyst-faecal mixture to 2.3 and 35 per cent, none of the agents examined showed any inhibitory effect on the sporulation of the oocysts.

When hermetically closed moisture chambers were used, good results were obtained with ammonia, formalin, Dekaseptol, DesL20, Izal, Lomasept, Salernil "spezial", and Triseptol. Lysococc, Eimeran-Neu, and Helasept failed to destroy more than 50 per cent of the oocysts. The good results with Eimeran-Neu and Helasept given in Table III, 4th position had been obtained due to an erroneous addition of 40 per cent more of the disinfectant as prescribed.

Table IV shows the results of the investigations evaluating the activity of the gaseous phase of ammonia and formalin. 3.6 and 10 per cent formalin and 3.7 per cent ammonia prevented the sporogony of about 96 to 100 per cent of the oocysts.

Discussion

Although the suspension test according to the modification of v.d. Gulden and v. Erp (1969) appears to be more related to practical conditions than the techniques of Enigk (1939), it seems not completely satisfactory,

because the volume of the agent used is about five to nine times the volume per unit area recommended by the suppliers for practical conditions. Preliminary experiments in which the attempt was made to use these recommended volumes showed that these conditions cannot be obtained in the laboratory using Petri dishes due to the surface tension of the agents. For this reason other methods were sought which might be more related to practical conditions. The carrier methods, already used in bacteriological investigations were applied. As it was not possible to wash the small amount of oocysts being used free of the agent it was assumed that theoretically in the case of a nonvolatile agent or using a hermetically closed moisture chamber the reaction time might be as long as three days according to the sporulation time. The longest evaporation period chosen was six hours to avoid any extreme drying effect which may influence the results of the experiments.

A comparison of the results shows that those of the carrier techniques do not in all cases correspond to the results of the suspension experiments and disagree in various cases with each other. Some agents that prevented sporogony in the suspension test as modified by V.D. GULDEN and V. ERP (1969) (ammonia, Dekaseptol, Helasept, Salernil "spezial") failed to affect the sporogony in nearly all oocyst-carrier techniques and vice versa (formalin, Izal). In contrast to this ENIGK (1939) found in his studies a similar effectiveness using the suspension test and the wooden plate method. This might be due to the greater volumes per unit area of the agents he used.

The reason for the disagreeing results using different methods may be due to the degree and rate of evaporation of the main components of the agents. Ammonium hydroxide proved to be very active in the suspension tests as well as in the tissue paper experiments with no evaporation time. If time was given to allow the ammonia to evaporate into a large space, as was done in the other carrier techniques, the disinfecting capacitiy was lost. A similar effect may be stated regarding the different results obtained with the carrier method (tissue paper) using hermetically and not hermetically closed moisture chambers. As there are no data obtainable in respect of the evaporation conditions of the disinfectants nor the solubility of their gaseous phases in water, this aspect, possibly explaining some of the unexpected results, cannot be discussed in this paper.

The results reported at Table IV otherwise show that the effectiveness of volatile agents is not dependent on a direct contact of the liquid phases with the oocysts.

Another observation made in the course of the present investigations was that the chemical and physical properties of the surfaces on which the oocysts are dropped may distinctly influence the results of the agents examined. Although the unwashed ceramic tiles were said to be untreated with any agent, we obtained only a poor sporulation result with some of the disinfectants. The

thoroughly washed ceramic tiles, however, gave good sporulation results using the same agents. Moreover in one of the experiments with tissue papers as oocyst carriers it was found that Dekaseptol had a high effectiveness if, instead of a glass Petri dish, a plastic one had been used. The areas where Dekaseptol had been applied to the oocysts and their vicinity were distinctly rough and opaque. In repetition experiments care should also be taken regarding the carrier's physical properties because e.g. in the case of ceramic tiles these seem to absorb much of the disinfectant, so that even after thorough washing they still smell strongly of some of the agents used.

In the case of the two series of experiments with cleaned ceramic tiles the difference in the results might be explained by the fact that the moisture chambers in the first experiment were too wet, a phenomenon not observed in the second experiment, where the sporulation rate was poorer.

Only in case of Izal, a definite decrease in its disinfectant activity was found when oocysts were mixed with faeces.

The agents tested might yet have other effects on the oocysts, which may not necessarily result in the inhibition of sporogony but render the oocysts more susceptible to environmental conditions, such as aging, humidity, dryness, sunlight and decaying debris. It was not possible to ascertain these effects with the methods used in the present investigations.

Some parameters have so far been neglected in the experiments: the volume of the evaporation room or moisture chamber, the temperature and eventually the presence of water. According to our findings these have to be considered in future evaluations of commercial disinfectants combatting exogenous stages of parasites. However, as long as there exist no regulations of the producers concerning these points, they may be neglected in praxis related experiments.

The purpose of the present investigations was to provide a discussion basis to assess the efficiency of experiments on disinfectants. The suspension test as modified by V.D. GULDEN and V. ERP (1969) seems to give more reliable results than the suspension tests applied in the past. This experiment is, however, not entirely satisfactory if used alone, since it leads too far away from the recommendations given by the suppliers for the practical use. It might be a useful and valuable primary screening method which has to be accompanied by other tests using the various oocyst-carrier techniques. It seems also necessary to determine the number of oocysts lost during the suspension test and to prove the viability of these lost oocysts in order to get satisfactory results independent of the density of the fluid.

In carrier-experiments the following factors which might affect the results should be considered:

Volume of the agent used per unit area of contaminated surface, or in the case of chambers, per unit volume of space;

Ratio between size of disinfected surface to space volume where evaporation takes place;

Time provided for evaporation into a large room before closing the moisture chamber;

Sort of the chamber used;

Porosity of the carrier and its possible preliminary treatment with chemicals;

Species of coccidia used during the experiments;

Presence or absence of debris;

Temperature at which the experiments are done.

Primary screening using unsporulated oocysts instead of sporulated ones seems to be useful, as this is much easier and cheaper. An agent which cannot destroy such oocysts may not be worth being examined with sporulated oocysts. Recommendations based on such screening results should only rely on a combination of different test methods, regarding also the higher resistance of sporulated oocysts.

Since it is not yet known if the reactions of different species of coccidia to chemical agents are identical, it might be necessary to examine all questionable species with the agent to be recommended.

Regarding the high resistance of the oocysts to physical and chemical agents (SCHNEIDER, AYENI and DÜRR, 1971/72a, b) it seems uncertain to develop disinfectants with 100 per cent efficiency under practical conditions as required in bactericidal and fungicidal investigations, however the best has to be tried.

SUMMARY

On the basis of own investigations practical and standardizable methods of examining the effectiveness of disinfectants on coccidian oocysts have been discussed. Two methods have been used as screening experiments for unsporulated oocysts of *Eimeria stiedai:*

I. The suspension test as modified by V.D. GULDEN and V. ERP (1969);

II. The oocyst-carrier method using:

a) pieces of tissue paper,

b) ceramic tiles as used for animal floors, and

c) glass slides.

The agents used included ammonium hydroxide, formalin and some of the commercial disinfectants.

The results show that the suspension test alone is not sufficient for examining the effect of disinfectants on exogenous stages of parasites. It is necessary that this method is supplemented by other more practice-oriented investigations, e.g. the oocyst-carrier experiments regarding the suppliers recommendations for practical use of the disinfectant. Standardized tests regarding also the physical properties of the disinfectants are to be developed.

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ROSENBERGER, G.: *Diseases of Cattle* (Krankheiten des Rindes). Co-authors: Professors G. DIRKSEN and M. STÖBER, Senior Lecturer H. D. GRÜNDEL. Publisher: Verlag Paul Parey, Berlin, 1970. 1418 pages, with 747 figures and illustrations and 28 colour plates. Linen cloth binding, price DM 350,—

After the publication in 1964 of "Clinical examination of cattle" by Professor Rosenberger and his colleagues of the reputed Clinic for Bovine Diseases at the Veterinary High School in Hannover, readers were looking forward with great expectations to this present volume. Nor will they be disappointed, for it is a compilation unprecedented in the world veterinary literature and it is of a size far greater than even the delay in publication would have lead one to suppose.

The adoption of teaching systems based on the different branches of veterinary medicine or on animal species has been a matter of continuous dispute and the reviewer is wondering whether a unanimous agreement can ever be reached on this point. In the organization of training, certain personal problems and objective circumstances are at least as relevant as tradition itself. It is, nevertheless, indisputable that the demand for veterinary experts with specialized training has become greater with the industrialization of animal breeding. Teaching of all branches of veterinary medicine is acceptable for the training of general practitioners, but specialist postgraduate courses and the related textbooks have to be necessarily restricted to single or selected species of animals.

Professor Rosenberger's book is equally valuable for undergraduate and postgraduate veterinarians.

There are essentially four parts, dealing in succession with organic, infectious, parasitic and metabolic diseases of cattle, the latter including also the description of various toxicoses. The tendency to concentrate non-epidemic non-infectious diseases in the first part of the book could not be pursued throughout. For example, enzootic bovine leucosis is described in the chapter dealing with organic diseases of the lymph nodes, though a later chapter is devoted to infectious diseases of the lymphatic system. Although the causative agent of bovine leucosis, supposedly an oncogenic virus, has not yet been isolated, the infectious nature of the disease has been verified by Professor Rosenberger himself.

For those not familiar with the system of grouping diseases according to animal species it may seem strange that though the book deals primarily with internal diseases, various procedures of surgery, epizootiological information and parasitic diseases are described in great detail. Naturally, there was no room in this already large book for obstetrics and reproduction biology.

In the individual chapters the diseases are as a rule discussed in the sequence of examinations followed in differential diagnosis. Nevertheless, the parasitoses manifested by cutaneous symptoms (demodicosis, etc.) are left to the end of the chapter.

The book begins with a short historical survey of bovine diseases and their treatment, and an outline of the aims and significance of veterinary hygiene in this respect. The tables included in this chapter show data on cattle populations in European countries and in other continents.

The discussion on organic diseases takes up 685 pages, more than one-third of the volume. Diseases of the hair, skin, subcutaneous connective tissue and cutaneous appendages (e.g. horns) are dealt with first. Though the fetlocks are cutaneous structures, discussion of their abnormalities is presented in another chapter as are local cutaneous changes (e.g. eczema of the tail).

The greater part of the chapter on diseases of the lymphatic system is devoted to leucosis. This extraordinarily well written part gives much information on the research work in this field carried out in Hannover. In the chapter on diseases of the hemopoietic system, blood and spleen, the highly illustrative figures and tables contribute much to the valus of the text and are particularly helpful in the discussion of cardiac failure and the endocarditis syndrome. Unfortunately, although the changes in the protein composition of the blood plasma are analyzed in detail, no information is presented on syndromes related to alteration of chemical composition of the blood (acidosis, alkalosis, etc.). Diseases of the upper airways (nasal passages, adnasal cavities, larynx, trachea), bronchi and lungs are dealt with in great detail. Two hundred pages are devoted to diseases of the digestive system which allows profound descriptions to be presented of pictures of great, practical importance, such as traumatic reticulitis, meteorism and obstruction of the oesophagus, aswell as of certain newly described conditions, such as the Hoflund syndrome, ruminal acidosis and diseases of the abomasum as well.

In keeping with the principles followed in compilation, the techniques of the surgical relief of these conditions are outlined in the appropriate chapters, as e.g. in context of intestinal invagination and abomasal displacement. Surprisingly, the pictures associated with paralysis of cerebral nerves and medulla oblongata are dealt with among the diseases of the digestive tract. Description of liver and biliary duct diseases is followed by discussion of abnormalities of the urinary tract and kidneys. Icterus and renal insufficiency are interpreted as comprehensive syndromes.

Description of diseases of the reproductive organs is completed by discussion of methods of sterilization and ovariectomy. Diseases of the extremities include affections of joints, tendons, tendinimous sheaths, bursae and peripheral nerves as well as symptomatology and treatment of bone fractures and muscle ruptures, congenital abnormalities. Care is also outlined in these chapters. The 50 pages on diseases of the fetlock are followed by sections on affections of the neck, trunk, thoracic region, navel, abdominal wall and tail. Descriptions of diseases of the nervous system and eye conclude this large first part of the book.

The part on infectious diseases also commences with descriptions of conditions manifested by cutaneous changes. Variola, papillomatosis, gas oedema and gas phlegmon, actinomycosis and trychophythiasis are treated in great detail. The section on infectious diseases of the lymphatic system includes much interesting information on mycotic infections of lymphatic organs. Respiratory diseases, such as parainfluenza, enzootic bronchopneumonia of adult cattle, rhinotracheitis, pleuropneumonia and pasteurellosis, are outlined in the chapter on respirators. In context of the enteric infections various oral inflammatory changes, mucosal disease, colibacillosis, salmonellosis, paratuberculosis, enterotoxaemia, vibrionic enteritis and enteric mycoses are discussed in more detail, and the same part deals with nephritis and pyelonephritis. Among diseases of the reproductive organs infectious vulvovaginitis, vibrio-induced abortion and brucellosis receive the most attention as do rabies, Aujeszky's disease, tetanus and listeriosis among the infectious diseases affecting the nervous system. The part on the infectious diseases affecting single organs is concluded with description of infectious keratoconjunctivitis. Turning to infectious diseases affecting several organs or the entire animal, one finds excellent descriptions of foot-and-mouth disease, necrotic nasal catarrh, rinderpest, anthrax, leptospirosis and rickettsiosis.

The section on parasitoses takes up about 100 pages and there is a profound sense for the practical context. First to be treated are protozoan infections, followed by those due to roundworms and tapeworms. Cutaneous parasitic affections are discussed at the end of the section.

In acknowledgement of their particular significance for cattle about 200 pages are devoted to metabolic and deficiency diseases including all necessary information for even the most exacting veterinarian. The section on toxicoses deals with diseases due to organic and inorganic poisons, drugs, deteriorated feed, mycotoxicoses and poisoning by plants and animals. The pictures of allergies and diseases due to physical agents, e.g. sunstroke, are also presented in this chapter. A concise summary of commonly applied therapeutic schedules is appended. It is clear from this survey of the book's contents that a textbook of great practical value is now available to veterinarians. Professor Rosenberger and his co-authors have rendered an invaluable service to veterinary medicine and science for which the reviewer feels obliged to express his thanks on behalf of the Hungarian veterinarians.

PROF. Z. HORVÁTH

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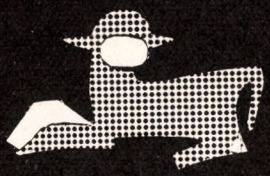

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TYPE A INFLUENZA VIRUS INFECTION IN GUINEA FOWLS

By

J. TANYI

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(Received February 10, 1971)

Investigations into avian influenza during the last decade have shown that other birds in addition to chicken and duck (PEREIRA et al., 1966, 1967; RINALDI et al., 1969) may harbour influenza virus strains of different serotypes. Positive isolations have been made from the common tern (*Sterna hirundo;* ROWAN, 1962), turkey (*Meleagris gallopago;* LANG et al., 1965; WELLS, 1963), quail (*Coturnix coturnix;* RINALDI et al., 1965), Japanese quail (*Coturnix coturnix japonica;* RINALDI et al., 1967), pheasant (*Phasianus colchicus;* RINALDI et al., 1967) and partridge (*Perdix perdix;* RINALDI et al., 1968).

This paper is a report of the first isolation of influenza virus from guinea fowls *(Numida meleagris).* The type A influenza virus concerned is the first avian strain ever isolated in Hungary. The epizootology, symptoms and pathology of the disease were studied in three field outbreaks and infection experiments were performed with the isolates on domestic poultry, mammals and laboratory rodents. The fourth egg passage of the first isolate was examned for centain diagnostically important properties and thermosensitivity.

Materials and methods

Virus isolation, agar gel diffusion precipitation tests, haemagglutination and haemagglutination inhibition tests, and preparation of convalescent sera for examination were carried out by the conventional methods (BEARD, 1970; PEREIRA et al., 1966, 1967). Reference antigens and antisera were kindly supplied by the World Influenza Centre (London), through the competent Division of the World Health Organization (Geneva). Specific antisera to the isolates were obtained from experimental infections to be described later. The fourth egg passage of the first isolate was used for infection experiments and for studies of the properties of the virus.

Epizootology, symptoms and pathology of the natural infection

The outbreaks occurred in two layer flocks and a rearing flock of one and the same farm, although the flocks were housed at a considerable distance

from one another. Both layer flocks became infected in an advanced stage of the laying period, under satisfactory feeding and management conditions, whereas management and climatic conditions were comparatively less advantageous for the growing birds.

In the first outbreak starting in May 1969, losses were initial and did not interfere with egg production, but they later rose abruptly and subsequently the daily egg yield declined from about 85% to 30%. Since neither a diet change, nor antibiotic treatment notably reduced the losses or improved the egg production over the next 3—4 weeks, the birds were slaughtered and marketed as table guinea-fowls.

The outbreak among the rearing guinea fowls began in November 1969 and took a prolonged course; the death rate was not too high, but it persisted with some fluctuations at an elevated level for a considerable time.

The third outbreak occurred during the early summer of 1970. In this case, losses rose somewhat already in the early laying period. Egg production grew only slowly to the 70% level (viz. far less than the maximum production), and then fell abruptly to 20—40%. A slow improvement began a few days later. Losses decreased, while the egg yield gradually climbed back over about one month to 70%, when it again dropped abruptly and the mortality rate rose once more. Subsequent egg production fluctuated between 30—40% and losses remained high. Rough estimates of the hatching rate indicated that it was as low as 40—50% for several weeks at the beginning of the outbreak, embryonic death occurring as a rule after two weeks of incubation. Dead embryos were bacteriologically sterile.

The birds died either without showing symptoms, or after a temporary general and respiratory illness (listlessness, cyanosis). Interestingly, almost exclusively females were affected.

Post mortem examination usually revealed different degrees of hyperaemia and occasionally a slight hypertrophy of all organs. Involvement of the internal reproductive organs was frequently seen: ova were degenerated, ovaries and oviducts were inflamed and haemorrhagic. Slight bleedings also occurred in the proventriculus and serous membranes. The serosae, above all of the abdominal cavity and air sacs, were fairly often coated by a delicate fibrin film. Degeneration of the liver (and rarely of other organs) and catarrhal enteritis were commonly found. Bacteriological examinations always had negative results.

In addition to virus isolation, haemagglutination inhibition (HI) tests were performed with 20 and 10 pooled serum samples from the two layer flocks, respectively, taken 3—4 weeks after the beginning of the outbreaks. Each serum sample (representing a pool from 2—5 birds) was treated with trypsin, heat and potassium periodate, the titres were always above 1 : 10 and most were 1 : 100. Separate examination of serum samples from hens and

roosters during the third outbreak showed titres of 1 : 20 — 1 : 160 and 1 : 10 — 1 : 20, respectively. Of 20 individual serum samples taken a few months after the onset of the outbreak, 16 still had low or medium high antibody titres.

Apparently healthy guinea fowls, ducks and hens from nearby flocks possessed no serum antibodies to the isolates, nor to standard avian influenza virus antigens.

Infection experiments

These were performed on poultry (chickens, ducks, turkeys, guinea fowls), mammals (sheep and swine) and laboratory rodents (mice and guinea-pigs). One-day-old birds were infected either intranasally and into the conjunctival sac with 100 or 10,000 ELD_{50}/0.2 ml virus, or intraabdominally with 10,000 ELD_{50}/0.2 ml. Two control groups were set up for the intranasally infected birds, using chicks from the same hatching; one group served as contact controls, the other was kept in isolation, but under similar conditions. Birds infected at 3—6 weeks of age were treated according to the same scheme. A few adult chickens and ducks were infected intranasally and into the conjunctival sac. From each poultry species a total of 30—50 day-old birds and 10—20 growing birds, divided into five uniform groups, were used. The birds were observed for 14—28 days. Blood samples were taken at intervals of 3—5 days, and as far as possible two birds of each group were killed at predetermined times to obtain blood or organs for virus isolation. One or more sheep and swine were infected at young or adult age with about 1,000 ELD_{50}/1 ml virus, administered either intranasally and into the conjunctival sac, or intravenously.

Altogether 50 mice and 20 guinea-pigs in five groups each were treated on the same schedule as poultry with regard to virus doses and infection routes.

Results

Chicken. No bird became infected in any of the age groups. Serum antibodies appeared in the infected and in-contact birds after 8 days at the earliest and generally at 12 days.

Duck. Infected and in-contact birds behaved similarly to chickens, except that ducks infected above 3 weeks of age did not produce demonstrable amounts of antibodies after 8—14 days.

Attempted virus isolations from emergency slaughtered and healthy appearing chickens and ducks were in all cases negative.

Turkey. One-day-old poults: Growth of infected and in-contact birds was considerably retarded compared to that of isolated controls, and some of the former died at 5—12 days of age, after showing listlessness and inappet-

ence for a few hours. Grossly, all dead poults showed a slight fibrinous inflammation of the air sacs, particularly of the thoracic sacs, and occasionally also of the sinuses. The virus could always be reisolated from spontaneously dead birds, but only rarely and irregularly from those which had been slaughtered for blood collection and displayed no gross lesions. Antibodies regularly appeared in the serum of surviving infected birds and emergency slaughtered birds from the 12th day after infection.

Growing poults: These showed no symptoms, but serum antibodies were demonstrable from the 8th day.

Guinea-fowl. Day-old age group: Catarrhal conjunctivitis and rhinitis developed after 3 days in birds inoculated intranasally and into the conjunctival sac, after about 4—5 days in intraabdominally inoculated birds and in-contact controls. From 5—6 days to 14 days the greater part of these groups died or had to be emergency slaughtered after showing severe general symptoms for a relatively short period. Antibodies appeared in the sera of survivors after 8 days. Spontaneously dead guinea-fowls as well as in-contact birds slaughtered after the first few days all had a fibrinous sinusitis and a few also exhibited fibrinous aerosacculitis.

Growing guinea-fowls infected at 3—4 weeks of age developed the disease 3—4 days after infection; they became listless and lost appetite. One intranasally-intraconjunctivally infected bird and one in-contact bird died showing severe general symptoms after 3 and 5 days, respectively. Apart from a general organic hyperaemia, no other gross lesions were found. The virus could be reisolated from both carcases. The remaining birds recovered after an illness of 2—3 days. HI antibodies appeared in the serum after 8 days. Intra-abdominally infected birds and in-contact controls possessed no demonstrable antibodies after 2 months, but in intranasally infected birds antibodies persisted for 3 months. The virus was always reisolated from spontaneously dead or emergency slaughtered birds with established gross lesions, but rarely if there were no gross changes, and then only from the lungs.

Mammals. Sheep and swine: Intraconjunctival and intravenous infection of young and adult animals neither caused symptoms nor did it evoke a demonstrable serological immune response.

Laboratory rodents. In previous immunization experiments one guinea-pig died showing symptoms of fibrinous pericarditis, pleuritis and acute catarrhal pneumonia 3 days after virus infection, while another died with semi-acute catarrhal pneumonia between 2—3 weeks. Virus was reisolated from lung lesions of both animals. Infection experiments were therefore also carried out with further guinea-pigs and also mice, according to the five-group schedule used for poultry.

Mice. Two mice from each group were killed every 5th day to obtain blood samples. Mice infected intranasally with the low virus dose (100 ELD_{50}/

0.2 ml) did not show symptoms. Among those infected with the higher dose (10,000 ELD_{50}/0.2 ml) a minor outbreak of paratyphoid developed, killing some of them between 3 and 10 days. Reisolation was not attempted from the carcases. Of the apparently healthy animals killed at 5 days those given the higher dose displayed acute catarrhal pneumonia; lesions were of pin-head size and the virus was reisolable from them. Mice given the lower dose, as well as the in-contact and unexposed controls possessed no antibodies, whereas those given the higher dose showed a demonstrable antibody level after 15 days.

Guinea-pigs. Intranasal infection with the higher dose (10,000 ELD_{50}/ 0.2 ml) caused a slight weight loss. Antibodies appeared 10 days after intranasal or intraabdominal infection with the high dose and at 15 days after inoculation of the lower intranasal dose as well as in the in-contact controls. Titres were highest in the group inoculated intranasally with the higher dose.

Virological examinations

The two strains isolated in 1969 originally showed a distinct antigenic relationship with the influenza virus strain Duck/England/62 and a less distinct relationship with Quail/Italy/1117/65 strain when tested by the HI test. These relationships became gradually reversed during subsequent transfers, until in the 8th passage, whereas the second became more pronounced. The strain isolated from the outbreak in 1970, however, was from the start related to the Italian quail strain only.

The fourth egg passage of the first isolate was used to study further the properties of the virus, because at this stage antigenic relationship to both the Duck/England/62 and the Quail/Italy/1117/65 strains still existed.

The virus grew readily in the allantoic cavity of chicken, guinea-fowl, goose and duck eggs. Embryonic death usually occurred between 72—144 hours, infrequently within as little as 24 hours. The average minimal lethal dose killed the embryo in about 120 hours. The HA titres of chorio-allantoic fluid varied from 1 : 8 to 1 : 2,048 and were inversely related with the infective titre of the inoculum and directly related with the time of death. The highest HA titres were measured in allantoic fluid from eggs that had been inoculated with a low-infectivity material and probably in related isolations and reisolations (e.g. from guinea-pig). In embryonated hen's eggs, haemagglutinins appeared in the embryo, yolk sac, amniotic and chorio-allantoic membrane. As long as the embryo was viable amniotic and yolk titres were scarcely measurable, if at all, but the chorio-allantoic membrane titre approximated that of the fluid. The titre of the yolk sac wall was mostly average, but in 10—20% of the cases it rose to a very high level (1 : 4,096), exceeding even

that of the allantoic fluid. Yolk sac and chorio-allantoic membrane titres also seemed to depend on the infective titre of the inoculum and on the time of embryonic death. The infective titre of the chorio-allantoic fluid varied between 10^6—10^8.

Thermosensitivity of the virus

Undiluted chorio-allantoic fluid retained its infectivity on exposure at 56 °C for 50 minutes, but lost it completely at 56 °C in 60 minutes, at 65° C in 30 minutes, and at 37 °C in 20 days. Infectivity was unaffected after several months' storage at room temperature and not demonstrably lower after one and a half years at —20 °C.

Haemagglutination titres remained unchanged after incubation at 37° and 56 °C for the times required to inactivate infectivity, but they tended to decrease slowly at lower temperatures.

Discussion

Isolations of three influenza virus strains from outbreaks of the disease in adult and rearing guinea-fowl flocks during 1969—1970 show that, like several other bird species, guinea-fowl are susceptible to natural avian influenza virus infection.

During successive transfer the surface antigenic activity of the first isolate changed rapidly towards the properties of a more stable strain, originally isolated in Italy from quails. The third isolate was nearly identical with the "transformed" first isolate. In view of the uncommon liability of influenza viruses to antigenic variation, the question may be posed whether such transformation depends on the biological-biochemical conditions in the laboratory or in the field.

The mortalities and decline of egg production that occurred during the outbreaks were in all probability due to several factors, of which influenza infection was only one. Despite the failure of attempts to identify at least some other factors, the uniformity of the clinical picture and its similarity with viral influenza of other avian species indicate that it played the decisive role.

The clinical manifestation of influenza itself depends on predisposing factors. Given populations of certain avian species have been shown to be symptomless carriers of influenza virus without possessing much antibodies, if any (Homme et al., 1970a, b). Clinical illness is elicited by a variety of often poorly understood factors, such as increased stress on the organism (e.g.

high egg production in genetically well established, appropriately fed layer flocks), management failures (e.g. high stocking density), abrupt climatic changes (e.g. cold stress), etc. As a rule the predisposing factors can be controlled only partially, because some are occasional or may be related to marketing or other economic transactions.

SUMMARY

In 1969—70 three outbreaks of influenza were observed in Hungary among guinea-fowl *(Numida meleagris)* in two layer flocks and a rearing flock, each consisting of 4,000—6,000 birds, a 50 km long farm unit. The disease caused a considerable depression of egg production and some mortality. Most birds died abruptly, without exhibiting clinical symptoms, but grossly they had hyperaemia and haemorrhages of internal organs, degeneration and inflammation of the inner reproductive organs, and a fibrinous inflammation of air sac serous membranes. Blood samples collected from two outbreaks contained haemagglutination inhibiting (HI) antibodies.

The fourth egg passage of the first isolate was pathogenic only for day-old and growing guinea-fowls and turkey poults, but it elicited an immune response in various bird species (chicken, duck, turkey, guinea-fowl) and laboratory rodents (mouse, guinea-pig) of different ages, infected with it directly or by contact.

Isolates from the first two outbreaks were antigenically related to the strains Duck/England/62 and Quail/Italy/65, whereas the isolate from the third outbreak was related to the second strain only. The first isolate gradually lost its relationship with Duck/England/62 during successive transfers until by the eighth passage it could no longer be differentiated from the third isolate. The thermosensitivity and certain other properties of the isolates were similar to those of related viruses.

These isolations of influenza virus are the first to be reported from guinea-fowl and the first avian influenza virus isolations in Hungary.

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QUANTITATIVE CHANGES OF CLOSTRIDIA IN THE INTESTINE OF EARLY-WEANED PIGS DISEASED IN COLI-ENTEROTOXAEMIA

By

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Anaerobic bacteria form a substantial component of the intestinal flora of pigs and have been implicated in the pathogenesis of swine gastroenteritis (MÉSZÁROS and PESTI, 1966; PESTI, 1970). As the isolation and cultivation of anaerobes from the intestine involve various technical difficulties, it seemed worthwile to compare the results obtained by various methods of isolation.

Materials and methods

The litters of two breeder sows from the herd of a state farm, a total of eighteen piglets, were used in the experiments. Farrowing took place under average winter conditions and one litter was weaned at 21 days of age, the other at 28 days. During the experimental period the animals received an antibiotic-containing feed (Phylaxia, Budapest) which had previously been examined bacteriologically. No prophylactic or symphylactic treatment was performed. For the purpose of bacteriological examination of the intestinal tract, the animals were killed in a predetermined sequence, depending on the time of weaning. One gramme samples of chyme (containing about 25% mucosal scrapings to promote cultivation of anaerobes) were collected from different segments of the intestine by as far as possible sterile technique, the segments being ligated in their original position before being cut out. The samples were homogenized in 9 ml buffer (MITSUOKA et al., 1965), containing 0.1% agar and 0.05% cystine-HCl and tenfold serial dilutions of this suspension were inoculated into the following media:

1. Semi-solid sulphite agar (SA) (TAKÁCS, 1967). Prior to inoculation, 1 ml of each dilution step was pipetted into a tube, heat-treated at 80 °C for 10 minutes and covered with 15 ml freshly prepared sterile medium warmed to 50–60 °C temperature. This procedure made possible the counting of thermoresistant spores in the test material, the heat treatment furnishing the temperature required for spore activation. The cultures were incubated for 72 hours at 39 °C.

2. SA + 1000 γ/ml D-cycloserine tartrate (Chinoin, Budapest). The addition of D-cycloserine permits selective isolation of all types of *Clostridium perfringens* (FÜZI and CSUKÁS, 1968) without heat treatment, thus enabling the demonstration of thermosensitive spores and vegetative forms. Clostridia growing in this medium will further on be referred to as "vegetative organisms" for the sake of simplicity. The cultures were incubated for 72 hours at 39 °C.

3. Reinforced Clostridial Medium (RCM). Streak plates were prepared from this medium to isolate clostridia and bacteroides (FULLER and LEV, 1964). The cultures were incubated for 4 days at 37 °C, under anaerobic conditions.

4. Lactose-eggyolk-milk medium + 200 γ/ml neomycin sulphate (WILLIS and HOBBS, 1959). Plates prepared from this medium were used for isolation and — after transfer to Holman medium with the antibiotic component omitted — identification of pure cultures.

Anaerobic conditions were provided in a glass jar of 15 litre volume, using pyrogallic acid and NaOH (PELCZAR, 1957). About 2—3 hours elapsed between sampling and setting up of the cultures in the anaerobic environment.

Complementary microscopic examinations and further culturing were performed, if required, to obtain more information.

Results

The methods described in the foregoing served above all for the isolation of clostridia and asporogenic anaerobes termed collectively as bacteroides.

Agar stabs in SA medium proved to be most suitable for the assessment of the quantitative relationships of isolated clostridia. The results obtained with high dilutions of 60 sulphite-reducing strains, previously transferred to Holman broth, are shown in Table I. Addition of D-cycloserine to SA further promoted clostridial growth. Several authors have suggested the use of neomycin-containing media for clostridium isolation from swine intestine. This is not advisable, especially when the material derives from animals, because neomycin suppresses the growth of type C *Clostridium perfringens* (NARAYAN, 1965) which has been shown to play an important role in the pathogenesis of swine gastro-enteritis (MÉSZÁROS and PESTI, 1966; PESTI, 1970).

The Willis—Hobbs lactose-eggyolk-milk medium was less suited than SA for clostridium isolation from chyme. Readings were more difficult than both SA and RCM and selectivity was unsatisfactory, owing probably to the high water vapour content in the glass jar, which may have altered the surface conditions of the culture.

For certain intestinal segments the clostridial counts established on RCM plates were several orders lower than those obtained on SA medium. This may have been due to the omission of the heat treatment required for the activation

Table I

Biochemical reactions of *Clostridium perfringens* isolated from alimentary tract of pig

Lactose-eggyolk-milk medium	Opalescence	+	
	Lactose	+	
	Pearly layer	—	
	Proteolysis	—	
Phenol red broth base (Difco B 92) pH = 7.8	Glucose	4.1	final pH after 6 days
	Lactose	4.6	
	Sucrose	4.1	
	Mannitol	7.4	
	Salicin	7.6	
Ellner's sporulation medium	Sporogonic	49	reading after 3 days
	Asporogonic	11	
RCM—blood agar	Strongly haemolytic	50	
	Weakly haemolytic	10	

of clostridium spores (ROBERTS, 1968) or, on the other hand, to the less vigorous anaerobic techniques used. After incubation for 4 days at 37 °C, the clostridia formed slightly wrinkled yellowish colonies about 0.5 cm in diameter on the RCM agar; the haemolytic activity of the colonies was non-uniform. The bacteria themselves were Gram variable, often filamentous rods which formed spores in Ellner's broth.

Asporogenic anaerobes also grew on the RCM agar, especially on plates seeded with samples from the posterior intestinal tract. These organisms formed small, dewdrop-like colonies having only a slight haemolytic activity, if any. At a magnification of 1 : 100, these colonies appeared slightly granular, with undulate edges, and they were often pitted owing to phage activity. Most colonies consisted of Gram-negative coccoids of varied size some of which had a granular cytoplasm, but a few colonies appeared as clumps of markedly polymorphous rods. Various authors who have used the same technique are of the opinion that these bacteria belong chiefly to the family Bacteroidaceae and some to the genus *Veillonella* (FULLER and LEV, 1964). On examining the intestinal flora of piglets weaned at an early age, KENWORTHY and CRABB (1963) found that the coccoid form of asporogenic anaerobes predominates up to 45 days of age. This is in good agreement with experience in the present studies.

The quantitative relationships of asporogenic anaerobes reported here fail to confirm the general view that their count in pig large intestine amounts to 10^9—10^{10}. This discrepancy was in all probability due to shortcomings of the anaerobic technique applied.

Since six of the 18 experimental piglets, three from each litter, developed clinically and bacteriologically verified *E. coli* enterotoxaemia, an opportunity

presented to compare the counts of the three groups of bacteria in healthy and diseased animals. Fig. 1 shows counts (log 10) for samples of chyme taken from different intestinal segments of healthy animals. The shapes of the curves clearly indicate that except for the duodenum and jejunum, which contained only spores, the counts for the vegetative forms of clostridia were 1—2 exponents higher compared to those for sporogenic forms. This is in good accor-

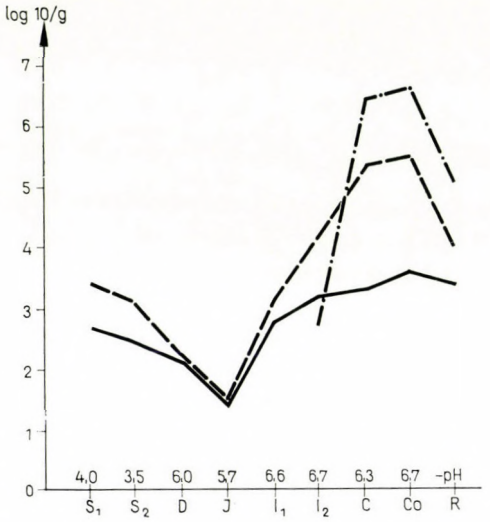

Fig. 1. Quantitative changes of certain anaerobic bacteria of the alimentary tract of healthy piglets. S_1, stomach (oesophageal part); S_2, stomach (pyloric part); D, duodenum; J, jejunum; I_1, ileum (proximal part); I_2, ileum (distal part); C, caecum; Co, colon; R, rectum. ——— clostridial spores; — — — vegetative clostridia; —.—.—.— bacteroides

dance with the observation (FULLER and MOORE, 1967) that lipids and bile acid salts may suppress clostridial growth in the small intestine. This inhibitive effect seems to have been stronger than the selective effect of gastric pH, despite the low acid resistance of clostridia. The counts of vegetative and sporogenic forms again differed markedly in the large intestine, owing very likely to the slower peristalsis and the absence of inhibitory effects in this segment.

The animals suffering from *E. coli* enterotoxaemia showed the known alterations of counts of coliforms and haemolytic *E. coli* strains (SOJKA, 1965; SZABÓ, 1964a, b). The quantitative relationships of the vegetative and sporogenic forms of clostridia are displayed in Fig. 2. Differences from the healthy state were spore counts fell by one exponent and counts of vegetative forms rose by 1—1.5 exponents compared to normal. Differences were less pronounced in the large intestine (Fig. 3) and could not be demonstrated by bacteriological examination of the faeces.

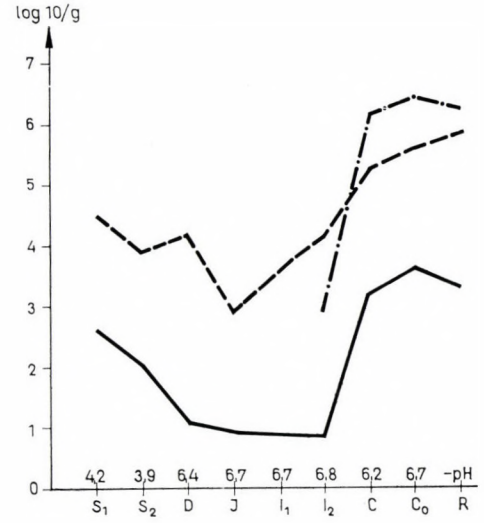

Fig. 2. Quantitative changes of certain anaerobic bacteria of the alimentary tract of scouring piglets

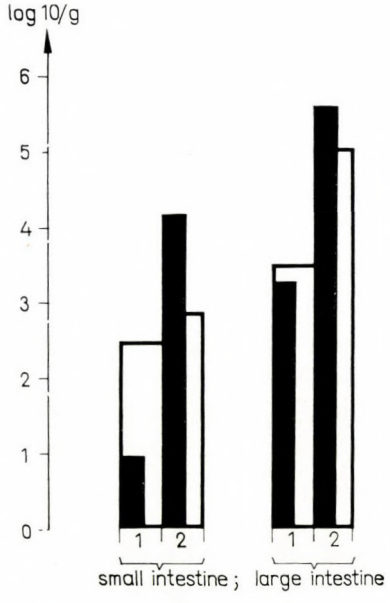

Fig. 3. Clostridial flora in the healthy and scouring piglets. 1, clostridial spores; 2, vegetative clostridia. ▭ healty piglets; ▬ scouring piglets

Discussion

Changes in the quantitative relationships of vegetative clostridia and spores in the different intestinal segments always reflect the local environmental factors controlling clostridial growth. Inhibitory factors present in the duodenum and jejunum destroy the vegetative phase and may even reduce spore counts. In pigs the destruction of vegetative clostridia takes place in the small intestine rather than in the stomach. Since all of the vegetative cells are missing in the small intestine of healthy piglets, intestinal clostridial counts probably depend on the ingestion of spores. The inhibitory factors seem to be less operative in the large intestine while the slow peristalsis promotes the activation of spores. In the piglets with *E. coli* enterotoxaemia — although the number of examined animals involved was, of course, too low to permit general conclusions — the relationship of spore activation clearly differed from those of healthy animals. The spore count fell, but this was due chiefly to a definite rise of the count of vegetative forms. It means a marked mobilization and activation of clostridia in the small intestine of piglets during the disease. This phenomenon was less pronounced in the large intestine (Fig. 3) and faecal examinations showed no indication of change. The disease process begins in all probability with the multiplication of pathogenic *E. coli* strains, the toxins of which alter the mucosa in favour of clostridial growth (MÉSZÁROS and PESTI, 1966; PESTI, 1970). This interpretation is supported by the observation that mobilisation was especially distinct in advanced coli-enterotoxaemia. The toxins released during clostridial growth are probably responsible for the enteritis which occasionally complicates this disease.

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SUMMARY

Comparison of methods of isolation of certain intestinal anaerobes revealed that in healthy pigs, the counts of vegetative clostridia and spores differ in the large intestine, owing to the influence of local inhibitory factors.

Pigs affected by enterotoxaemia, however, showed an activation of clostridia in the small intestine, resulting in the rise of vegetative forms and fall of spore counts. It is suggested that the toxins released during clostridial multiplication play a role in the enteritis which occasionally complicates enterotoxaemia.

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RÖNTGENANATOMIE DER HIRNSCHÄDELKNOCHEN DES HUNDES

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Diese Arbeit bildet den vierten, ergänzenden Teil der unter dem Titel »Röntgenanatomie der Hirnschädelknochen des Pferdes, des Rindes und des Schweines« veröffentlichten Mitteilungen. Im Vergleich zu den vorangehenden Arbeiten hat sich diese Aufgabe als wesentlich komplizierter erwiesen und erforderte eingehendere Studien, da nämlich die Schädelknochen der einzelnen Hundearten — im Gegensatz zu den übrigen Haustieren — bedeutende Abweichungen erkennen lassen.

Nach der allgemeinen, der Länge des Schädels entsprechenden Gruppierung, werden die Hundearten folgendermaßen aufgeteilt: dolichocephale Hunde (Windhund, Setter, Doberman, deutscher Schäferhund, Neufundländer, usw.), brachycephale Hunde (Buldogg, Mops, Boxer, Dachshund, usw.) und schließlich die einen Übergang zwischen den angeführten beiden Gruppen bildenden mesocephalen (hypsicephalen und turricephalen) Hundearten.

Auf dem Hundeschädel macht das zwischen der Länge von Hirn- und Gesichtsschädel bestehende Verhältnis — unabhängig von der Art — im allgemeinen 1 : 1 aus.

Das Schädeldach des dolichocephalen Hundes ist durch einen äußeren, hohen, in Pfeilrichtung verlaufenden Kamm, der Crista sagittalis externa charakterisiert — und dies im Gegensatz zum Schädel des mesocephalen oder noch eher des brachycephalen Hundes, auf dem ein derartiges Gebilde nur rudimentär oder überhaupt nicht vorzufinden ist. Beim brachycephalen Hund ist der Gehirnschädel umfangreicher als der Gesichtsschädel, was gleichzeitig bedeutet, daß sich auch die Proportion 1 : 1 ändert. Beim mesocephalen, besonders aber beim brachycephalen Hund ist der Hirnschädel rundlich, fast kugelartig, bei der letzterwähnten Art weicht deshalb der Jochbogen beiderseitig stark aus. Beim brachycephalen Hund ist — wegen der sich seitwärts vorwölbenden Wand der Schädelhöhle — auch die Schläfengrube seicht. Die Stirn-Nasen-Profillinie zeigt bei den dolichocephalen Hunden eine mäßige, bei den brachycephalen dagegen eine ausgeprägte Wölbung.

Der lange Gesichtsschädel der dolichocephalen Hunde dominiert gegenüber dem Hirnschädel. Die Proportion zwischen der Schädellänge und der

Breite der beiden Jochbogen beträgt 1,0 : 0,6—0,65. Die Länge des Hirnschädels (bis zur Sutura frontonasalis) verhält sich zur Länge der Nasenbeine wie 1,0 : 0,6—0,7. Auf dem Schädel des dolichocephalen Hundes besteht zwischen der kräftigen Crista sagittalis externa und der Entwicklung der Muskulatur eine gerade Proportion. Diese Crista ist nämlich der Treffpunkt der beiden mächtig entwickelten Mm. temporales.

Die brachycephalen Hunde haben einen kurzen Gesichtsschädel. Die zwischen Länge und Breite des Schädels bestehende Proportion macht 1,0 : 0,84—0,90 aus, während die zwischen der Länge von Hirnschädel und Gesichtsschädel registrierbare Proportion 1,0 : 0,30—0,36 beträgt (SCHWARZE, 1960). Die Crista sagittalis externa fehlt, dagegen lassen sich auf dem Schädeldach zwei in entgegengesetzter Richtung verlaufende Lineae temporales beobachten; aus dem Schädeldach entspringt auch der M. temporalis; die Bestandteile der Lineae temporales sind die Crista frontalis und die Crista parietalis (Crista frontalis externa). Bei einigen Hundearten ist der Schädel in Mangel der Linea temporalis vollkommen kugelförmig. Die Stirngrube (Fossa frontalis) ist schwach ausgebildet oder fehlt. Bei domestizierten Tieren bildet das Schädeldach unmittelbar vor den Augenhöhlen, an der Nasenwurzel eine ebene Fläche, die Glabella (ZIMMERMANN, 1939). Der Schädel junger dolichocephaler Hunde ist dem der Brachycephalen ähnlich.

Zwischen diesen beiden Grundformen nimmt der Schädel des mesocephalen Hundes eine Mittelstellung ein. Der Hirnschädel ist kugelförmig, zwischen den beiden Lineae temporales befindet sich eine breite Wandfläche. Die Längs- und Breitenmaße belaufen sich auf 1,0 : 0,75 (beim Spitz), die Proportion zwischen der Länge von Hirnschädel und Gesichtsschädel beträgt 1,0 : 0,44. Der Entwicklungsstand der Fossa frontalis ist unterschiedlich, die Nase verhältnismäßig gebogen (Spitz, Dachshund). Angesichts des ziemlich langen Hirnschädels reihte SCHWARZE (1960) den Bulldogg und den deutschen Boxer — trotz des kurzen Gesicht-Nasenabschnitts — unter die dolichocephalen Hunde ein.

Literatur

Mit der Röntgenanatomie der Schädelknochen des Hundes haben sich neuestens HARE (1958), STEINBECK (1962), FINKGRÄF (1961) und SCHEBITZ und WILKENS (1967) befaßt. Anhand seiner bei dolichocephalen (deutscher Schäferhund) und brachycephalen (englischer Bulldogg) Hunden durchgeführten Untersuchungen demonstrierte HARE (1958) die größeren knochengebildeten Schatten und gleichzeitig die Aufnahmepositionen auf Röntgenaufnahmen und Röntgenzeichnungen.

Wie das bei den amerikanischen Verfassern (MILLER u. Mitarb. 1964) üblich ist, werden die als Textillustration dienenden Schädelaufnahmen und Konturzeichnungen (in frontobasaler Strahlenrichtung) in der Arbeit von HARE (1958) in einer Weise dargestellt, als ob man das fragliche Objekt von der Körperlage des Tieres her betrachten würde, d. h., daß auf den Röntgenbildern das hintere, nackenseitige Ende des Schädels nach unten und das vordere, mundseitige Ende nach oben blickt. Diese Darstellungsweise unterscheidet sich von der bei der deutschen Schule gebräuchlichen (ELLENBERGER und BAUM, 1943; NICKEL u. Mitarb., 1954; SCHEBITZ und WILKENS, 1967), die den Schädel so veranschaulicht, wie man gewöhnlich dem Schädel

des untersuchten Tieres gegenübersteht, d. h. daß man den Schädel nicht vom Nacken, sondern von der Mund-Nasenöffnung her betrachtet. Bei Anwendung dieser Methode befindet sich auf den Röntgenbildern der nackenseitige Teil oben und der mundseitige Teil unten. Wir hielten uns in unseren bisherigen röntgenanatomischen Mitteilungen und auch in dieser Arbeit an die Darstellungsweise der deutschen Schule.

HARE (1958) hat seine Aufnahmen in ventrodorsaler (mandibulofrontaler) Strahlenrichtung verfertigt und bei der transversalen Strahlenrichtung die sinistrodextrale Strahlenrichtung angewandt. Unseres Erachtens ist die dorsoventrale (frontomandibulare) Strahlenrichtung vorteilhafter als die ventrodorsale, da die zahlreiche Einzelheiten zeigende Schädelbasis näher zum Aufnahmefilm liegt als der weniger Details enthaltende frontale Teil, so daß man von den vielen winzigen Einzelheiten ein schärferes Bild gewinnt.

STEINBECK (1962) untersuchte den Schädel des Airedale-Terriers; diese Art nimmt zwischen den dolichocephalen und brachycephalen Hundearten eine Mittelstellung ein. Die Aufnahmen wurden in dorsoventraler, ventrodorsaler sowie zweiseitiger sinistrodextraler, dextrosinistraler Strahlenrichtung verfertigt. Nach Ansicht des Verfassers besteht im Falle einer großen Fokus-Film-Entfernung zwischen den dorsoventralen und ventrodorsalen Aufnahmen kein wesentlicher Unterschied.

BARONE und GUELFI (1966) beschrieben das in sagittaler und transversaler Strahlenrichtung verfertigte normale Röntgenbild des Hundeschädels. Vom praktischen Standpunkt aus hat sich die ersterwähnte Strahlenrichtung als geeigneter erwiesen. Nach den Verfassern soll der Hauptstrahl — um ein verzerrungsfreies Bild zu gewinnen — auf die sog. frankfurter bzw. deutsche Ebene senkrecht geleitet werden. Die frankfurter Ebene reicht von der Margo infraorbitalis der Orbita durch den Arcus zygomaticus bis zum oberen Rand des Porus acusticus externus. Der Aufnahmefilm muß dieser Ebene parallel sein.

Der sich auf 198 Seiten erstreckende, zweisprachige Röntgenanatomische Atlas von SCHEBITZ und WILKENS (1967) enthält Röntgenaufnahmen lebender Pferde und Hunde. Der besondere Wert der Arbeit ergibt sich daraus, daß das Verfasserteam aus einem Anatomen und einem Kliniker (Chirurg) besteht. In dem mit vielen Röntgenaufnahmen und Röntgenkonturzeichnungen illustrierten Atlas wird sowohl die deutsche, als auch die angelsächsische Nomenklatur angewandt.

SCHÜLER (1968) analysierte den Katzenschädel und zwar nicht nur in senkrechter, sondern auch in schräger Projektion. Obwohl Verfasser die senkrechte Projektion als vorteilhafter betrachtet als die schräge, da Verzerrungen geringer und Orientation einfacher sind, war er mehrmals gezwungen, auch die schrägen Strahlenrichtungen in Anspruch zu nehmen, so z. B. — wie er schrieb — bei den Zahnaufnahmen, im Interesse der Vermeidung der einander bedeckenden Projektionen der Schatten der beiderseitigen Backenzähne.

Bei der Zusammenstellung unserer Arbeit, der kurzen, deskriptiven, unter Berücksichtigung der röntgenanatomischen Standpunkte verfertigten Beschreibung der Hirnschädelknochen, stützten wir uns auf die hervorragende Monographie von MILLER, CHRISTENSEN und EVANS (1964). Die ausführliche, gründliche Erörterung leistete zur Homologisation der Knochenschatten eine wertvolle Hilfe.

Methodik

Unter den röntgenanatomischen Untersuchungen des Knochengerüsts ist das Studium der Schädelknochen eine der kompliziertesten Aufgaben. Schon wegen der häufigen Superposition der Schatten können unmöglich Aufnahmen verfertigt werden, auf denen ein jedes Detail mit derselben Intensität dargestellt ist.

Die Anatomie der Hirnschädelknochen haben wir — ebenso wie in unseren früheren Arbeiten — unter Berücksichtigung röntgenanatomischer Gesichtspunkte beschrieben. Jene wichtigen anatomischen Charakteristika,

die größtenteils auch auf den Röntgenaufnahmen in Erscheinung treten und auch eine praktische Bedeutung besitzen, sind in der Arbeit kurz angegeben.

Die Erlernung des Materials der Röntgenanatomie ist anhand von Röntgenaufnahmen und Röntgenkonturzeichnungen sowie aufgrund des die Schatten erklärenden Textes möglich, obwohl das letzterwähnte eigentlich nicht unbedingt notwendig ist. Die Mehrzahl der röntgenanatomischen Arbeiten enthält nur Röntgenaufnahmen und Röntgenkonturzeichnungen, ohne daß der Schatten der betreffenden Gebilde beschrieben wäre; die ausführliche Beschreibung der Röntgenschatten ist aber in der Röntgenanatomie ebenso wichtig, wie in der deskriptiven Anatomie, da nämlich der regelwidrige Schatten irgendeines Knochens ansonsten nicht mit Sicherheit zu diagnostizieren ist. Fehlt der erklärende Text, wird man betreffs der Zugehörigkeit des fraglichen Knochens, selbst angesichts der sogar die kleinste Einzelheit darbietenden Aufnahme, unsicher. Um diesen Fehler auszuschalten, haben, wir auch diese Arbeit mit einem schattenerklärenden Text ergänzt.

Die Röntgenaufnahmen wurden von den mazerierten Schädeln dolichocephaler, mesocephaler und brachycephaler Hunde — ohne Berücksichtigung der Hundeart — verfertigt. Auf der vom lebenden Tier verfertigten Röntgenaufnahme verlieren sich die Schatten der kleineren, jedoch wichtigen Schädelabschnitte häufig in den Schatten der Weichteile, und sogar auf der mazerierte Schädel darstellenden Aufnahme bleiben die Schatten einiger wichtiger anatomischer Details unsichtbar.

Im Laufe der analysierenden Untersuchungen spielt bei der Bewertung der Röntgenschatten auch das Einbildungsvermögen eine gewisse Rolle. Um diesen Faktor zu eliminieren und Irrtümer auszuschalten — die zu begehen es mannigfaltige Möglichkeiten gibt — haben wir unsere Aufnahmen von in verschiedenen Richtungen zersägten Schädeln verfertigt. Der Röntgenologe muß — ob Untersuchung, oder Beschreibung — stets maximale Exaktheit bestreben. Bereits die kleinste Änderung der Richtung bzw. des Fußpunktes des Hauptstrahles kann eine wesentliche Modifizierung des Röntgenschattens zur Folge haben. Dies wird auch durch die Tatsache unterstützt, daß sich die Schattenverhältnisse, dementsprechend ob die Aufnahme vom ganzen Schädel, oder nur von dem Gehirn- bzw. Gesichtsschädel verfertigt worden war, wesentlich voneinander unterscheiden. Vergleicht man die Röntgenatlasse der verschiedenen Verfasser, so lassen sich, was die Bewertung der Lokalisation bzw. der Schatten einiger Löcher und Öffnungen anbelangt, nicht selten einander widersprechende Angaben vorfinden. Nebst der unterschiedlichen Festlegung der Fußpunkte spielen hierbei auch subjektive Gesichtspunkte eine wichtige Rolle. Auch im Laufe unserer Untersuchungen hat sich die Bewertung der Schatten häufig als problematisch erwiesen und gerade deshalb lag es nicht in unserer Absicht, die Stelle sämtlicher auf dem Röntgenbild kaum wahrnehmbarer Schatten eines jeden anatomischen Gebildes zu bezeichnen.

In Anbetracht des Gesagten haben wir als bedeutende modifizierende Faktoren den Fußpunkt der Hauptstrahlen in zwei Strahlenrichtungen festgelegt. Die Aufnahmen wurden in dorsoventraler, frontobasilarer (Norma frontalis) und transversaler, temporaler (Norma temporalis, sinistrodextralis, dextrosinistralis) Strahlenrichtung verfertigt. Die dorsoventraler, Strahlenrichtung schien geeigneter zu sein als die basofrontale (mandibulofrontale), da sich die stärker gegliederten Teile des Schädels auf der Schädelbasis befinden, so daß man von ihnen in Filmnähe ein schärferes Bild erhält. Den Fußpunkt des Hauptstrahles haben wir auf dem Schädel der dolichocephalen und mesocephalen Hunde in dorsoventraler Strahlenrichtung in der Medianebene, in oder etwas vor die Naht der Scheitel- und Stirnbeine angebracht, d.h. an jene Stelle, wo als Fortsetzung der Crista sagittalis externa die beiden Lineae temporales entspringen. Es sei jedoch erwähnt, daß wegen des individuell unterschiedlichen Entwicklungszustands der Scheitel- und Stirnbeine der sichere Halbierungspunkt der Hirnschädellänge nur schwer festzustellen ist. Auf dem Schädel des brachycephalen Hundes fällt der Fußpunkt des Hauptstrahles, ebenfalls in der Medianebene, in die Naht der Scheitel- und Stirnbeine, zwischen die beiderseits divergierenden Lineae temporales. In temporaler oder transversaler Strahlenrichtung gelangt der Fußpunkt des Hauptstrahles auf dem Schädel der dolichocephalen bzw. mesocephalen Hunde dem oberen Rand des Jochbogens entlang, in Richtung der von der Ausgangsstelle der Lineae frontales gefällten Senkrechte in die Naht der Scheitel- und Schläfenbeinschuppen (Sutura squamosa). Auf dem Schädel des brachycephalen Hundes fällt der Fußpunkt des Hauptstrahles in den Treffpunkt des Schuppenabschnitts der Scheitel-, Stirn- und Schläfenbeine. Da der Entwicklungsgrad der Schädelhöhle, Lokalisation bzw. Wölbung des Jochbogens den einzelnen Individuen und Rassen entsprechend die Stelle der Fußpunkte wesentlich beeinflussen, sind die oben angeführten Angaben nur annähernd genau.

Wir wandten die Fachwörter der neuen anatomischen Nomenklatur (NAV) an, und folgten bei der Gruppierung der Schädelknochen, angesichts unserer früheren röntgenanatomischen Arbeiten, der alten Einteilung.

Angesichts dessen, daß auf den von Röntgenaufnahmen verfertigten Reproduktionen die feineren Teilschatten zumeist verschwinden, empfiehlt es sich, das Bildmaterial der Arbeit in Form von Photographien beizufügen. Die von den vorderen Fußwurzelknochen des Rindes verfertigten Photographien von Funk (1966) sind fast ebenso schön, wie die originalen Röntgenaufnahmen. Funk (1966) hat sich bei Verfertigung seiner Bilder dem elektrischen Verfahren, der sog. Logetronographie bedient. Auf den Bildern sind die Kontraste weniger scharf, und die Teilschatten treten hervor (s. noch Schebitz, 1960 und Schüler, 1968).

Deskriptive Anatomie der Hirnschädelknochen

(unter Berücksichtigung röntgenanatomischer Gesichtspunkte)

Die Grundlage der Untersuchungen, auf denen die Angaben des deskriptiven anatomischen Kapitels beruhen, bildete die Hundeanatomie von MILLER—CHRISTENSEN—EVANS (1964) (Norma frontalis, norma temporalis, norma basilaris). Im Mittelpunkt unserer Untersuchungen stand der Schädel des dolichocephalen Hundes, wobei sich auch zur Erörterung der auf dem Schädel des mesocephalen bzw. brachycephalen Hundes beobachtbaren Unterschiede eine Gelegenheit bot.

Dorsale Fläche des Hirnschädels

(Planum dorsale, Norma frontalis)

Der Schädel des Neugeborenen ist fast kugelförmig. Auf dem Schädel des ausgewachsenen dolichocephalen Hundes erhebt sich die bereits gut entwickelte Crista sagittalis externa (Abb. 1, 3, 4, *1*), die etwa 1 cm vor der Sutura coronalis (Abb. 1, 3, 4, *2*) in die Linea temporalis übergeht (Abb. 1, *3*), welche ihrerseits auf den Processus zygomaticus (Abb. 1—5, *4*) verläuft. Auf dem Schädel des brachycephalen Hundes ist die Crista sagittalis externa (Abb. 2, 5, *5*) äußerst kurz und geht nach vorne in die nach rechts und nach links divergierende, gut entwickelte Linea temporalis (Abb. 2, *3*) über; die Ausgangsstelle befindet sich beim zwischen die Scheitelbeine eindringenden Fortsatz (Processus interparietalis) des Hinterhauptbeines. Die dorsale Ebene begrenzt von hinten den schräg verlaufenden, unterschiedlich entwickelten Genickkamm (Crista nuchae) (Abb. 1—5, *6*), welcher die oberen und unteren Schädelflächen voneinander absondert.

Die an den beiden Seiten der Crista sagittalis externa verlaufenden Fossae temporales (Abb. 1—5, *7*) begrenzen medialwärts und vorne beim dolichocephalen Hund die Crista sagittalis externa und die Linea temporalis, beim Brachycephalen dagegen hauptsächlich die Linea temporalis. Die Basis der Schläfengrube bilden, den drei Knochen entsprechend, drei Teile: die hinten liegende Pars parietalis, die etwas davor lokalisierte Pars frontalis und die lateral befindliche Pars temporalis.

Zwischen den beiden Lineae temporales liegt in der Medianebene bzw. in der Querebene des hinteren Endes der Nasenbeine die Fossa frontalis (Abb. 1, *8*); diese Stelle, die Glabella, entspricht der Ebene der Lamina cribrosa; die Ausbreitung dieses fünfeckigen Gebildes hängt mit der Größe der Stirnhöhle zusammen.

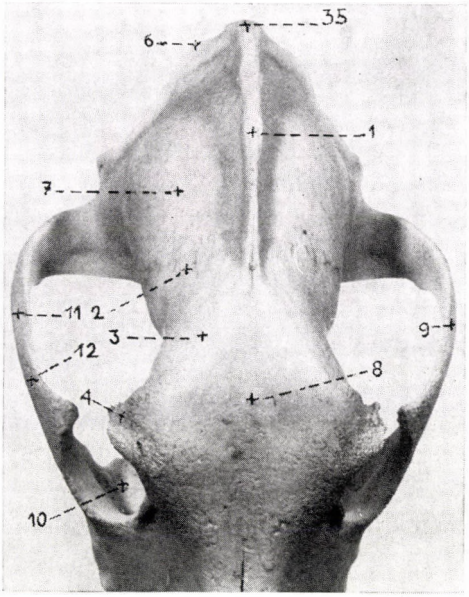

Abb. 1

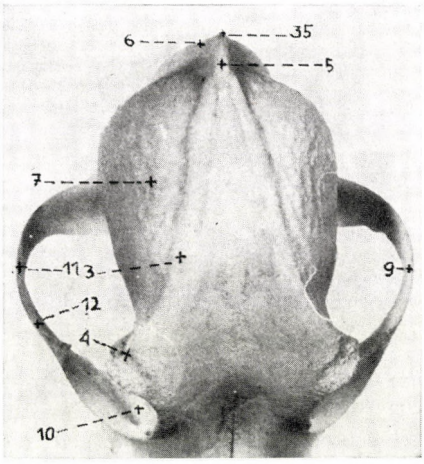

Abb. 2

Abb. 1 und *2*. Die Hirnschädelknochen des dolichocephalen und des brachycephalen Hundes (Norma frontalis). 1, Crista sagittalis externa (dolichocephaler Hund; Abb. 1); 2, Sutura coronalis (Abb. 1); 3, Linea temporalis; 4, Processus zygomaticus ossis frontalis; 5, Crista sagittalis externa (brachycephaler Hund; Abb. 2); 6, Crista nuchae; 7, Fossa temporalis; 8, Fossa frontalis; 9, Arcus zygomaticus; 10, Orbita; 11, Processus zygomaticus ossis temporalis; 12, Processus temporalis ossis zygomatici; 35, Protuberantia occipitalis externa

Laterale Fläche des Hirnschädels

(Planum laterale, Norma temporalis)

Die Charakteristika dieser Fläche sind der sich vorwölbende Arcus zygomaticus (Abb. 1—7, *9*) und die Orbita (Abb. 1—5, *10*). Der stark entwickelte Jochbogen bildet zwischen Hirnschädel und Gesichtsschädel lateral und dorsal eine konvexe Knochenbrücke; der lateromediale Abschnitt des vorderen Zweidrittels und der dorsoventrale hintere Drittel sind flach; seine Bestandteile sind der Processus zygomaticus des Schläfenbeins (Abb. 1—7, *11*) und der Processus temporalis des Jochbeins (Abb. 1—7, *12*). Beim hinteren Ende befindet sich der Porus acusticus externus (Abb. 3—5, *13*) und ventral davon die Bulla tympanica (Abb. 3—7, *14*), welche sich von der ventralen Seite her besser erkennen läßt. Der breite, flache, verhältnismäßig kleine Processus jugularis (Abb. 3—7, *15*) verläuft caudoventral hinter der Bulla tympanica und seitwärts der Condyli occipitales des Hinterhauptbeines (Abb. 3—7, *16*). Der nach vorne und seitwärts gerichtete Eingang der Orbita ist auf dem Schädel des brachycephalen Hundes rund und auf dem der Dolichocephalen unregelmäßig oval. Im hinteren-inneren Teil der Augenhöhle befinden sich hintereinander drei Öffnungen: der Canalis opticus (Abb. 5, *17*), die Fissura orbitalis (Abb. 4, *18*) und das Foramen alare rostrale (Abb. 3, 4, und 6, 7, *19*); dazu gesellen sich noch die anterodorsal lokalisierten, winzigen Foramina ethmoidalia (Abb. 3, 5, *20*). Auf der medialen Wand der Augenhöhle sondert sich die Crista orbitalis ventralis in einem dorsal gebogenen Bogen ab, sie bedeutet die obere Grenze der Ursprungsstelle des M. pterygoideus.

Ventrale Fläche des Hirnschädels

(Planum ventrale, Norma basilaris)

Diese erstreckt sich vom Foramen magnum bis zum freien Rand der horizontalen Gaumenplatte (Margo liber). Die hintere Grenze bilden die beiden Condyli occipitales (Abb. 3—7, *16*) mit der interkondylären Inzisur. Proximal der ventralen Fläche befindet sich, teils zwischen den beiden Partes tympanicae, die Pars basilaris ossis occipitalis (Basioccipitale) (Abb. 6, 7, *21*). Das mit der Oberfläche der Bulla tympanica medial zusammenhängende Tuberculum musculare (Abb. 6, 7, *22*) ist eine niedrige, sagittale, längliche Protuberantia mit ungleichmäßiger Oberfläche. In der von der Bulla tympanica (Abb. 3—7, *14*), dem Condylus occipitalis (Abb. 3—4, *16*) und dem Processus jugularis (Abb. 3—4, *15*) umgebenen Fossa condylaris ventralis (Abb. 6—7, *23*) öffnet sich in Form eines winzigen, runden Lochs das Foramen hypoglossi

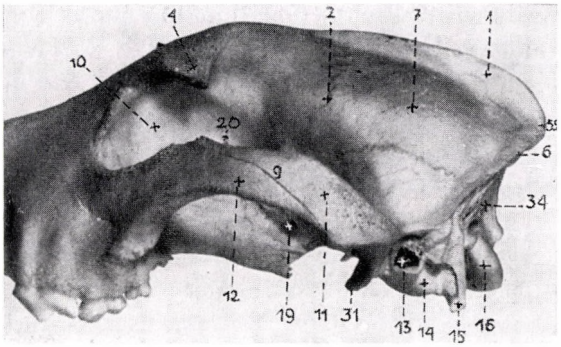

Abb. 3

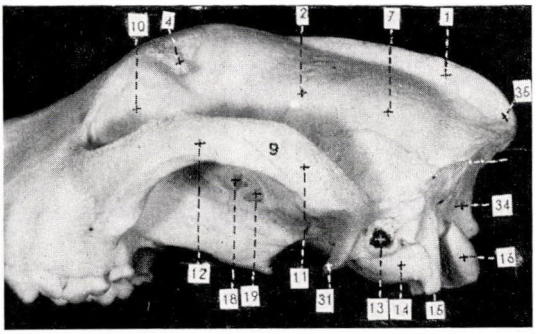

Abb. 4

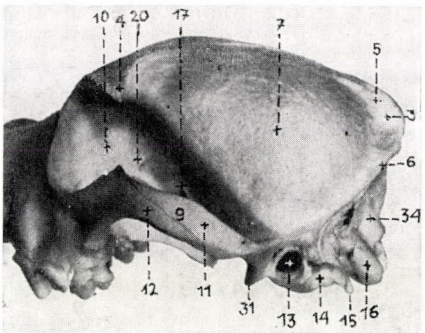

Abb. 5

Abb. 3, 4 und *5*. Die Hirnschädelknochen des dolichocephalen und des brachycephalen Hundes (Norma temporalis). 1, Crista sagittalis externa (dolichocephaler Hund; Abb. 3, 4); 2, Sutura coronalis (Abb. 3, 4); 4, Processus zygomaticus ossis frontalis; 5, Crista sagittalis externa (brachycephaler Hund; Abb. 5); 6, Crista nuchae; 7, Fossa temporalis; 9, Arcus zygomaticus; 10, Orbita; 11, Processus zygomaticus ossis temporalis; 12, Processus temporalis ossis zygomatici; 13, Porus acusticus externus; 14, Bulla tympanica; 15, Processus jugularis; 16, Condylus occipitalis; 17, Canalis opticus (Abb. 5); 18, Fissura orbitalis (Abb. 4); 19, Foramen alare rostrale (Abb. 3, 4); 20, Foramen ethmoidale (Abb. 3, 5); 31, Processus retroarticularis; 34, Squama occipitalis; 35, Protuberantia occipitalis externa

(Abb. 6, 7, *24*). Zwischen diesem Gebilde und der Bulla tympanica in Sutura petrooccipitalis nehmen das länglich, querlaufende Foramen jugulare (Abb. 6, 7, *25*) und Foramen caroticum externum (Abb. 6, 7, *26*) Platz. Der Processus jugularis ist mit der hinteren Fläche der Bulla tympanica verwachsen. Zwischen der Bulla tympanica und dem Körper des Hinterhauptbeines liegt — lateral vom Foramen caroticum externum — der Semicanalis tubae auditivae (Tuba auditiva ossea) (Abb. 6, 7, *27*). Das umfangreichste Loch auf diesem Gebiet ist das der Fossa mandibularis medial liegende Foramen ovale (Abb. 6, 7, *28*); unmittelbar davor befindet sich das Foramen alare caudale (Abb. 6, 7, *29*). Die Fossa mandibularis ist eine glatte Gelenkfläche (Abb. 6—7, *30*) auf der hinteren-unteren Fläche des Arcus zygomaticus. Dem Processus retroarticularis medial (Abb. 3, 7, *31*) öffnet sich in Form einer winzigen, eirunden, trichterförmigen Öffnung die Fissura petrotympanica *(Glaseri)* (Abb. 6, 7, *32*). Die auf der hinteren Fläche des Fortsatzes befindliche kleine Furche bildet einen Teil des Foramen retroglenoideum (Abb. 6, 7, *33*).

Dort wo der Temporalflügel des Keilbeins und das Basisphenoidale zusammentreffen, befindet sich der kurze Canalis alaris (Abb. 6, 7, *19*), während vom Körper des Keilbeins, dem Processus pterygoideus sowie dem Os pterygoideum umgeben der Canalis pterygoideus — zu dem der kurze Sulcus pterygoideus führt — liegt.

Hintere Fläche des Hirnschädels

(Planum nuchale; MILLER)

Die hintere Fläche des Hirnschädels hat die Form eines unregelmäßigen Dreiecks. Die Seitenteile sind die Squama occipitalis (Abb. 3, 4, *34*) mit den Condyli (Abb. 3, 4, *16*) und den Processus jugulares (Abb. 3—7, *15*), während den medioventralen Teil die Pars basilaris (Abb. 6, 7, *21*) mit der Incisura condylaris bildet. Die lateralwärts liegende Crista nuchae (Abb. 1—5, *6*) trennt dieses Gebiet von der Fossa temporalis ab. Die Protuberantia occipitalis externa (Abb. 1—5, *35*) befindet sich auf dem hinteren Ende der Crista sagittalis externa. Das Foramen magnum ist breit und häufig asymmetrisch (Abb. 6, 7, *36*). Die Fossa condylaris ventralis (Abb. 6, 7, *23*) grenzt lateral die Gelenkknorren vom Processus jugularis ab. Im vorderen Teil der Fossa befindet sich das Foramen hypoglossi (Abb. 6, 7, *24*). Auf dem Schädel von Jungtieren nimmt lateral vom Processus jugularis die Sutura occipitomastoidea Platz. Der Processus mastoideus — der eigentlich die Pars mastoidea des Schläfenbeins ist — befindet sich dorsal vom Foramen stylomastoideum (Abb. 6, 7, *37*), welches Gebilde vor dem Processus jugularis und oberhalb der Bulla tympanica liegt.

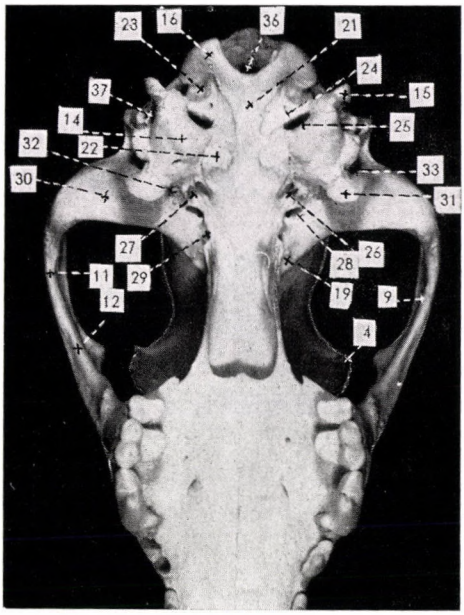

Abb. 6

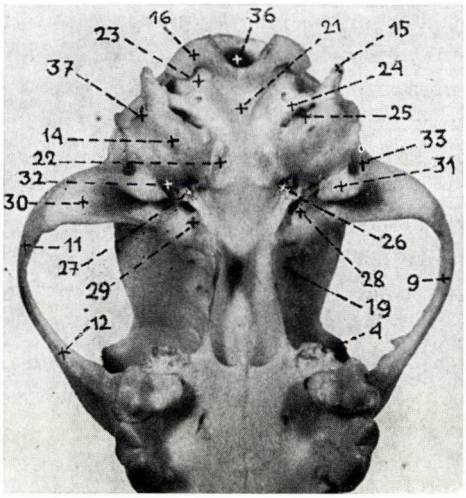

Abb. 7

Abb. 6 und *7*. Die Hirnschädelknochen des dolichocephalen und des brachycephalen Hundes (Norma basilaris). 4, Processus zygomaticus ossis frontalis; 9, Arcus zygomaticus; 11, Processus zygomaticus ossis temporalis; 12, Processus temporalis ossis zygomatici; 14, Bulla tympanica; 15, Processus jugularis; 16, Condylus occipitalis; 19, Foramen alare rostrale; 21, Pars basilaris ossis occipitalis; 22, Tuberculum musculare; 23, Fossa condylaris ventralis; 24, Foramen hypoglossi; 25, Foramen jugulare; 26, Foramen caroticum externum; 27, Semicanalis tubae auditivae; 28, Foramen ovale; 29, Foramen alare caudale; 30, Fossa mandibularis; 31, Processus retroarticularis; 32, Fissura petrotympanica (Glaseri); 33, Foramen retroarticulare; 36, Foramen magnum; 37, Foramen stylomastoideum

Röntgenanatomie der Hirnschädelknochen

1. Dorsoventrale Strahlenrichtung

Die hintere Grenzlinie des Schädels bildet die in unregelmäßigem konvexem Bogen seitwärts und nach vorne gerichtete Schattenlinie der auf dem *Os occipitale* liegenden Crista nuchae (Abb. 8—14, *1*). Von hinten begrenzt das Gebiet die einen hellen Schatten gebende Squama occipitalis (Abb. 8—11, *2*), deren verkürzter Schatten sich in den Schatten der Pars basilaris bzw. der Pars lateralis projiziert; der in Richtung des Schädeldachs fallende Abschnitt gibt einen einfachen Schatten von feiner, trabekulärer, schaumiger Struktur. Der sich in der Medianebene dahin projizierende breite, dunkle, streifenartige Schatten der Crista sagittalis externa mit verschwommenem Rand (Abb. 8—11, *3*) teilt den oben beschriebenen Schatten in zwei Teile. Das hintere freie Ende endet im die Protuberantia occipitalis externa nachahmenden Schatten (Abb. 8—11, *4*). Der das Foramen magnum von oben begrenzende Abschnitt der Hinterhauptbeinschuppe ergibt einen dunklen additionalen Schatten, in dem sich die dunklen, scharfumrandeten Schatten der beiden Tubercula nuchalia befinden (Abb. 8—11, *5*). Der auf der inneren (der Schädelhöhle zu liegenden) Fläche der Squama occipitalis befindliche Processus tentoricus gibt keinen entschiedenen Schatten, da der intensiv dunkle Schatten der Crista sagittalis externa den Fortsatz — den auch der Hauptstrahl senkrecht betrifft — bedeckt. Der Schatten des in der Medianebene lokalisierten Abschnitts des Hinterhauptkörpers erinnert an ein gleichschenkliges Dreieck und ist von feinschwammiger Struktur (Abb. 8—11, *6*). Der helle, länglichrunde Schatten des Foramen magnum (Abb. 8—11, *7*) ist teils durch dem Schatten des Hinterhauptbeins bedeckt; auf der Röntgenaufnahme tritt auch die durch den hinteren Rand des Basiooccipitale gebildete Grenzlinie des vorderen-unteren Rands der Öffnung (Abb. 8—11, *8*) in Erscheinung, welche auch die Fossa intercondylaris (ZIMMERMANN, 1939) begrenzt. In der Nähe des vorderen Basiooccipital-Endes ist der eirunde, eine schaumige Struktur und einen unregelmäßigen Rand aufweisende Schatten der beiden Tubercula muscularia (Abb. 8—11, *9*) ersichtlich. Gegen die Mitte des Körpers kann mitunter der Schatten des in der Medianebene liegenden, über eine ungleichmäßige Oberfläche verfügenden Tuberculum pharyngeum erkannt werden (Abb. 9, *9a*).

Die die Basis der hinteren Schädelgrube bildenden Impressio pontina und Impressio medullaris sind in dieser Strahlenrichtung nicht ersichtlich.

Da beim Hund das Foramen lacerum fehlt, besteht das Foramen jugulare aus dem lateralen Rand des Hinterhauptbeinkörpers und dem Felsenbein; dieser länglichrunde, mit dem hinteren Ende lateralwärts ausweichende, helle Lochschatten (Abb. 8—11, *10*) befindet sich am Ansatz der Schatten der Paukenhöhle (Abb. 8—11, *48*) und des Drosselfortsatzes (Abb. 8—11, *11*). Der Schatten des dem Foramen lacerum orale entsprechenden Foramen ovale

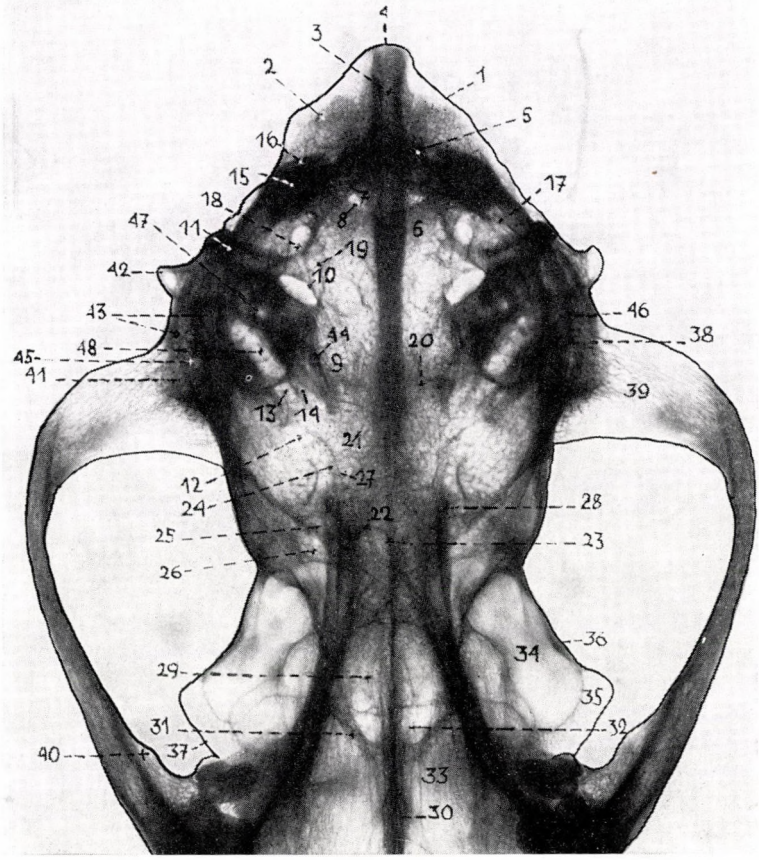

Abb. 8. Röntgenbild der Hirnschädelknochen des dolichocephalen Hundes. Dorsoventrale Strahlenrichtung. 1, Crista nuchae; 2, Squama occipitalis; 3, Crista sagittalis; 4, Protuberantia occipitalis externa; 5, Tuberculum nuchale (Abb. 8, 10, 11); 6, Pars basilaris; Os basioccipitale; 7, Foramen magnum; 8, Unterer Rand des Foramen magnum; 9, Tuberculum musculare; 9a, Tuberculum pharyngeum (Abb. 9); 10, Foramen jugulare; 11, Processus jugularis; 12, Foramen ovale; 13, Öffnung der Tuba Eustachii; 14, Foramen caroticum externum; 15, Condyli occipitales; 16, Jugum condyli occipitalis; 17, Fossa condylaris ventralis; 18, Canalis condylaris; 19, Canalis nervi hypoglossi (Abb. 8, 10, 11); 20, Synchondrosis (synostosis) sphenooccipitalis; 21, Os basisphenoidale; 22, Orbitale Öffnung des Canalis orbitalis (Abb. 8, 10); 23, Lamina perpendicularis (Abb. 8, 10, 11); 24, Canalis alaris; 25, Foramen alare rostrale (Abb. 8, 10, 11); 26, Fissura orbitalis (Abb. 8, 11); 27, Foramen rotundum (Abb. 8, 11); 28, Processus pterygoideus; 29, Os ethmoidale; 30, Septum nasi; 31, Lamina cribrosa; 32, Fossa ethmoidea (Abb. 8, 10, 11); 33, Ethmoturbinalia; 34, Sinus frontalis; 35, Processus zygomaticus ossis frontalis; 36, Linea temporalis; 37, Vorderer Rand des Processus zygomaticus (Abb. 8, 11); 38, Squama temporalis; 39, Processus zygomaticus ossis temporalis; 40, Processus frontalis ossis zygomatici (Abb. 8, 10, 11); 41, Processus retroarticularis; 42, Processus occipitalis ossis temporalis; 43, Pars petrosa et pars tympanica; 44, Crista partis petrosae (crista petrosa) (Abb. 8, 9, 11); 45, Foramen retroarticulare; 46, Canalis temporalis lateralis; 47, Porus acusticus internus; 48, Cavum tympani

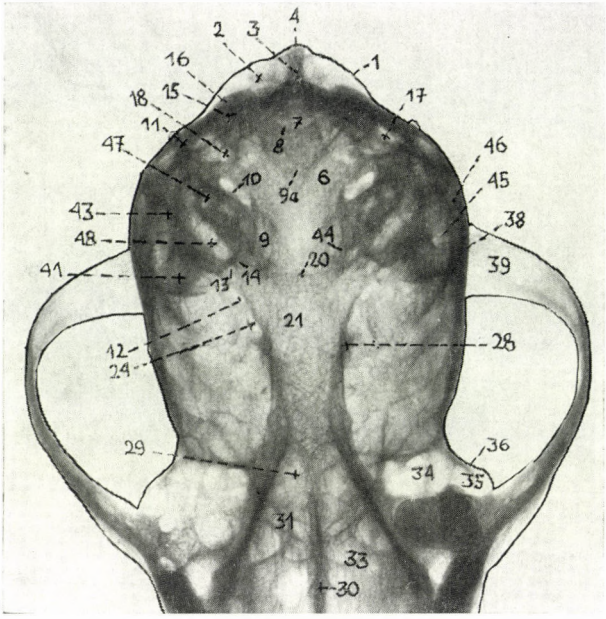

Abb. 9. Röntgenbild der Hirnschädelknochen des brachycephalen Hundes. Dorsoventrale Strahlenrichtung. Erläuterungen s. bei Abb. 8

(Abb. 8—11, *12*) nimmt im Verhältnis zur Öffnung der Hörtrompete vorwärts und nach außen Platz (Abb. 8—11, *13*). Fast zusammengeschmolzen mit dem Foramen ovale bzw. unmittelbar davor läßt sich der verschwommene Schatten des Foramen alare caudale beobachten. In der Nachbarschaft der Tubaöffnung ist medial der Schatten des Foramen caroticum externum ersichtlich (Abb. 8—11, *14*).

Die Condyli occipitales geben einen dunklen Schatten, der die Form eines unregelmäßigen Vierecks hat (Abb. 8—11, *15*). Ihre hintere scharfe Grenzlinie bildet das Jugum condyli occipitalis (Abb. 8—11, *16*) (Kovács u. Mitarb., 1964). Die Schatten der beiden Gelenkknorren nehmen den länglichrunden Schatten des Foramen magnum in die Mitte (Abb. 8—11, 7). Die vordere Grenze der Condylusschatten bildet der helle Schatten der wohlentwickelten Fossa condylaris ventralis (Abb. 8—11, *17*). An deren Grund kommt die Canalis condylaris in Form eines longitudinalen Schattens zum Vorschein (Abb. 8—11, *18*). Der ventralen Knopfgrube lateral tritt der dunkle, dichte Schatten des bereits erwähnten breiten, flachen, verhältnismäßig kurzen, knollenartig endenden Processus jugularis in Erscheinung (Abb. 8—11, *11*). Verschwommen kann auch der winzige, rundliche Schatten des Kanals, genauer gesagt der Öffnung des N. hypoglossus beobachtet werden (Abb. 8, 10, 11, *19*).

Zwischen dem Hinterhauptbeinkörper und dem Keilbeinkörper ist der in Querrichtung verlaufende, die beiden Tubercula muscularia überbrückende dunkle, streifenartige, über einen verschwommenen Rand verfügende Schatten der Synchondrosis (Synostosis) sphenoccipitalis gut zu sehen (Abb. 8—11, *20*).

Das *Basisphenoidale* und *Praesphenoidale* ergeben auf dem mesocephalen Schädel einen sich nach vorne verschmälernden, mehr oder minder dreieckförmigen Schatten mit feinschaumiger Struktur (Abb. 8 —11, *21*), den auf dem dolichocephalen Schädel der Schatten der Crista sagittalis externa in der Medianlinie halbiert (Abb. 8, *3*).

Beim Hund befindet sich im Keilbeinkörper kein Sinus sphenoideus (MILLER, 1964); es gibt zwar kleinere Höhlen sowohl im Basi-, als auch im Praesphenoidale, diese treten aber auf dem Röntgenbild kaum in Erscheinung.

Bei den Karnivoren kann der Schatten der schwach entwickelten Crista orbitosphenoidea auf dem Röntgenbild überhaupt nicht und der der orbitalen Öffnung des Canalis opticus kaum abgegrenzt werden (Abb. 8—11, *22*). Der Schatten des Rostrum sphenoideum der schwach entwickelten Crista galli übergeht in den Schatten der Lamina perpendicularis des Siebbeins (Abb. 8, 10, 11, *23*).

An der Grenze des Temporalflügels und des Körpers des Keilbeins verläuft vom Schatten des Foramen ovale (Abb. 8—11, *12*) der verhältnismäßig breite bogenförmige Schatten des Canalis alaris (Abb. 8—11, *24*) nach vorne und auswärts in Richtung der Schatten des Foramen alare rostrale (8—10, *25*) und der Fissura orbitalis (Abb. 8—11, *26*). Auf der Röntgenaufnahme erscheint der Schatten des von der Schädelhöhle her in den Canalis alaris führenden Foramen rotundum (Abb. 8—11, *27*). Zwischen dem Temporalflügel und der Pars tympanica des Felsenbeins ist der Schatten des Foramen caroticum externum ersichtlich (Abb. 8, 9, 11, *14*).

Der Processus pterygoideus des Keilbeins und das dazugehörende Flügelbein sowie der Hamulus desselben ergeben einen am hinteren Ende tuberkelartigen und sich nach vorne verschmälernden lamellösen Schatten (Abb. 8—11, *28*); die doppelte-dreifache Konturlinie ergibt sich aus den medialwärts gebogenen, konvex-konkaven Fortsätzen.

Der Schatten des *Os ethmoidale*, welcher Knochen die vordere Wand der Schädelhöhle sowie die Basis der Nasenhöhle bildet, und gleichzeitig die Schädelhöhle von der Nasenhöhle trennt, befindet sich an der Grenze von Schädel- und Nasenhöhle (Abb. 8—11, *29*) bzw. zum Teil in die Nasenhöhle projiziert. In seinem Schatten nimmt auch ein Teil des Schattens des umfangreichen Sinus frontalis Platz. Unter den Siebbeinplatten ist die Lamina mediana s. perpendicularis gut dargestellt (Abb. 8, 10, 11, *23*), indem ihr Schatten in den des Septum nasi (Abb. 8—11, *30*) übergeht. Die Lamina cribrosa des Siebbeins gibt einen intensiv dunklen, streifenartigen Schatten

(Abb. 8, 10, 11, *31*), da der Hauptstrahl die Kante des Gebildes betrifft. Unter den Siebbeinlöchern kommt in dorsoventraler Strahlenrichtung der Schatten der größeren Löcher zum Vorschein.

Die durch die Lamina cribrosa umgrenzten Fossae ethmoideae (Abb. 8, 10, 11, *32*) lassen sich gut darstellen (die Riechkolben des Hundes sind hochentwickelt). Der Schatten der Siebbeinlabyrinthe (Abb. 8—11, *33*) tritt auf der Basis der Nasenhöhle, in Form feiner, dünner, sagittaler Linienschatten in Erscheinung.

Beim Hund ist das *Os interparietale* bereits vor der Geburt mit der Hinterhauptbeinschuppe verwachsen, folglich sondert sich der Schatten des Knochens nicht ab. Obschon der Processus tentoricus wohl entwickelt ist und einen großen Teil des Tentorium cerebelli osseum bildet, kann sein Schatten auf dem Schädel des dolichocephalen Hundes vom breiten Schatten der Crista sagittalis externa nur schwerlich differenziert werden, da der Hauptstrahl das fragliche Gebilde seiner Fläche senkrecht betrifft; auf der sagittalen Aufnahme gewinnt man einen gut differenzierbaren Schatten (s. unten).

Das lamellen- bzw. schalenförmige *Os parietale* erreicht der Hauptstrahl in dieser Strahlenrichtung senkrecht, so daß sein Schatten in den der

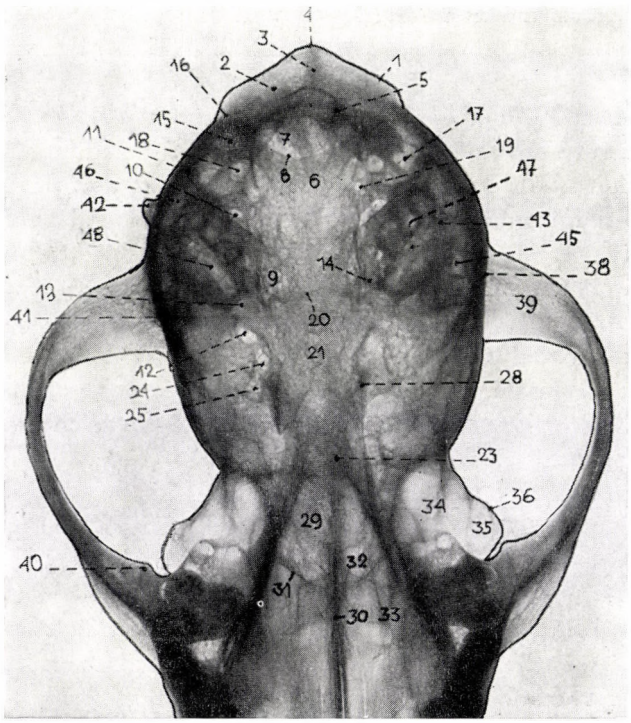

Abb. 10. Röntgenbild der Hirnschädelknochen des mesocephalen Hundes. Dorsoventrale Strahlenrichtung. Erläuterungen s. bei Abb. 8

Schädelbasisknochen einschmilzt; beim dolichocephalen Hund kann ausschließlich der intensive, breite, dunkle streifenförmige additionelle Schatten der Crista sagittalis externa (Abb. 8, *3*) beobachtet werden. Der Schatten der die Fortsetzung des vorderen Endes der Crista sagittalis externa bildenden Linea temporalis tritt auf der Röntgenaufnahme entweder nicht oder verschwommen in Erscheinung; das vordere Crista-Ende bezeichnet den Anfangsteil der beiden Lineae temporales.

In dorsoventraler Strahlenrichtung gibt das *Os frontale* einen schwer analysierbaren Knochenschatten, auf dem fast alleinigen pneumatisierten Knochen erscheint dagegen der helle Schatten des umfangreichen Sinus frontalis (Abb. 8—11, *34*) deutlich umgrenzt. Der Sinus dringt in alle drei Teile des Stirnbeins und auch in den Processus zygomaticus ein (Abb. 8—11, *35*).

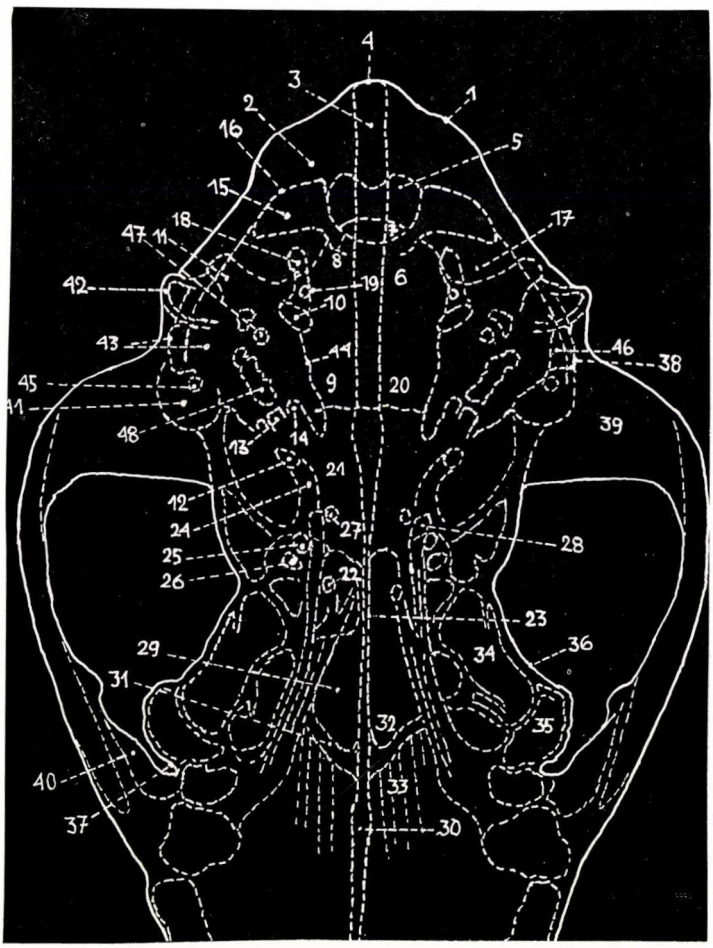

Abb. 11. Röntgenkonturzeichnung der Hirnschädelknochen des dolichocephalen Hundes. Dorsoventrale Strahlenrichtung. Erläuterungen s. bei Abb. 8

Die beiderseitigen Sinus frontales sind in der Medianebene miteinander benachbart, die beiden werden durch das Septum sinuum frontalium voneinander getrennt. Der Schatten des größeren frontolateralen Sinusabschnitts liegt außerhalb des Schattens der vorderen Schädelwand, der Schädelbasisknochen und besonders des Praesphenoidale, so daß dieses Gebiet durch einen deutlich differenzierten, auch die Septa darstellenden, hellen, pneumatischen Schatten gekennzeichnet ist. Der Schatten des sich in den Schatten der Schädelbasisknochen projizierenden hinteren-inneren Teiles sondert sich eher nur auf den von der Schädelbasis verfertigten Aufnahmen ab; der eher die Bezeichnung »Recessus« verdienende Schatten befindet sich an den beiden Seiten der Medianebene.

Von der Linea temporalis ist nur der terminale Abschnitt (Abb. 8—11, *36*) sichtbar; dieser bildet die hintere Grenzlinie des zum Stirnbein gehörenden Processus zygomaticus.

Der eine stumpfe Spitze nachahmende Schatten des Processus zygomaticus (Abb. 8—11, *36*) liegt vor der Schädelhöhle, und hinter dem vorderen Ende des Jochbogens; da der Sinus frontalis in Form eines Recessus in das Gebilde eindringt, ist der Schatten pneumatisch.

Die Grenzlinie des vorderen Randes des Processus zygomaticus — welche zum Teil durch den Schatten der Molaren bedeckt ist — bedeutet die Margo supraorbitalis der Orbita (Abb. 8—11, *37*).

Der sich intensiver vorwölbende Teil der Pars temporalis des Stirnbeins trägt in Form einer additionalen Schattenlinie zur Bildung des Schädelwandschattens bei.

Der Schatten des auf der Pars orbitalis des Stirnbeins liegenden winzigen, mitunter doppelten Foramen ethmoideum (»canalis ethmoideus«) verliert sich zumeist im Schatten des Sinus frontalis (auf der latero-lateralen Aufnahme ist der Schatten deutlich sichtbar).

Beim brachycephalen Hund ist der Sinus frontalis nur in geringem Maße entwickelt, dessen ungeachtet (Abb. 9, *34*) ist aber sein Schatten ausgeprägt.

Die Squama temporalis des Schläfenbeins, *Os temporale*, gibt den Schatten der sich am meisten vorwölbenden Wand der Schädelhöhle (Abb. 8, 11, *38*). Da der Hauptstrahl die Schuppe tangential trifft, gewinnt man einen auf eine dichtere Kompakte weisenden gleichmäßig breiten, dunklen additionellen Schatten. Der Schatten der Schädelwand ist an jener Stelle am breitesten, wo der Processus zygomaticus von der Schuppe ausgeht. Die äußere Grenzlinie des Schädelwandschattens ist scharf, die in Richtung der Schädelhöhle blickende dagegen verwaschen und ungleichmäßig.

Der von der Squama mit breiter Basis ausgehende Processus zygomaticus ist frontobasilar flach, so daß der senkrecht fallende Hauptstrahl von der Fortsatzwurzel einen hellen Schatten mit feinschaumiger Struktur (Abb. 8—11, *39*) ergibt. Der vordere und hintere Rand ahmt einen Kompaktaschatten nach

Der Fortsatz beschreibt einen etwas nach vorne gebeugten Bogen, um sich in seiner Fortsetzung zu verschmälern und auf eine Weise zu drehen, daß seine Flächen bereits seitwärts blicken, d. h. daß die Seite des Fortsatzes flach ist; der Schatten ist charakteristisch. In der Nähe des vorderen Jochbogen-Endes beim inneren Rand kommt in Form eines den Eindruck eines kleinen Fortsatzes bzw. einer Spitze erweckenden Schattens der Jochfortsatz des zum Jochbein gehörenden Processus temporalis (Abb. 8, 10, 11, *40*) zum Vorschein.

Die Jochfortsatz-Wurzel verfügt sowohl vorne, als auch hinten über einen ausgeprägten Rand; am Ansatz des Fortsatzes sondert sich der Schatten des stark entwickelten Processus retroglenoidalis ab (Abb. 8—11, *41*). Bei den Karnivoren lassen sich das Tuberculum mandibulare und die Fossa mandibularis in dieser Strahlenrichtung auf der Röntgenaufnahme nicht differenzieren.

Auf dem Röntgenbild des dolichocephalen Hundeschädels breitet sich der Schatten des wohlentwickelten Processus occipitalis — das ist der dorsalwärts gerichtete, hinter dem Jochbogen liegende Fortsatz der Schläfenbeinschuppe — über die Grenze der lateralen Wand der Schädelhöhle hinaus (Abb. 8, 10, 11, *42*), während sich der weniger entwickelte Fortsatz des brachycephalen Hundes im Schatten des umfangreichen, kugelförmigen Schädels befindet.

Unmittelbar hinter der Jochbogenwurzel tritt in Form eines Vier- oder Rechtecks mit abgerundeten Ecken der helle schattenfleckige, einheitliche, kompakte, dunkle, massive Schatten der Pars petrosa und der Pars tympanica in Erscheinung (Abb. 8—11, *43*); lateralwärts davon befindet sich der Schatten des äußeren Gehörgangs, während die hintere Grenze der dunkle, knollige Schatten des Processus jugularis bildet. Auf der medial lokalisierten Pars petrosa sind der sich in die Schädelhöhle erhebende scharfe Schatten der Crista partis petrosae (Crista petrosa) (Abb. 8, 9, 11, *44*) und am Ansatz desselben der Schatten des Foramen caroticum externum (Abb. 8—11, *14*) ersichtlich.

Am äußeren Rand des erwähnten, größtenteils massiven Schattens der Pars petrosa, am Ansatz der Wurzel des Processus retroglenoidalis kann der helle, rundliche Schatten des Foramen retroglenoideum (Abb. 8—11, *45*) und dahinter, einen gestrichelten Kanal nachahmend, der Schatten des Canalis temporalis (Canalis temporalis lateralis; Abb. 8—11, *46*) erkannt werden. Der Schatten des in der Nähe des Foramen retroglenoideum lokalisierten Porus acusticus externus läßt sich in dorsoventraler Strahlenrichtung weniger deutlich darstellen. Im Schatten der Pars petrosa, vor dem auffallend charakteristischen Foramen jugulare, kommt der helle rundliche Schatten (Abb. 8—11, *47*) des Porus acusticus internus zum Vorschein. Das Cavum tympani ergibt einen longitudinalen, durch feine Septa gegliederten

hellen Schatten (Abb. 8—11, *48*) mit charakteristisch pneumatischer Struktur; an der vorderen-inneren Grenze nimmt der Schatten der Öffnung der Hörtrompete (Abb. 8—11, *13*) Platz.

2. Transversale (sinistrodextrale) Strahlenrichtung

Die hintere Grenzlinie des Schädelschattens bildet das *Os occipitale*, genauer gesagt die Gelenkknorren und die Schuppe des Hinterhauptknochens (Abb. 12, 13, 15, *1*, *2*). Der untere Abschnitt dieser Grenzlinie ergibt sich aus dem das Foramen occipitale umrahmenden scharfen Rand der beiden Kondyli, dort wo sich Gelenkfläche und innere Fläche des Knorrens im Übergangsrand treffen. Der oberhalb der Schatten der Kondyli nach hinten gebeugte Schatten ist die Projektion des oberen Randes des Foramen magnum (Abb. 12—15, *3*), dessen eigentliche Basis die beiden Tubercula nuchalia bilden. Auch die Grenzlinie des scharfen Randes der die Fossa condylaris dorsalis von hinten umrahmenden Kondyli ist deutlich sichtbar (Abb. 12—15, *4*). Der helle, kanalförmige Schatten des Canalis condylaris — welches Gebilde beim Hund die Kondyli durchbohrt — kann im Schatten der Kondyli wahrgenommen werden; auf diesem Gebiet befindet sich in der Nähe des Schattens der Fossa condylaris ventralis auch der Schatten des Foramen jugulare (Abb. 12—15, *6*). Vor dem Schatten der Kondyli liegen die Schatten der Processus jugulares (Abb. 12—15, *7*); sie haben ein kegelartig abgerundetes Ende und sind in Richtung der Basis breiter. Die auf der äußeren Fläche des Processus jugularis befindliche, mit scharfem Knochenrand umrahmte, zum Muskelursprung dienende Furche kann auf dem Röntgenbild des Fortsatzes mitunter deutlich erkannt werden; in den Schatten der Fortsätze projiziert sich quer der untere Rand der Gelenkknorren.

Die ungleichmäßige Grenzlinie der Schuppe der Pars nuchalis (Abb. 12—15, *8*) erreicht die Grenzlinie der Pars parietalis durch die Protuberantia occipitalis externa (Abb. 12—15, *9*). Beim dolichocephalen Hund ist der Schatten der Pars parietalis von grobschaumiger und der der Pars parietalis von feinschaumiger Struktur. Der Genickkamm bildet eine von der Protuberantia occipitalis ausgehende, bilateral, anteroventral, im Schatten des Schuppenabschnitts bis zum vorderen-unteren Ende des Felsenbeins verlaufende Grenzlinie von unregelmäßiger Breite; da die beidseitigen Ränder einander im allgemeinen nicht bedecken, ist diese Linie zumeist verdoppelt (Abb. 12—15, *10*). Der helle, eine schaumige Struktur aufweisende Schatten der auf der Pars parietalis befindlichen Crista sagittalis externa sondert sich beim dolichocephalen Hund vom massiven Schatten des Schädeldachs deutlich ab (Abb. 12—15, *11*).

Die Facies cerebralis der Hinterhauptbeinschuppe hat einen Schatten mit ungleichmäßiger Grenzlinie, während der darauf befindliche Processus

tentoricus (Tentorium cerebelli osseum) einen etwas verschwommenen, hellen Schatten mit unregelmäßigem Rand und schwammiger Struktur ergibt (Abb. 12—15, *12*). Oberhalb des Processus tentoricus sind die Schatten des Canalis transversus meatus temporalis (Abb. 12—15, *13*) sowie des aus diesem Gebilde ausgehenden und sich beiderseitig fortsetzenden Canalis temporalis lateralis ersichtlich; der letzterwähnte Kanal öffnet sich durch das Foramen retroglenoideum.

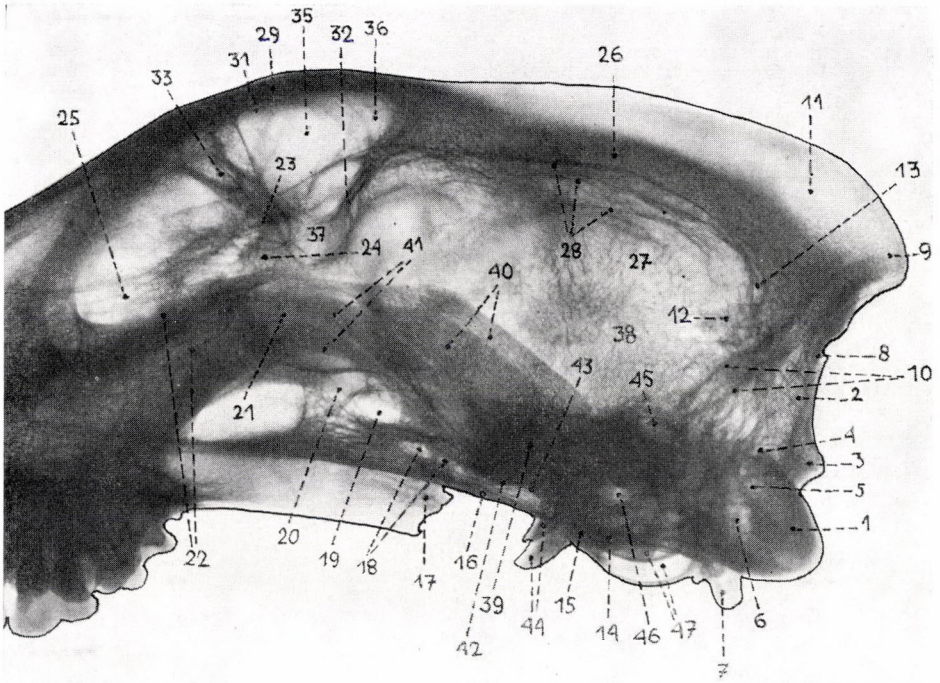

Abb. 12. Röntgenbild der Hirnschädelknochen des dolichocephalen Hundes. Sinistrodextrale Strahlenrichtung. 1, Condyli occipitales; 2, Squama occipitalis; 3, Tuberculum nuchale; 4, Fossa condylaris dorsalis; 5, Canalis condylaris; 6, Foramen jugulare; 7, Processus jugularis; 8, Pars nuchalis squamae occipitalis; 9, Protuberantia occipitalis externa; 10, Crista nuchae; 11, Crista sagittalis externa; 12, Processus tentoricus (Tentorium cerebelli osseum); 13, Canalis transversus meatus temporalis; 14, Pars basilaris; Os basioccipitale; 15, Tuberculum musculare; 16, Os sphenoidale; 17, Processus pterygoideus ossis ethmoidalis; 18, Foramen alare caudale; 19, Fissura orbitalis; 20, Canalis opticus; 21, Foramen ethmoideum (Abb. 12); 22, Lamina cribrosa; 23, Lamina interna ossis frontalis (Abb. 12, 14, 15); 24, Foramina ethmoidalia (Abb. 12, 15); 25, Ethmoturbinalia; 26, Addizionierter Schatten der Crista sagittalis externa (Abb. 12, 14, 15); 27, Planum temporale ossis parietalis; 28, Juga cerebralia; 29, Pars nasofrontalis ossis frontalis (Abb. 12, 14, 15); 30, Pars nasofrontalis ossis frontalis (brachycephaler Hund) (Abb. 13); 31, Innere Grenze der Kompakta der Pars nasofrontalis; 32, Lamina interna ossis frontalis; 33, Processus zygomaticus ossis frontalis; 34, Canalis ethmoideus (foramen ethmoideum; Abb. 13); 35, Sinus frontalis; 36, Kaudomedialer Teil des Sinus frontalis; 37, Recessus sinus frontalis (Abb. 12, 14, 15); 38, Schatten von spongiöser Struktur der lateralen Schädelwand (Abb. 12, 14, 15); 39, Radix processus zygomatici ossis temporalis; 40, Processus zygomaticus ossis temporalis; 41, Grenzlinie der falschen Naht des Schläfenbein- und Jochbeinfortsatzes; 42, Tuberculum articulare (Abb. 12, 14, 15); 43, Fossa mandibularis (Abb. 12, 14, 15); 44, Processus retroarticularis; 45, Felsenbein (Pyramidum); 46, Meatus acusticus externus; 47, Cavum tympani

Die Grenzlinie der äußeren Fläche des flachen, kurzen, breiten Basioccipitale tritt im zusammengesetzten Schatten — obwohl es durch den Processus jugularis und der Pars tympanica des Schläfenbeins bedeckt ist — mit der verhältnismäßig dichten Kompakta (Abb. 12—15, *14*) deutlich hervor; den verschwommenen dunklen Schatten des auf dem Basioccipitale liegenden Tuberculum musculare (Abb. 12—15, *15*) findet man beim vorderen Rand der Pars tympanica.

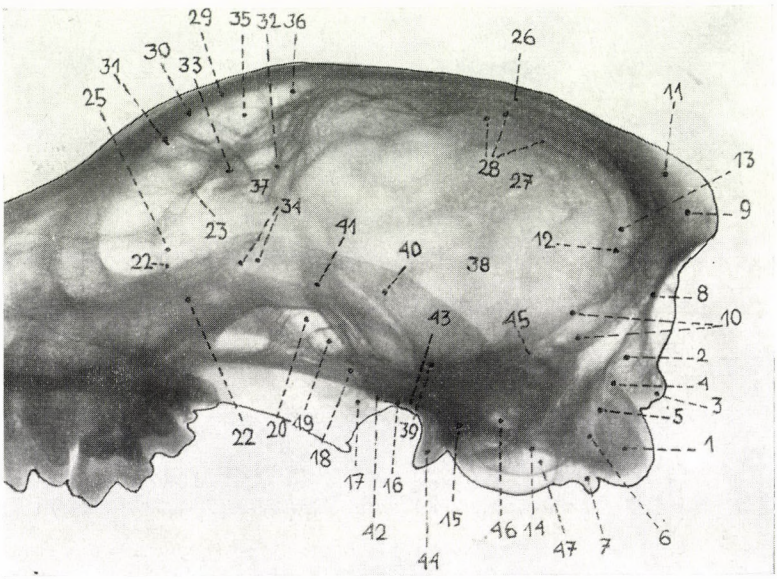

Abb. 13. Röntgenbild der Hirnschädelknochen des mesocephalen Hundes. Sinistrodextrale Strahlenrichtung. Erläuterungen s. bei Abb. 12

Die äußere Fläche des vor dem Hinterhauptbein lokalisierten *Os sphenoidale* bildet in transversaler Strahlenrichtung eine scharfe Grenzlinie (Abb. 12—15, *16*); die poröse Substanz seines verhältnismäßig dicken Körpers wird durch die Wurzel des Jochbogens größtenteils bedeckt; das erwähnte Gebilde bedeckt auch den temporalen und teils den orbitalen Flügel des Keilbeins, der Processus pterygoideus nimmt dagegen, als auf die Knochengrenze deutender Schatten (Abb. 12—15, *17*) teilweise außerhalb des Schädelbasisschattens Platz. Die dorsale und zerebrale Flächen des Keilbeinkörpers umrahmt eine abwechselnd schärfere, dunklere oder hellere verschwommene Grenzlinie; da der hintere Teil durch den dunklen Schatten der Wurzel des Processus zygomaticus bedeckt ist, sind weder die Sella turcica, noch das Dorsum sellae oder der Processus clinoideus ersichtlich (nicht einmal auf der Röntgenaufnahme des der Medianebene entlang halbierten Schädels). Angesichts dessen, daß der Hauptstrahl die Längsachse der Jochbogenwurzel

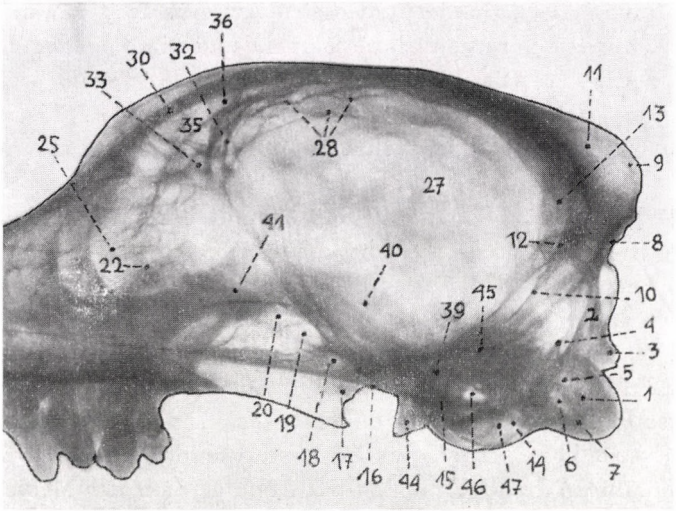

Abb. 14. Röntgenbild der Hirnschädelknochen des brachycephalen Hundes. Sinistrodextrale Strahlenrichtung. Erläuterungen s. bei Abb. 12

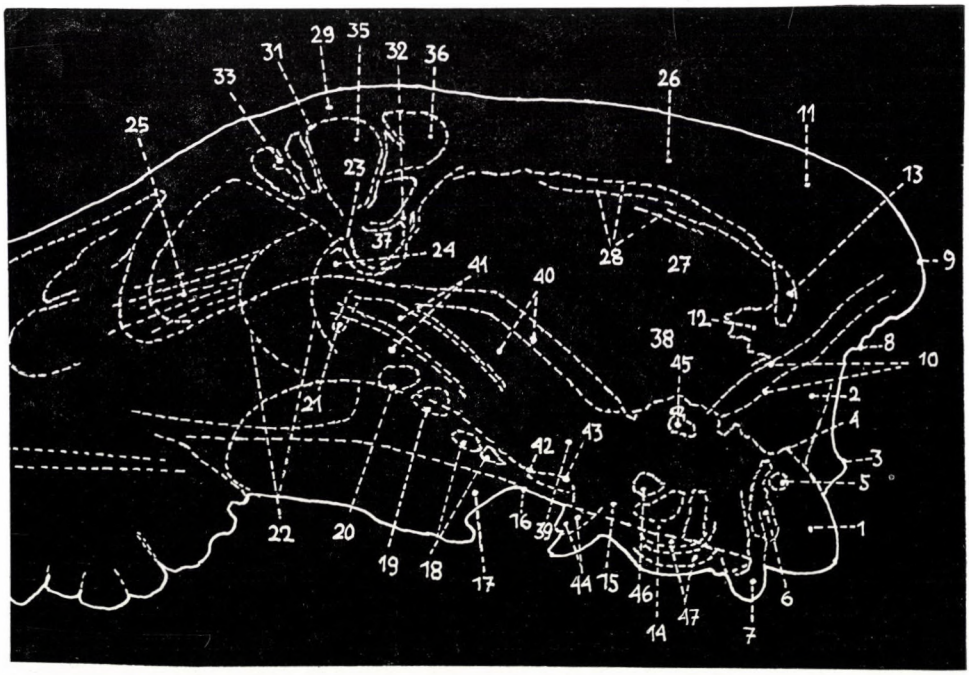

Abb. 15. Röntgenkonturzeichnung der Hirnschädelknochen des dolichocephalen Hundes. Sinistrodextrale Strahlenrichtung. Erläuterungen s. bei Abb. 12

tangiert, ist ihr Schatten dunkel und dicht. Am Ansatz der Wurzel befinden sich die Schatten des Foramen alare rostrale und ein Teil der des Foramen alare caudale (Abb. 12—15, *18*). Vor und oberhalb des Foramen alare rostrale befindet sich der Schatten der Fissura orbitalis (Abb. 12—15, *19*), während über und vor dieser Spalte der Schatten des Canalis opticus (Abb. 12—15, *20*) anzutreffen ist; die äußere und innere Öffnung des Canalis opticus sondern sich voneinander nicht ab. Das auf dem temporalen Flügel befindliche Foramen ethmoideum gibt einen kurzen, kanalförmigen Schatten (Abb. 12, *21*); das schwach entwicklte Rostrum sphenoideum ist auf der Röntgenaufnahme nicht wahrzunehmen.

Der in transversaler Strahlenrichtung am deutlichsten dargestellte Teil des die vordere Wand der Schädelhöhle bildenden *Os ethmoidale* ist die Lamina cribrosa mitsamt den darauf befindlichen Löchern; die sehr feine Schattenlinie ist nach vorne konvex, nach hinten konkav und hat einen verschwommenen ungleichmäßigen Rand (Abb. 12—15, *22*); auf dem der Medianebene entlang halbierten Schädel ist dieser Schatten ausgeprägter. Die Schattenlinie übergeht nach oben und nach hinten in den Schatten der zum Sinus frontalis gehörenden Lamina interna (Abb. 12, 13, 15, *23*). Auf den sagittal liegenden lateralen Teilen der Lamina cribrosa lassen sich die Siebbeinlöcher (besonders auf dem halbierten Schädel) in Form zahlreicher winziger, kreisförmiger, heller Schatten (Abb. 12—15, *24*) beobachten.

Die Ethmoturbinalia erscheinen in Form von sich radiär von der Lamina cribrosa etwas verzweigende, in Richtung der Nasenhöhle verlaufende feine Linien (Abb. 12—15, *25*); sie kreuzen den Schatten der Stirnhöhle; am ausgeprägtesten sind sie auf dem Schädel des brachycephalen Hundes.

Das *Os interparietale*, das beim Hund bereits vor der Geburt mit der Hinterhauptbeinschuppe verwächst, hat einen gut entwickelten Processus tentoricus; der Schatten dieses Gebildes ist sichelförmig, mit fein spongiöser Struktur und hebt sich in Form eines spitz zulaufenden Fortsatzes (Abb. 12—15, *12*) in den Schatten der Schädelhöhle hinein. Auf der Basis des Fortsatzes ist der helle Schatten des quer verlaufenden Canalis transversus meatus temporalis ersichtlich (Abb. 12—15, *13*); auf dem Schädel des brachycephalen Hundes tritt dieses Gebilde deutlicher in Erscheinung, als beim mesocephalen Hund.

Auf den Plana parietalia der das Schädeldach und teilweise auch die Seitenwand des Schädels bildenden beiden *Ossa parietalia* gibt die in der Medianebene liegende, mächtig entwickelte Crista sagittalis externa einen dunklen additionellen Schatten von dicht spongiöser Struktur (Abb. 12, 13, 15, *26*), der an beiden Seiten in einen hellen Schatten mit ähnlich spongiöser Struktur übergeht (Abb. 12—15, *11*). Der Schatten des Planum parietale setzt sich verschwommen im eine schaumige Struktur aufweisenden Schatten des Planum temporale fort (Abb. 12—15, *27*). Auf der der Schädelhöhle zu

fallenden Fläche des Scheitelbeins sind den longitudinalen Furchen der Großhirnhemisphären entsprechend, die kräftig entwickelten longitudinalen Juga cerebralia ersichtlich, welche intensiv dunkle, in parallelen Bogen verlaufende linienförmige Schatten (Abb. 12—15, *28*) ergeben. Auf der halbierten und mit scharfem Licht durchleuchteten Schädelwand befinden sich die Juga cerebralia sichtlich den zwischen den einzelnen Großhirwindungen verlaufenden Furchen entsprechend; auch die SYLVIUSsche Fissur läßt sich deutlich erkennen. Der sich nach vorne stufenweise verschmälernde Schatten der Crista sagittalis externa endet beim Ausgangspunkt der beiden Lineae temporales in spitzem Winkel.

Auf dem Schädel des mesocephalen und noch eher des brachycephalen Hundes ist die Crista sagittalis externa lediglich in der Nähe der Crista nuchae ausgeprägt, an jener Stelle, wo der helle, einen kleinen Kamm nachahmende Schatten mit fein spongiöser Struktur (Abb. 13, 14, *11*) zum Vorschein kommt. Auf der dorsalen Wand des brachycephalen Schädels ergibt das Schädeldach in der Fossa frontalis einen, einer massiven Kompakta ähnlichen, gleichmäßig dicken, dunklen Schatten, welcher sich im Schatten der das Schädeldach bildenden Scheitel- und Stirnbeine befindet.

Die Pars nasofrontalis des *Os frontale* ist beim dolichocephalen Hund, der Linea temporalis entsprechend, stark gewölbt und tritt auf der Röntgenaufnahme in Form eines dicken additionalen Schattens (Abb. 12, 13, 15, *29*) in Erscheinung. Auf dem brachycephalen und mitunter auch auf dem mesocephalen Schädel ergibt dieses Gebilde einen die Stirnhöhle kreuzenden dicken, scharfkantigen, gleichmäßig breiten, dunklen Kompaktaschatten (s. oben, Abb. 14, *30*). Die äußere Stirnbeinplatte erscheint auf dem Röntgenbild als dicker, dunkler Kompaktaschatten mit einer nach außen scharfer, und in Richtung der Stirnhöhle verschwommener Grenze (Abb. 12, 13, 15, *31*). Die Lamina interna des Stirnbeins, die die Stirnhöhle von unten umrahmt, geht vom Schatten der Siebplatte aus und verläuft in Wellenlinienform nach rückwärts (Abb. 12—15, *32*).

Unter den Fortsätzen des Stirnbeins ergibt der Processus zygomaticus, im Gegensatz zum Processus nasalis, einen deutlich bewertbaren Röntgenschatten (Abb. 12—15, *33*). Der Processus zygomaticus, in den die Stirnhöhle in Form eines Recessus eindringt, erscheint auf dem Röntgenbild — und zwar besonders ausgeprägt auf der den halbierten Schädel darstellenden Aufnahme — im Schatten der Stirnhöhle, in Form eines dreieckigen pneumatischen Schattens. Der Processus zygomaticus und die Pars nasofrontalis nehmen einigermaßen auch in der Bildung des Schattens des Anulus orbitalis teil. In der senkrecht fallenden Strahlenrichtung gibt die Pars orbitalis keinen abgrenzbaren Schatten, der Schatten des an dieser Stelle befindlichen, manchmal doppelten Foramen ethmoideum, genauer gesagt »Canalis ethmoideus« (Abb. 13, *34*), — die einen die Pars orbitalis quer kreuzenden Kanal bildet — tritt

jedoch in Erscheinung. Der in den Schatten der Pars temporalis projizierte Schatten des Sinus frontalis verliert sich in der senkrecht fallenden Strahlenrichtung.

Der Sinus frontalis gibt beim dolichocephalen Hund einen umfangreichen Schatten (Abb. 12—15, *35*), während auf dem mesocephalen und brachycephalen Schädel der Schatten weniger umfangreich ist. Die Grenze des kaudomedialen Abschnitts der Stirnhöhle befindet sich beim dolichocephalen Hund am vorderen Ende der Crista sagittalis externa, beim Initialabschnitt der beiden Lineae temporales (Abb. 12—15, *36*); die Grenzlinie, die sich ausschließlich auf das Stirnbein ausbreitet, erreicht das vordere Ende der Pars nasofrontalis auf den beiden Seiten der Siebplatte, die vordere, etwas verschwommene Grenze verliert sich in dieser Strahlenrichtung gewissermaßen im Schatten der Ectoturbinalia bzw. der Nasenmuschelhöhlen. Im Schatten des Sinus frontalis lassen sich die Schatten der die Zellen abscheidenden Knochensepta beobachten. An den beiden Seiten der Medianebene projiziert sich in den Schatten der Schädelhöhle ventral je ein Recessusschatten (Abb. 12, 13, 15, *37*).

Die Schuppe des *Os temporale*, welche der Hauptstrahl senkrecht betrifft, ergibt anstatt eines selbständigen Schattens, einen Teil des eine spongiöse Struktur aufweisenden Schattens der Schädelwand (Abb. 12, 13, 15, *38*). Auf dem Röntgenbild des halbierten Schädels läßt sich als Grenze des erwähnten Schattens ein unregelmäßiger, halbkreisförmiger, dunkler Kondenzstreifen erkennen. Der von der Schuppe lateralwärts ausgehende Processus zygomaticus hat, da der Hauptstrahl seine Längsachse betrifft, einen dunklen, dichten Schatten mit verwaschener Grenze (Abb. 12—15, *39*), der sich vom ebenfalls dichten, auf winzige pneumatische Höhlen weisenden Schatten des dahinter liegenden Felsenbeins kaum unterscheiden läßt. Auf der oberen und unteren Kante des ausgeprägten temporalen Jochfortsatzes tritt ein Kompaktaschatten mit feinspongiöser Struktur in Erscheinung (Abb. 12—15, *40*), der sich nach vorne, wie ein spitzer Fortsatz, stufenweise verschmälert. Der Jochfortsatz des Stirnbeins und der temporale Fortsatz des Jochbeins treffen sich in einer hellen Schattenlinie (Abb. 12—15, *41*). Dort wo der transversale Abschnitt des Jochfortsatzes in den sagittalen Abschnitt übergeht, sondert sich — insbesondere auf dem halbierten Schädel — der ausgeprägte, höckerartige Schatten des Tuberculum articulare (Abb. 12, 13, 15, *42*) ab. Auf dem Röntgenbild läßt sich auch die Fossa mandibularis (Abb. 12, 13, 15, *43*) erkennen, obwohl das Gebilde bei den Karnivoren weniger entwickelt ist. Der intensive, einen vorwärts gebeugten Hacken nachahmende, den Schatten der Schädelbasis überschreitende Schatten des Processus retroglenoideus (Abb. 12—15, *44*) tritt auf den drei Schädeltypen gleich deutlich in Erscheinung. Der Schatten des den äußeren Gehörgang umgebenden Processus aboralis schmilzt mit dem dunklen Schatten des Felsenbeins zusammen und befindet sich im oberen

Teil desselben. Die wellenförmige Schattenlinie der Crista nuchae passiert anteroventral den dunklen Schatten des Felsenbeins (Abb. 12—15, *45*) und verliert sich demnach im Schatten des Processus retrotympanicus. Der Schatten des äußeren knöchernen Gehörganges umfaßt den umfangreichen, rundlichen, hellen Schatten des äußeren Gehörganges (Abb. 12—15, *46*) ringartig. Im Felsenbein befinden sich mehrere rundliche Schatten, welche die Öffnung des inneren Gehörganges, das Foramen stylomastoideum, den Canalis temporalis und schließlich den pneumatischen Schatten der Paukenhöhle repräsentieren; das letzterwähnte Gebilde umrahmt in Form einer dünnen, dunklen Schattenlinie die dünne Wand der Pars tympanica. In der Pars petrosa läßt sich die Höhle des Labyrinthus osseus unterscheiden. Der verhältnismäßig umfangreiche helle Schatten der Paukenhöhle (Abb. 12—15, *47*) projiziert sich in den Schatten der die Schädelbasis bildenden Knochen; die Höhle wird durch den Hinterhauptbeinknochen halbiert. Die untere Hälfte der Höhle liegt außerhalb des Schattens der Schädelbasis, während der hintere Rand an den Processus jugularis stößt.

ZUSAMMENFASSUNG

Von den Hirnschädelknochen verschiedener Hundearten (dolichocephaler, mesocephaler, brachycephaler Hunde) wurden mit weitem Fokus-Film-Abstand Röntgenaufnahmen aus der dorsoventralen und sinistrodextralen Strahlenrichtung gemacht. In beiden Strahlenrichtungen wurde der Fallpunkt der Zentralstrahlen bestimmt. Um die röntgenanatomischen Verhältnisse der Knochen besser verständlich zu machen, wird die deskriptive Anatomie der Schädelknochen des Hundes unter Berücksichtigung der röntgenanatomischen Gesichtspunkte kurz besprochen. Die Autoren beschreiben den Röntgenschatten der Knochen aus den erwähnten beiden Strahlenrichtungen und berücksichtigen hierbei besonders jene Hirnschädelknochenteile, denen vom klinischen und praktischen Gesichtspunkt Bedeutung zukommt. Röntgenaufnahmen und Röntgenkonturzeichnungen illustrieren die ausführliche Beschreibung; in den Aufnahmen und Zeichnungen sind die im Text beschriebenen Schatten und Konturlinien mit Zahlen bezeichnet.

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BEOBACHTUNGEN ZUR EXCYSTATION VON SPOROZOITEN DER KOKZIDIENART EIMERIA STIEDAI

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Untersuchungen über eine in vitro-Excystation von Oocysten wurden schon seit langem durchgeführt. METZNER (1903) stellte fest, daß der die Excystation auslösende Wirkstoff im Pankreassaft enthalten wäre. Aufgrund zahlreicher weiterer Experimente wissen wir heute, daß als exogene Faktoren im wesentlichen eine Sauerstoffverminderung und eine Kohlendioxydanreicherung sowie daran anschließend eine Inkubation in einem Trypsin-Galle-Gemisch bei Temperaturen, die der Körpertemperatur der jeweiligen Wirtsart entsprechen, eine Rolle spielen (zusammenfassende Lit. s. bei KRIEG, 1971). Eine teilweise Auflösung und ein Herausquellen des Stieda- und Substiedakörpers der Sporozysten nach Zusatz von Trypsin und Galle beobachteten ROBERTS u. Mitarb. (1970b) bei *E. callospermophili* und *E. larimerensis*.

Weniger ist über eine aktive Beteiligung der Sporozoiten bei der Excystation bekannt. METZNER (1903) beobachtete die Excystation bei Kaninchenkokzidien und verwies auf Eigenbewegungen der Sporozoiten in den Sporozysten bei der Excystation. Letztere gehe mit einer Einschnürung der Sporozoiten einher und ruckweise vonstatten. Das zugespitzte Vorderteil des Sporozoiten erscheine zuerst außerhalb der Sporozyste und verbreitere sich sofort danach. Weitere zwei bis drei Bewegungen führten ihn ganz heraus. Ähnliche Beobachtungen an Kaninchen-, Geflügel- und Rinderkokzidien stammen von SMETANA (1933), GOODRICH (1944), IKEDA (1960), DORAN und FARR (1962), DORAN und Mitarb. (1962), FARR und DORAN (1962), NYBERG und HAMMOND (1964), NYBERG und Mitarb. (1968) sowie LOTZE und LEEK (1968), wobei immer wieder die über den Körper des Sporozoiten hinweggleitende Einschnürung erwähnt wird.

Zur weiteren Klärung von Einzelheiten der Excystationsvorgänge bei *E. stiedai* wurden sowohl Direktbeobachtungen als auch Filmaufnahmen herangezogen.

Material und Methodik

Für die Untersuchungen wurden etwa 4—8 Wochen alte, sporulierte *E. stiedai*-Oocysten benutzt, die bis zum Untersuchungszeitpunkt bei ca. 4 °C in Kaliumbichromatlösung aufbewahrt worden waren. Zur Excystation wurden die Sporozysten durch Zermörsern aus den Oocysten freigesetzt und nach einmaliger Filtration durch Glasperlen von ca. 100 μ Durchmesser oder

* Mit Unterstützung der Deutschen Forschungsgemeinschaft.

durch Augenwatte (KRIEG, 1971) in physiologischer Kochsalzlösung mit 0,25% Trypsinzusatz (ROSE, 1959) oder 8% Galle- und 0,2% Trypsinzusatz (KRIEG, 1971) suspendiert. Zur Direktbeobachtung der Excystationsvorgänge wurden die Präparate im hängenden Tropfen oder im Deckglaspräparat auf einen Wärmetisch mit etwa 39 °C gebracht und im Hellfeld betrachtet.

Für die Filmaufnahmen erfolgte die Excystation im Deckglaspräparat und Mikroskop-Heizschrank nach HEUNERT (1962) bei 36 °C.

Die Filmaufnahmen wurden mit einer Askania — Z — Kamera unter Verwendung eines Zeiss — WL — Mikroskops (Objektive: Neofluar und Planachromat 100×, Fotookulare 5 und 6,3×) auf 35 mm Normalfilm durchgeführt. Es wurden Phasenkontrast- und Differential-Interferenzkontrastbeleuchtung nach Normarski verwendet. Die Aufnahmegeschwindigkeit betrug 2, 4 und 24 Bilder pro Sekunde. Die Auswertung erfolgte bei bis zu 3000-facher Vergrößerung.

Ergebnisse

a) Verhalten der Sporozoiten in den Sporozysten

In den Sporozysten lagen die Sporozoiten stets so, daß sich das Vorderende des einen Sporozoiten neben dem Hinterende des anderen befand. Die bei Hellfeldbeleuchtung oft opaken und nur geringgradig durchscheinenden Sporozystenhüllen begannen sich kurze Zeit nach dem Trypsinzusatz aufzuhellen und waren nach einigen Minuten meist nur noch mit Mühe zu erkennen. Die Mikropylenöffnung[1] war durch einen in der Interferenzkontrastbeleuchtung kompakt aussehenden, ovoiden Körper verschlossen, dessen Durchmesser in der Breite 2,3 $\pm$ 0,1 μ und in der Höhe 1,5 $\pm$ 0,2 μ (n = 21) betrugen. Frühestens knapp 1 Minute nach dem Zusatz der Trypsin-Galle-Lösung begann er von der Spitze her heller zu werden und war nach einer weiteren $^1/_2$ Minute kaum mehr zu erkennen. Stattdessen setzten sich die Konturen der Sporozystenhülle an seiner ehemaligen oberen Begrenzung nur undeutlich fort, während an seiner unteren Begrenzung eine sich konkav in die Sporozyste hineinwölbende Schicht in Erscheinung trat, die in kontinuierlichem Zusammenhang mit der Sporozystenhülle stand und auch etwa deren Struktur und Dicke aufwies (Abb. 1, *1—3* und Abb. 2, *1—2*).

Nach unterschiedlichen Zeiten (s. Tab II), oft zur gleichen Zeit, in der der Stiedakörper heller wurde oder nicht mehr sichtbar war, begannen sich

[1] Im folgenden wird bis auf weiteres der Begriff Mikropyle für die durch das Stieda-Körperchen verschlossene Ausschlupföffnung der Sporozoiten aus der Sporozyste verwendet. Eine Normierung dieses und anderer Begriffe entsprechend dem von LEVINE (1969) vorgeschlagenen Schema erscheint erstrebenswert.

die Sporozoiten in den einzelnen Sporozysten zu bewegen.[2] Langsam begann das spitzere, nicht den refraktilen Körper enthaltende Ende des Sporozoiten — im folgenden stets als das Vorderende bezeichnet — an der Innenwand der Sporozyste entlang zu gleiten. An einem der Pole angelangt, verharrte es einen

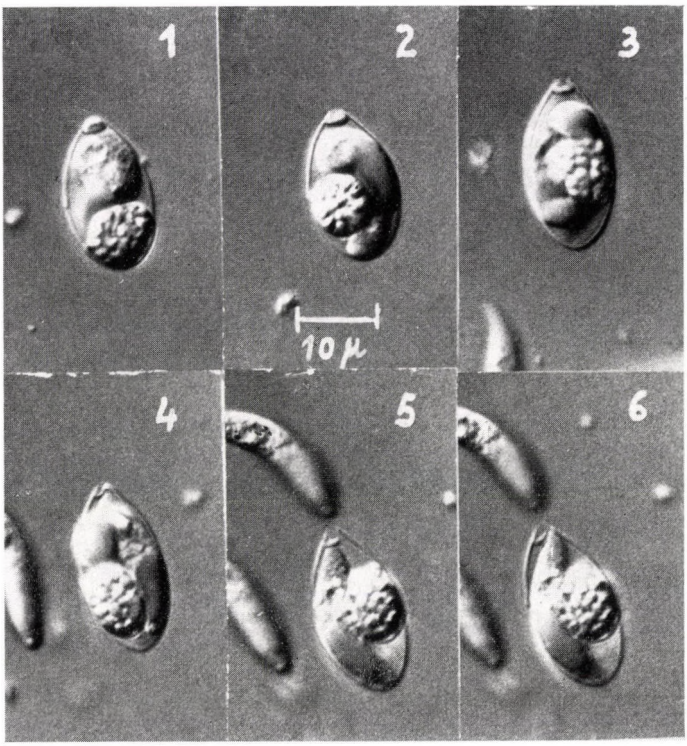

Abb. 1. Auflösung des Stiedakörpers und Verhalten der die Mikropyle verschließenden Schicht. 1—3, Auflösung des Stiedakörpers und Sichtbarwerden des zweiten Mikropylenverschlusses; 4, Der erste Sporozoit drückt die Verschlußschicht der Mikropyle nach außen; 5, Nach der Excystation des ersten Sporozoiten ist der Mikropylenverschluß an der rechten Seite durchbohrt; 6, Der zweite Sporozoit zwängt seine Spitze durch die bestehende Öffnung

Moment, um sich danach entweder unter Stumpferwerden oder mit einer sich ausbildenden stabartigen Verdünnung abzubiegen. Weiter an der Wand entlanggleitend wurde die Biegung des Sporozoiten immer stärker. Schließlich betrug sie nahezu 180° und erfaßte bis zur Mitte des Sporozoitenkörpers fortschreitend immer größere Teile desselben, bis sich nach Überwindung eines toten Punktes der ganze Körper in einer schnelleren gleitenden Bewegung

[2] Ein Durchschnittswert läßt sich, ebenso wie bei fast allen folgenden Zeitangaben aufgrund der großen Streubreite und der Tatsache, daß oft viele Sporozoiten selbst nach bis zu 4-stündiger Beobachtung keine Lebenszeichen erkennen ließen, nicht angeben.

wieder streckte, wobei auch der hintere, den refraktilen Körper enthaltende Körperteil gegen einen inneren elastischen Widerstand etwas abgebogen wurde (Abb. 3).

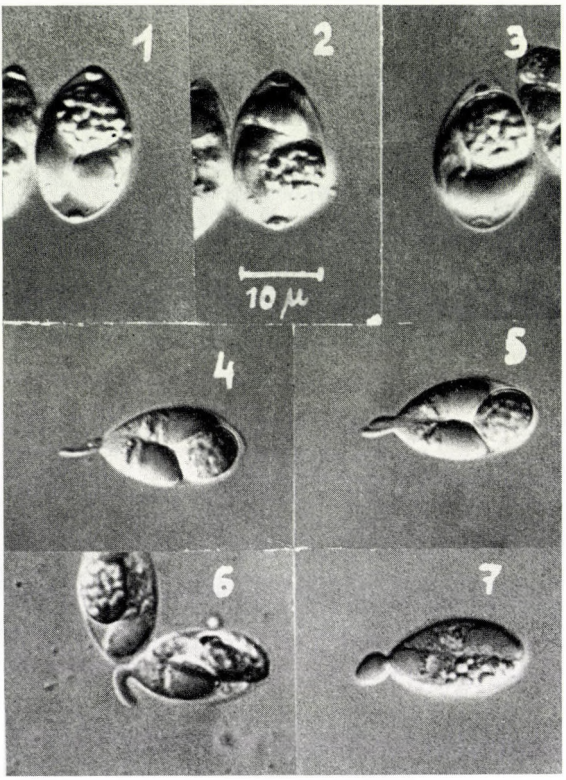

Abb. 2. 1—3, Sporozyste mit einem zweiten Stiedakörper. 1, Zu Beginn der Inkubation mit Trypsin und Galle; 2, Der obere Stiedakörper hat sich vollständig aufgelöst, der untere beginnt sich zu lösen; 3, Nach der Excystation des ersten Sporozoiten ist von dem unteren Stiedakörper nur noch die Begrenzung zu sehen; 4—5, Gleichzeitiger Excystationsversuch beider Sporozoiten einer Sporozyste; 6, Abknicken der verdünnten Apikalregion des zuerst excystierenden Sporozoiten; 7, Excystation eines Sporozoiten mit dem refraktilen Körper voran

Die für eine derartige Wendung benötigte Zeit schwankte zwischen 1 Minute und knapp 4 Sekunden. Vor dem Beginn einer erneuten Wendung wurde häufig eine deutliche Ruhepause eingelegt. Nur ganz selten, meist am stumpferen Sporozystenpol, wurde eine zweite Wendung ohne Unterbrechung angeschlossen. Die höchste Zahl solcher Wendungen, die bei einem Sporozoiten beobachtet werden konnten und die von nur kaum wahrnehmbaren Pausen von 1—2 Sekunden Dauer unterbrochen waren, betrug 15 hintereinander.

Die Bewegungen erfolgten überwiegend in Richtung der Längsachse der Sporozyste, seltener mehr oder weniger schräg dazu in deren Mitte. Sie wurden von einem oder beiden Sporozoiten, abwechselnd oder gleichzeitig,

gegenläufig oder hintereinander hergleitend, durchgeführt. Die anfängliche Lagebeziehung der Sporozoiten zueinander wurde nicht mehr eingehalten, sondern ergab sich aus den vollführten Wendungen. Der als kompaktes Gebilde vorhandene oder in einzelne Granula zerfallene Sporozystenrestkörper geriet fast stets in Bewegung, vereinzelt begann sich die ganze Sporozyste zu drehen. Eine Deformierung der Sporozystenwand erfolgte nicht.

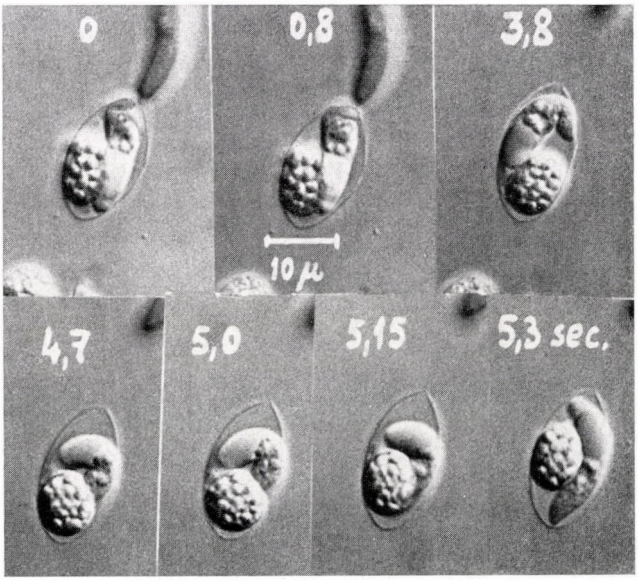

Abb. 3. Wendung eines Sporozoiten in der Sporozyste; Angabe der fortlaufenden Zeit in Sekunden

Ab und zu legten die Sporozoiten längere Ruhepausen ein, in denen einer oder auch beide zusammen sich mit ihrem Vorderende dem Mikropylenverschluß anlegten. Mit gelegentlich leicht tastenden Bewegungen suchte das sich manchmal wieder stabartig verdünnende und 1—2mal abgeknickte Vorderende offensichtlich nach einem Ausgang (Abb. 4, *1—2*). In einigen Fällen konnte beobachtet werden, wie einer der Sporozoiten sein Vorderende durch die Mikropyle wiederholt (in einem Fall innerhalb von knapp 2 Minuten neunmal) bis zu 2,5 μ weit nach außen streckte und wieder zurückzog. Es konnte dabei nicht festgestellt werden, ob der die Mikropyle verschließende Teil der Sporozystenhülle bei diesem Vorgang perforiert wurde bzw. war oder nicht. Die durchschnittliche Dauer dieses Ausstreckens betrug etwa 7 Sekunden, als längste Zeit wurden 15 Sekunden, als kürzeste 4,5 Sekunden gemessen. Im Anschluß an derartige Schlüpfversuche kam es nicht immer zu einer Excystation.

b) Excystation

Aus dem Verhalten der Sporozoiten konnten keine Rückschlüsse auf den Beginn der Excystation gezogen werden. Von den beiden sich in den Sporozysten bewegenden Sporozoiten verweilte einer erneut etwas länger am Mikropylenpol. Sein Vorderende, dessen Verhalten bereits oben beschrieben wurde, schien diesmal intensiver gegen den Verschluß der Mikropyle zu drücken. Ganz vereinzelt war auch zu bemerken, wie dieser leicht nach außen (konvex) ausgebeult wurde. Plötzlich, ohne daß irgendein Übergang zu sehen gewesen wäre, erschien außerhalb der Mikropyle ein dornähnlicher, ca. 0,7—1,2 μ breiter Fortsatz, der sich langsam stabähnlich verlängerte (Abb. 2, 4 und Abb. 4, 3). Bei den zuerst schlüpfenden Sporozoiten konnte seine Länge schließlich bis zu einem Viertel der normalen Sporozoitenlänge von $16{,}6 \pm 1{,}5\ \mu$ (n = 11) erreichen. Gleichzeitig wurde gelegentlich beobachtet, wie sich der in der Sporozyste verbliebene Teil des Sporozoiten verdickte und gegen die Mikropyle drückte (Abb. 4, *4*).

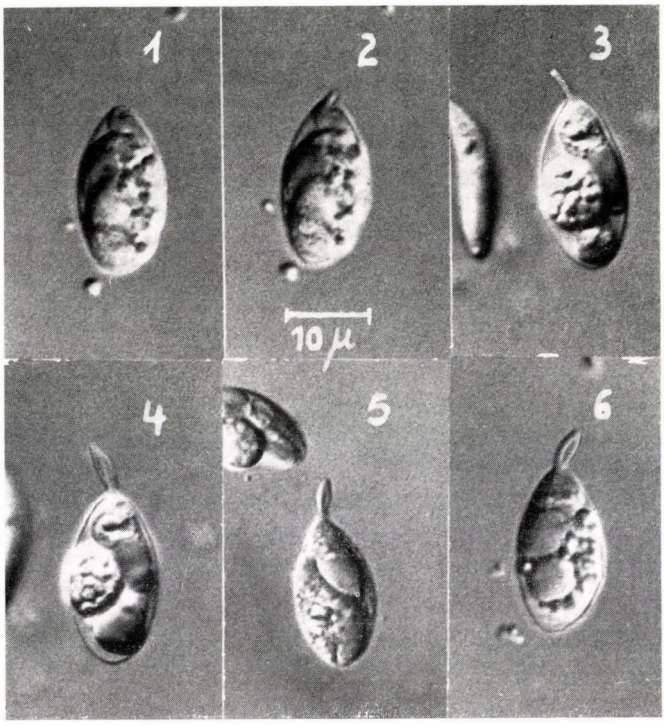

Abb. 4. Einzelphasen des Excystationsbeginns erster Sporozoiten. 1—2, Abknicken in der Sporozyste; 3, Herausstrecken der stabartig verdünnten Apikalregion; 4, Pressen des noch in der Sporozyste befindlichen Sporozoitenkörpers gegen die Mikropyle und dadurch Eindellung der Kernregion; 5—6, Blattartige Verbreiterung der herausgestreckten Apikalregion

In der Regel zeigte der außerhalb der Mikropyle befindliche vorderste Teil des Sporozoiten keine Bewegungen, nur vereinzelt konnte ein passives oder aktives Abbeugen beobachtet werden (Abb. 2, *6*). In einigen Fällen verbreiterte sich dieser stabartige Fortsatz etwas und zeigte in Interferenzkontrastbeleuchtung das Bild eines manchmal von 1 oder 2 Rippen durchzogenen lanzettförmigen, leicht gewölbten Blattes (Abb. 4, *5—6*).

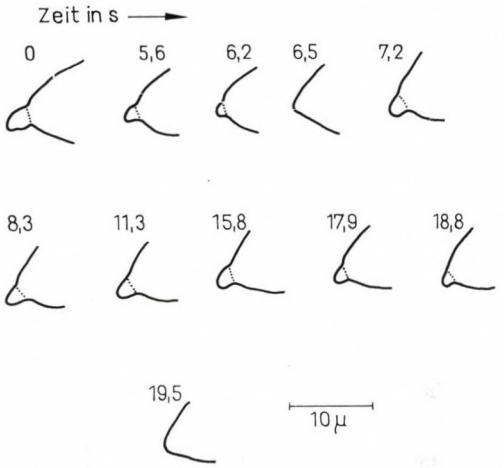

Abb. 5. Excystationsversuche des ersten Sporozoiten durch wiederholtes Vorstrecken und Zurückziehen seines Vorderendes durch die Mikropyle der Sporozyste; Angabe der fortlaufenden Zeit in Sekunden

Nach einiger Zeit begann der nach außen ragende Stab durch nachströmendes Zellplasma dicker zu werden. Erst gestielt schlank lanzettförmig nahm er an Dicke langsam weiter zu, gewann länglich tropfenförmige Gestalt und allmählich war zu bemerken, daß die Zytoplasmamasse des Sporozoiten innerhalb der Sporozyste abnahm. Wie durch eine sanduhrartige Einschnürung floß das Zytoplasma des Sporozoiten kontinuierlich durch den engen Ring der Mikropyle, dessen Durchmesser von ca. 1,2 μ etwa 1/4 bis 1/3 der normalen Sporozoitendicke (4,5$\pm$0,7 μ; n = 11) betrug. Selbst beim Hindurchgleiten des refraktilen Körpers war keinerlei Veränderung der Weite zu sehen.

Der in der Sporozystenhülle verbliebene Teil des Sporozoiten nahm von einer längsovalen Form über die einer Kugel bis zu der eines winzigen Tropfens alle Übergangsformen an, bis er schließlich ganz verschwand. Der nun außen liegende, kommaförmig gebogene Sporozoit verweilte noch unterschiedlich lange direkt vor der Mikropylenöffnung, bis er sich schließlich mit einer meist kurzen, kaum wahrnehmbaren Bewegung von der Sporozyste entfernte.

Die kürzeste ermittelte, für einen derartigen Schlüpfvorgang des ersten Sporozoiten benötigte Zeit betrug 4 Sekunden, die längste 13 Minuten

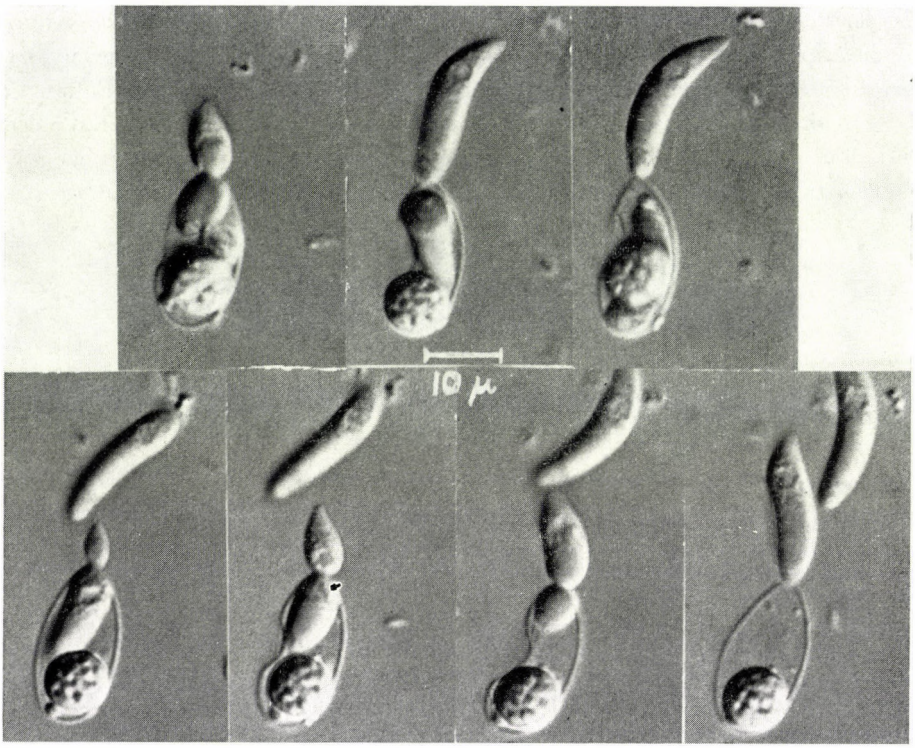

Abb. 6. Reversible Eindellung der Sporozystenhülle im Verlauf der Excystation des ersten und zweiten Sporozoiten

(s. auch Tab. I). Die zum Durchtritt des Vorderendes benötigte Zeit war fast stets länger als die für die hintere Hälfte benötigte.

Der zweite Sporozoit änderte sein Verhalten während des Schlüpfvorganges und nach der Excystation des ersten kaum. Seltener sofort, meist nach kurzer Zeit und ein oder zwei Wendungen folgte er dem ersten Sporozoiten und glitt auf die bereits beschriebene Weise aus der Sporozyste hinaus. In einigen Fällen bewegte er sich jedoch noch mehr als 30 Minuten lang (Ende der Beobachtungszeit) lebhaft in der Sporozyste umher, ohne Anzeichen einer beginnenden Excystation zu zeigen.

Die zum Schlüpfen benötigte Zeit war meist geringer als die des ersten Sporozoiten. Sie variierte in den beobachteten Fällen zwischen 4 Sekunden und 11 Minuten (Tab. I). In der Regel wurde auch der für den ersten Sporozoiten nahezu typische stabartige Fortsatz nicht so deutlich ausgebildet, sondern der außerhalb der Mikropyle befindliche Teil nahm eher tropfenförmige Gestalt an.

Von dem die Mikropyle verschließenden Teil der Sporozystenhülle war meist bereits nach der Excystation des ersten Sporozoiten kaum noch etwas

Tabelle I

Excystationsdauer einzelner Sporozoiten nach Zusatz von Trypsin und Galle zu einer Sporozystensuspension

Kleinste meßbare Zeiteinheit in Sekunden	Excystationsdauer für den 1. Sporozoiten in Sekunden	2.	Zwischenzeit von der Beendigung der Excystation des 1. bis zum Beginn der Excystation des 2. Sporozoiten in Sekunden
0,25	9,5	7	5
0,5	11	10	5
0,5	20	7,5	0
0,5	14	8,5	0
0,5	15 (rückwärts)	—	—
0,25	4	4	0
0,25	8	9	4
0,25	19,25	16,25	5
0,25	20	—	>15
0,25	16	16,75	3,75

zu sehen; in zwei Fällen war die Schicht erhalten und wies an einer Seite ein Loch auf (Abb. 1, 5). Nach der Excystation des zweiten Sporozoiten war sie in keinem Fall mehr nachweisbar. Eine Öffnung der leeren Sporozystenhülle an der Stelle der Mikropyle war deutlich nur mit Phasenkontrastbeleuchtung, bei Interferenzkontrastbeleuchtung nur schwach sowie im Hellfeld kaum zu sehen.

c) Experimentelle Variationen und von der Regel abweichendes Verhalten der Sporozoiten

Bei einer Inkubation der Sporozysten in reiner Trypsinlösung waren die Bewegungen der Sporozoiten in ihnen in der Regel langsamer und es wurden weniger häufig Wendungen durchgeführt als in reiner Galle oder in einer Trypsin-Galle-Lösung. Die Excystation begann später (s. Tab. II) und der Excystationsvorgang dauerte im allgemeinen länger (kürzeste Excystationszeit für den ersten Sporozoiten 49 Sekunden, für den zweiten Sporozoiten 11 Sekunden). Auffällig war, daß Sporozoiten, die in Trypsinlösung durch besonders lebhafte und schnelle Bewegungen in den Sporozysten auffielen, oft sehr lange, zweitweise über 1 Stunde lang, beobachtet werden konnten, ohne daß der Schlüpfvorgang begann, während andererseits viele Sporozoiten excystierten, ohne daß irgendeine vorangegangene Bewegung zu beobachten gewesen wäre.

In reiner Galle begannen die Sporozoiten dagegen sehr rasch mit ihren Bewegungen, jedoch blieb die Excystationsrate minimal (Tab. II). Erst nach Trypsinzusatz war es ihnen möglich, die Sporozysten zu verlassen.

Erfolgten die Excystationsversuche auf dem Heiztisch in hypertonischer Kochsalzlösung oder nach längerer Einwirkungszeit der Trypsin-Galle-Lösung auf die Sporozysten bei Zimmertemperatur, so wurde die zuerst nach außen gestreckte verdünnte Apikalregion sowohl bei dem ersten als auch bei dem zweiten Sporozoiten wesentlich deutlicher ausgebildet.

In verschiedenen Fällen war im Verlaufe der Excystation sowohl des ersten als auch des zweiten Sporozoiten eine fortschreitende Eindellung der Sporozystenhülle zu bemerken, die in dem Moment, in dem die Excystation beendet war, in die ursprüngliche Form zurückschnellte (Abb. 6).

Die Sporozoiten excystierten in der Regel mit dem Vorderende voran und ohne merkbare Eigenbewegungen. Jedoch fielen bei den Untersuchungen im hängenden Tropfen selten, im Deckglaspräparat häufiger Sporozysten auf, bei denen ein mehr oder weniger großer Teil des Hinterendes des ersten Sporozoiten außerhalb der Mikropyle, sein Vorderende dagegen innerhalb derselben zu sehen war (Abb. 2, 7). Dies war insbesondere dann der Fall, wenn das Deckglas erst aufgesetzt wurde, nachdem die Trypsinlösung einige Zeit auf die Sporozysten eingewirkt hatte oder wenn ein geringer Druck auf das Deckglas ausgeübt wurde. In vielen dieser Fälle glitt der Sporozoit dann mit dem Hinterende voran kontinuierlich aus der Sporozyste heraus, wobei immer wieder der »Mikropylenring« über seinen Körper hinwegglitt. Bei längerer Beobachtung konnte man jedoch auch vereinzelt bemerken, wie der im Freien befindliche Teil des Sporozoiten, in einem Fall nahezu 2/3 seines Körpers, ganz allmählich kleiner wurde und der Sporozoit sich in die Sporozyste zurückzog. Hier vollführte er eine oder mehrere Wendungen in der bereits beschriebenen Weise und verließ darauf entweder sofort oder im Anschluß an den anderen Sporozoiten die Sporozyste.

Nur vereinzelt war bei den excystierenden Sporozoiten eine Eigenbewegung zu sehen, die selten aus Abbeugen, häufiger aus Gleitbewegungen bestand, wobei im letzteren Falle stets die gesamte Sporozyste mitgezogen wurde.

d) Einzelbefunde

Im folgenden seien noch einige seltenere oder Einzelbeobachtungen mitgeteilt:

Gelegentlich erschien plötzlich neben dem außerhalb der Mikropyle befindlichen stabartigen Fortsatz des ersten Sporozoiten das Vorderende des zweiten (Abb. 2, *4—5*). Im allgemeinen zog sich dieser zwar wieder zurück, vereinzelt excystierte jedoch auch der zweite Sporozoit vor dem ersten, wobei dieser seine Spitze entweder zurückzog oder in der bereits eingenommenen

Tabelle II

Reaktion der Sporozoiten in den Sporozysten auf unterschiedliche Medien bei Verwendung einer gereinigten Sporozystensuspension im Deckglaspräparat

Medium	auf dem Wärmetisch bei ca. 39 °C				bei Zimmertemperatur (ca. 25 °C)		
	NaCl physiol.	Galle	Trypsin	Trypsin u. Galle	Galle	Trypsin	Trypsin u. Galle
erste Bewegungen in den Sporozysten nach Min.	nach 30 keine	1,25	4,5	1	3	nach 5 keine	nach 5 Min. bei einem 2. Sporozoiten
erste freie Sporozoiten nach Min.	nach 30 keine	9	4	ca. 1,5 je nach Anwärmgeschwindigkeit	nach 15 keine	10	5
Bewegungsintensität in den Sporozysten nach 10 Min.	keine	sehr lebhaft in $\leqslant$ 30% der Sporozysten	kaum	—	nur ganz vereinzelt u. träge	keine	keine
Excystationsrate nach 10 Min in %	0	< 0,1	ca. 10	80	0	< 1	< 2
Reaktion nach späterem Zusatz von Trypsin	—	nach 1/2 Min. Excystationsbeginn	—	—	—	—	—
Reaktion nach späterem Zusatz von Galle	—	—	nach 1 Min. zunehmende Bewegung u. Excystation	—	—	—	—
Reaktion nach anschließender Erwärmung auf 39 °C	—	—	—	—	Bewegungsrate nach 5 Min. kaum erhöht, 1/2 Min. nach weiterem zusätzlichem Trypsinzusatz Beginn zahlreicher Excystationen	nach 6 Min. erste Bewegung, nach 10 Min. 1—2% excystiert	nach 1/2 Min. lebhafte Bewegung, nach 1 Min. zahlreiche beginnende Excystationen

Lage verblieb. Einmal (bei mehreren hundert beobachteten Excystationen) fiel ein bereits zur Hälfte excystierter, scheinbar »steckengebliebener« Sporozoit auf. Nach einiger Zeit erschien neben ihm der stabartige Fortsatz des zweiten, sich in der Sporozyste bewegenden Sporozoiten, dieser quetschte sich heraus und im Anschluß daran beendete auch der erste Sporozoit seinen Durchtritt.

In einem Fall entsand der Eindruck, als ob die Verschlußschicht der Mikropyle durch einen sich heftig bewegenden Sporozoiten an den ihr gegenüberliegenden Pol verschoben worden sei. Eine genauere Analyse der Einzelbilder des Filmes ergab jedoch, daß an dieser Stelle, offensichtlich als Mißbildung, von Anfang an eine stiedakörperähnliche Verdickung vorhanden gewesen war. Durch die Trypsineinwirkung war diese Masse ebenfalls gelöst worden und es blieb eine dünne, sich konkav in die Sporozyste hineinwölbende Schicht zurück, die zufälligerweise erst in dem Moment deutlich in Erscheinung trat, in dem die eigentliche Verschlußschicht durch die Sporozoitenbewegungen so nach außen gedrückt worden war, daß sie nicht mehr erkennbar war (Abb. 2).

Vereinzelt wurde beobachtet, wie ein excystierter Sporozoit einen aus der Mikropyle herauskommenden (Schleim-?) Faden hinter sich herzog, der sich einmal regelrecht straffte. Plötzlich kontrahierte sich dann dieser Faden, zog den daran hängenden Sporozoiten ein kurzes Stück zurück, riß von ihm ab und schnurrte auf etwa ein Drittel seiner maximal erreichten Länge zusammen. Der zweite Sporozoit excystierte im Anschluß daran, ohne daß sich an diesem fadenartigen Gebilde etwas veränderte. Ein anderes Mal war bei einem zweiten Sporozoiten ein ähnlicher, von dem Restkörper ausgehender Strang zu sehen, der den vor der Mikropyle liegenden Sporozoiten an einer freien Gleitbewegung hinderte bzw. ihn nach jedem Versuch dazu wieder zurückzog, bis er nach etwa 4 Minuten abriß und der Sporozoit auf diese Weise frei wurde.

Diskussion

Trotz aller bisher durchgeführten Untersuchungen ist das Phänomen der Excystation von Sporozoiten aus Sporozysten und aus Oocysten noch nicht grundlegend geklärt. Alle vorliegenden Angaben basieren auf empirischen Studien und erfassen in der Regel nur Teilgebiete.

Metzner (1903) gelang bereits die Excystation aus unzerstörten *E. stiedai*-Oocysten durch Inkubation in Darmsaft von Hunden oder Kaninchen. Seine Ergebnisse waren in der folgenden Zeit nicht immer reproduzierbar und heute ist bekannt, daß zur Excystation aus Oocysten eine spezielle Vorbehandlung derselben (»conditioning« [Lotze und Leek, 1968]) notwendig ist, die bisher nicht eindeutig geklärte Veränderungen der Oocystenhülle und -mikropyle auslöst. Als schnellere und elegantere Methode zur Gewinnung

von Sporozoiten wird z.Zt. jedoch die Excystation aus Sporozysten bevorzugt, die durch mechanische Zerstörung der Oocysten (Zermörsern eines möglichst trocken abzentrifugierten Oocystensediments [KRIEG, 1971]) und anschließendes Filtrieren sowie Abzentrifugieren der Suspension leicht angereichert werden können. Durch Zusatz von Galle und Trypsin bei gleichzeitiger Erwärmung schlüpfen innerhalb kurzer Zeit nahezu alle Sporozoiten, die dazu in der Lage sind. ROSE (1959) und DÜRR (1970/71) excystierten ohne Gallezusatz, letzterer mit wechselndem Erfolg, bis KRIEG (1971) feststellte, daß Trypsin ohne Galle nur solange wirksam ist, wie die Sporozystensuspension nicht zu sehr verdünnt wird. Er schließt daraus auf bisher unbekannte excystationswirksame Substanzen in den Oocysten oder an den Oocystenhüllen, was in gewisser Übereinstimmung mit der Annahme von JACKSON (1962) über die Aktivierung eines Enzymsystems im Oocysteninhalt von *E. arloingi* durch einen im Pansen befindlichen Faktor stehen würde. Ein Zusatz von Galle zu der Trypsinlösung beschleunigt und verbessert sowohl das Excystationsergebnis als auch die Bewegung der Sporozoiten in den Sporozysten erheblich (Tab. 2; ROBERTS und Mitarb., 1970b; KRIEG, 1971). Auch in reiner Galle findet unter gewissen Voraussetzungen eine Excystation statt, nur ist sie erheblich langsamer (Tab. 2; HIBBERT und Mitarb., 1969; KRIEG, 1971), während Gallensalze auf die Sporozoiten nur bewegungsstimulierend wirken (ROBERTS und Mitarb., 1970a).

Die von einigen Autoren berichtete Aktivierung der Sporozoiten in indifferenten Medien bei Zimmertemperatur allein durch die Einwirkung von Licht bzw. durch eine Zerstörung der Oocysten (METZNER, 1903; v. WASIELEWSKI, 1904; MARQUARDT, 1963; ROBERTS und Mitarb., 1970b) wurde bei *E. stiedai* bisher nicht beobachtet. HIBBERT und HAMMOND (1966, 1968) stellten bei verschiedenen *Eimeria*-Spezies ein Temperaturoptimum zwischen 39 und 41 °C für den Excystationsvorgang fest.

Außer von METZNER (1903), der eine ruckweise Excystation schildert, wird von keinem der Autoren auf eine in dieser Art sichtbare Beteiligung der Sporozoiten hingewiesen. SMETANA (1933) betont allerdings noch einmal, daß die Excystation nach der passiven Zerstörung der Mikropylensubstanz aktiv von den Sporozoiten selbst vollendet werde. Eine von DORAN (1966) zunächst angenommene, excystationsfördernde enzymatisch wirksame Sekretion der Sporozoiten wird in einer späteren Publikation (DORAN und VETTERLING, 1969) jedoch abgelehnt.

Der Excystation voraus ging in jedem Falle, in dem darauf geachtet wurde, eine Auflösung des Stiedakörpers, u.U. nach einem Anschwellen desselben (DORAN und FARR, 1962; FARR und DORAN, 1962; NYBERG und HAMMOND, 1964; HAMMOND und Mitarb. 1970; ROBERTS und Mitarb., 1970a; ROBERTS und Mitarb., 1970b). Die Sporozoiten begannen mit ihren Bewegungen in den Sporozysten bereits vor oder auch erst nach dem Verschwinden des

Stiedakörpers (DORAN und FARR, 1962; FARR und DORAN, 1962; NYBERG und HAMMOND, 1964; ROBERTS und Mitarb., 1970b). HAMMOND und Mitarb., (1970) sowie ROBERTS und Mitarb. (1970b) berichten über einen zusätzlich vorhandenen Substiedakörper bei *E. callospermophili*, *E. larimerensis* und *E. utahensis*, der nach dem Verschwinden des Stiedakörpers und vor der Excystation der Sporozoiten aus der Mikropyle herausquillt. Bei *E. stiedai* konnte keine eindeutige Beziehung zwischen dem Bewegungsbeginn der Sporozoiten in den Sporozysten und dem Verschwinden des Stiedakörpers gefunden werden. An Stelle des Substiedakörpers war eine gelegentlich leicht verdickte Schicht zu sehen, die von den Sporozoiten offensichtlich perforiert werden mußte. Ihr genauerer Aufbau wäre durch elektronenmikroskopische Untersuchungen zu klären.

METZNER (1903) spricht bei *E. stiedai* als Einziger von einem »pfriemenartigen Fortsatz« bzw. einem »äußerst zugespitzten Vorderteil« der Sporozoiten bei den Bewegungen in der Sporozyste und bei der Excystation. Dieses Gebilde entspricht vermutlich dem bei den eigenen Untersuchungen gefundenen, als stabartiger Fortsatz bezeichneten Gebilde der Apikalregion, das auch bei anderen *Eimeria*-Arten bisher nicht beschrieben wurde.

In zumindest teilweiser Übereinstimmung mit den obigen Angaben ließe sich für das Excystationsgeschehen von *E. stiedai*-Sporozoiten aus freien Sporozysten bei in vitro-Versuchen die nachfolgende Hypothese aufstellen, die durch die bisher mitgeteilten Befunde erhärtet werden kann. Es muß jedoch beachtet werden, daß Schlußfolgerungen und Vergleiche aus den einzelnen Literaturangaben aufgrund der fast bei jeder Arbeit anderen Methodik (Excystation aus freien Sporozysten oder aus unterschiedlich vorbehandelten Oocysten in den verschiedensten Medien und bei unterschiedlichen Temperaturen) nur mit äußerster Vorsicht gezogen werden können.

Das Excystationsgeschehen ist ein komplexer Vorgang, der in der Regel durch das gemeinsame Einwirken mehrerer, verschiedenartiger Faktoren ausgelöst wird, jedoch schon durch ein oder zwei Faktoren ausgelöst werden kann. Es kommen in Frage: Die Anwesenheit von Trypsin und Galle, eine gewisse Mindesttemperatur, eine bisher nicht definierte chemische Substanz im Oocysteninhalt, eine aktive mechanische und eventuell auch enzymatisch-sekretorische Beteiligung der Sporozoiten sowie in Einzelfällen ein von außen auf die Sporozoiten einwirkender Druck.

Trypsin löst im wesentlichen den Stiedakörper auf und aktiviert zusammen mit der Galle und dem unbekannten System im Oocysteninhalt die Sporozoiten zu Eigenbewegungen. Inwieweit der Galle oder einzelnen Gallensalzen außer ihrer bewegungsstimulierenden Wirksamkeit eine Beeinflussung des Stiedakörpers zuzuschreiben ist, muß vorläufig offenbleiben. Ungeklärt bleibt bisher auch, warum bei Verwendung einer reinen Trypsinlösung viele Sporozoiten trotz ihrer manchmal lebhaften Bewegungen nicht excystieren.

Als Hauptfunktion der Wärme wäre eine Abkürzung sowie eventuell erst eine Ermöglichung der stattfindenden enzymatischen Reaktionsabläufe anzusehen.

Einen Hinweis auf eine aktive mechanische Beteiligung der Sporozoiten bei der Excystation geben neben der deutlichen Ausbildung der stabartig verdünnten Apikalregion des ersten Sporozoiten die Fälle, in denen Vor- und Rückwärtsbewegungen der Sporozoitenspitze in der Mikropylenöffnung, Suchbewegungen vor Beginn der Excystation in der Sporozyste sowie das Wiederhineingleiten von Sporozoiten in die Sporozyste nach einer zufälligen unvollständigen Rückwärtsexcystation beobachtet wurden.

Die Annahme einer enzymatischen Sekretion der Sporozoiten muß bis zu einer weiteren elektronenmikroskopisch-histochemischen Untersuchung vor, während und nach der Excystation eine Hypothese bleiben.

Ein von außen auf die Sporozyste ausgeübter Druck (bei in vitro-Versuchen durch das Deckglas, in vivo durch die Darmbewegungen und den Darminhalt) kann zu einer passiven Excystation führen, die in auffälligster Weise rückwärts erfolgt. Einzelbeobachtungen (Dürr, 1970/71), die sogar auf einen in der Sporozyste herrschenden Überdruck schließen lassen, bedürfen erst weiterer Untersuchungen.

Ein von Ikeda (1960) bei *E. tenella* zeichnerisch dargestelltes Ausbeulen der Sporozystenhülle durch die sich bewegenden Sporozoiten wurde bei *E. stiedai* nicht beobachtet. Dagegen kam es im Verlauf der Excystation gelegentlich zu reversiblen Eindellungen der offensichtlich elastischen Sporozystenwand, die sich durch ein zu schnelles Hinausgleiten der Sporozoiten und dadurch gleichzeitigen hermetischen Abschluß der Mikropyle erklären ließen, was zu einem Unterdruck in der Sporozyste führen würde.

Die in der Literatur für die Excystation der Sporozoiten verschiedener *Eimeria*-Arten (Metzner, 1903; Smetana, 1933; Nyberg und Hammond, 1964; Nyberg und Mitarb., 1968; Roberts und Mitarb., 1970b) angegebenen, von 2 bis 70 Sekunden schwankenden Zeitspannen stimmen unter Berücksichtigung der sowohl methodisch bedingten als auch individuellen Streubreiten mit der Mehrzahl der für *E. stiedai* gefundenen überein.

Im Gegensatz zu Roberts und Mitarb. (1970b), die über eine langsamere Excystation des zweiten Sporozoiten im Vergleich zu dem ersten bei *E. callospermophili* und *E. larimerensis* berichteten, excystierte bei *E. stiedai* der zweite Sporozoit bis auf wenige Ausnahmen stets wesentlich schneller als der erste. Welche Faktoren für diesen unterschiedlichen Befund verantwortlich sind, kann nicht gesagt werden. Ob die von Roberts und Mitarb. (1970b) gefundenen Relationen zwischen der relativen Größe des Substiedakörpers und der Excystationsgeschwindigkeit für andere Arten ohne Substiedakörper modifiziert werden können (z.B. bezogen auf die Weite der Mikropyle (?)), müßte in weiteren Untersuchungen geprüft werden.

Außerdem wäre zu überprüfen, ob die für verschiedene *Eimeria*-Arten berichteten unterschiedlichen Excystationsraten bei in vivo- (FARR und DORAN, 1962) und in vitro- (ROBERTS und Mitarb., 1970b) Versuchen wirklich artspezifische Eigenschaften und, wie von FARR und DORAN (1962) angenommen, vom Ansiedlungsort im Darm abhängig sind. Die Ergebnisse von ROBERTS und Mitarb. (1970b) bei den beiden, sich etwa an der gleichen Stelle entwickelnden Arten *E. callospermophili* und *E. larimerensis* (TODD und HAMMOND, 1968a, b) würden eher gegen diese These von FARR und DORAN (1962) sprechen.

Die gleichzeitige Excystation zweier Sporozoiten, wie sie bereits von NYBERG und HAMMOND (1964) bei *E. bovis* sowie DORAN und FARR (1962) bei *E. acervulina* beschrieben wurde, scheint trotz der von letzteren Autoren festgestellten relativen Häufigkeit eine Ausnahme zu sein. Die von ROBERTS und Mitarb. (1970b) berichtete Excystation einiger *E. larimerensis*-Sporozoiten in U-Form mit der konvexen Seite voran dürfte eine bisher nicht festgestellte Abnormität sein, die u.U. durch die bei dieser Art relativ weite Mikropyle ermöglicht wird.

Elektronenmikroskopische Untersuchungen an *E. stiedai*-Sporozoiten liegen bisher nicht vor. Eine fundierte Korrelation zwischen den beobachteten Bewegungs- und Excystationsmechanismen und der Feinstruktur der Sporozoiten soll daher erst erfolgen, wenn die entsprechenden, derzeit in Gang befindlichen Arbeiten (DÜRR und SCHOLTYSECK, in Vorbereitung) abgeschlossen sind.

ZUSAMMENFASSUNG

An Hand von Lebendbeobachtungen und Filmaufnahmen wurde die in vitro-Excystation von *Eimeria stiedai*-Sporozoiten aus Sporozysten untersucht. Bei Inkubation in einem Trypsin-Galle-Gemisch bewegen sich die Sporozoiten vor dem Beginn der Excystation in den Sporozysten in der Regel lebhaft. Die Excystation wird durch Suchbewegungen des Sporozoitenvorderendes an der Sporozystenmikropyle eingeleitet. Die auffälligste Erscheinung im Verlauf der Excystation ist eine stabartig aus der Mikropyle herausragende Verdünnung der Apikalregion des zuerst excystierenden Sporozoiten, die eine Länge von fast 4 μ erreichen kann. Die Excystation des ersten Sporozoiten nimmt fast stets mehr Zeit in Anspruch als die des zweiten. Vereinzelt wurden Sporozoiten beobachtet, die mit dem Hinterende voran excystierten oder die beide gleichzeitig versuchten, aus der Sporozyste zu schlüpfen.

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A CLINICAL STUDY OF RUBARTH'S DISEASE

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Canine virus hepatitis was first described as an independent disease by RUBARTH in 1947. Subsequently, post mortem diagnosis of the condition was reported from several parts of the world and detailed studies were carried out on its incidence and gross and microscopic lesions.

Several authors have dealt with the clinical course of both natural (BISCHOFF, 1952; COFFIN and CABASSO, 1953; SCHULZE and HAUKE, 1955) and experimental canine virus hepatitis (GÄRTNER, 1955; LARIN, SKULSKI and ORBELL, 1958; LINDBLAD and PERSSON, 1962); SCHEU (1953) even reported the detection of subclinical cases by serological tests (CF).

Descriptions of the symptoms are fairly uniform, but opinions have been divergent about whether the fever runs a uniphasic or biphasic course. In complementary laboratory tests, BISCHOFF (1952), COFFIN and CABASSO (1953) and GÄRTNER (1955) determined the blood picture, clotting and bleeding times, erythrocyte sedimentation rate as well as serum non-protein nitrogen, blood sugar and serum bilirubin levels, and abnormal urine components. HOE (1961) and LINDBLAD and PERSSON (1962) measured SGOT, SGPT, alkaline phosphatase and the changes of serum protein fractions in different stages of experimental Rubarth's disease. According to GÄRTNER (1966) fever, debility, sensitivity of the syphoid region, leucopenia, prolongation of bleeding and clotting times and proteinuria are pathognomonic for the early phase. SCHULZE and HAUKE (1955) recommended liver biopsy as the diagnostic method of choice.

Clinical observations and laboratory findings have seldom been correlated however, and information is particularly scanty about the early diagnosis of the condition. Opinions are also divergent about the effective therapy. GÄRTNER (1955) established that treatment with specific immune serum is advantageous in all stages of the disease, but others (KIRK, 1968; RÖHRER, 1968) regard its effect doubtful, on the grounds that the virus soon disappears from the blood stream and once it has become established intracellularly, it is no longer neutralizable by the serum. CHRISTOPH (1962) remarked that caution be exercised with serum therapy, because large quantities of exogenous protein burden the liver cells and thereby exacerbate the disease. Most investigators have proposed a complex symptomatic treatment.

As numerous cases of Rubarth's disease have been encountered recently in this clinic, detailed studies were carried out to obtain more information on its symptoms and the diagnostic usefulness of routine laboratory tests.

Experimental

Thirty-six clinically ill dogs served as subjects. Six of the 36 dogs were younger than one year, two of them only 4 months old. The 14 dogs between one and two years old formed the largest group; 10 dogs were three years old,

six dogs older. Diagnosis was established on the basis of both symptoms and laboratory tests. Clinical examinations included, apart from temperature measurements, inspection for mucosal haemorrhages, liver enlargement and icterus, which we regard as the main symptoms. In a few cases liver biopsy was also performed, using the Vim-Silvermann needle or Manghini's aspiration technique, but later it was omitted owing to its often fatal consequences (blood loss).

Leptospirosis and toxoplasmosis were excluded by serological and histological examinations. In fatal cases, gross lesions were studied and nuclear inclusions were demonstrated to reinforce the diagnosis.

Detailed laboratory examinations could be carried out in 19 of the 36 cases. Quantitative blood cell counts, differential count, clotting time, serum bilirubin, GPT and GOT levels, and abnormal urine components were regularly determined. Blood sugar level was additionally measured in nine dogs and alkaline phosphatase activity in six.

The results are tabulated along with the main clinical observations. The severity of icterus, mucosal haemorrhage and liver swelling as well as the amounts of protein, bilirubin and blood in the urine are graded on a scale $+-+++$. The day of illness on which the measurement was made is specified along with the data. The febrile course was uniphasic. At the beginning of observation, the temperature of six dogs had already normalized, while the rest showed various degrees of temperature elevation. All dogs showed a sensitivity of the xyphoid region.

Leukocyte counts fluctuated between the levels characteristic of leukopenia and leukocytosis, depending on the duration of the condition. The erythrocyte count usually fell. The differential count indicated a slight neutrophilia. The clotting time of blood samples taken after the 3rd—4th day of the disease was prolonged, and SGOT, SGPT, serum bilirubin and alkaline phosphatase levels rose considerably. All but one of the nine dogs whose blood glucose level was determined showed a notable hyperglycaemia. The urine was yellow coloured from the high bilirubin concentration and it contained protein and often also blood. The urine sediment comprised white and red blood cells, renal epithelial cells and cylindrical casts of renal tubules.

Twenty-two of the 36 dogs died, including both the very young puppies and the dogs more than 4 years old. Recovery rate was highest between 1/2—1 1/2 years of age.

Discussion

The certain diagnosis of canine virus hepatitis requires a thorough knowledge of the nature and symptoms of the disease and several differential examinations. However, the latter are not always possible in clinical practice,

as dogs are often brought in when in the moribund stage of an acute disease and many of them die while still being examined. Several such cases were identified as Rubarth's disease at post mortem examination. This, and the relatively large number of clinically recognizable cases encountered in this clinic during the past two years suggest that Rubarth's disease is next in frequency to canine distemper.

Of the 36 dogs examined 31 were from 6 months to 3 1/2 years old. This is in conformity with the general opinion that Rubarth's disease chiefly affects young dogs, although the former view that the highest incidence is between 2 and 4 months of age needs revising.

Three forms of the disease can be distinguished: hyperacute, acute and subacute. As the hyperacute form kills the dog within 24—72 hours after the onset of symptoms, such cases are rarely encountered by the veterinarian. Dogs apparently healthy in the evening are not infrequently found dead next morning so that the dog's owner usually suspects poisoning. The hyperacute course is characterized by an abrupt rise of temperature to 40—41 °C, extreme debility, repeated vomiting and increasing thirst. The mucous membranes appear slightly pale and the swollen tonsils exhibit reddening. The liver hypertrophies considerably, owing to serous inflammation, and its margins become rounded. The most typical laboratory finding is leucopenia: leukocyte counts may fall to 4,500 or even as low as 1,800. Other routine laboratory tests usually show no abnormality; the urine occasionally contains small amounts of protein and bilirubin and the clotting time may be slightly longer.

The acute course takes 3—5 days. The dogs are extremely listless, stuporous, and suffer from vomiting, diarrhoea and thirst. Temperature elevation is mostly medium-high, less often high, although infrequently there may be no fever at all. Icterus is first noticed on the sclera and conjunctiva and occasionally also on other mucous membranes; it usually becomes distinct by the 5th day. Haemorrhages often appear on the conjunctiva and oral (Fig. 1) and pharyngeal mucosa. The often yellow integument exhibits petechial haemorrhages or even suffusion particularly in the inguinal region and scrotum. Some animals show an oedematous swelling in the throat or inguinal region. The tonsils are swollen and pink to vivid red. Other characteristic symptoms are sensitivity of the xyphoid region and liver hypertrophy, the liver parenchyma becoming so friable that even a cautious palpation may cause it to rupture. Three dogs of our material died from spontaneous liver rupture. Exsanguination is indicated by a porcelain whiteness of the conjunctiva. Respiration is quick and slightly laborious, the pulse quick, but weak. Bradycardia develops if there is a distinct icterus. The intestines contain fluid and gas, and occasionally tonic-clonic convulsions set in.

The urine takes on an ochre yellow colour from the large amount of excreted bilirubin. Proteinuria may be very marked and leukocytes and renal

Table I

Results of physical

	Day after onset	Temperature °C	Icterus	Mucosal haemorrhage	Liver enlargement	Blood picture						Clotting time (min)
						Red cells mm³ (mill)	White cells mm³ (thousand)	Differential count (%)				
								Ly	Ne	Eo	Mo	
Acute form	3.	40.8	—	++	++	5,2	5,8	8	92	—	—	5
	3.	39.7	—	++	+	4,2	1,0	38	68	2	2	5
	4.	39.6	+	—	++	2,8	6,2	28	72	—	—	12
	4.	40.5	—	—	++	3,8	2,1	12	84	2	2	5
	4.	37.8	+++	++	++	4,2	5,1	25	73	—	2	7
	4.	40.0	+	++	++	4,8	6,2	18	82	—	—	7.5
	5.	40.4	+++	—	+	6,2	7,0	21	76	2	1	8
	5.	37.5	++	—	++	6,5	3,2	30	66	2	2	8
	5.	39.6	++	—	++	7,0	4,2	22	76	1	1	6.2
Subacute form	6.	39.0	—	++	++	5,9	7,2	6	92	2	—	6.5
	6.	38.5	+++	—	++	3,2	12,0	15	85	—	—	6.5
	7.	39.5	++	+	++	4,2	13,6	20	78	1	1	8.5
	7.	40.6	++	++	++	5,9	11,4	30	65	3	2	7.5
	7.	38.9	+++	++	++	4,4	29,3	24	74	—	2	10
	8.	40.6	+++	+++	++	5,8	10,25	17	83	—	—	6.5
	10.	38.0	+++	+++	+	3,7	19,0	18	80	2	—	8
	10.	38.5	+++	++	+	4,6	28,0	3	94	3	—	8
	10.	39.5	+	+	+++	6,8	7,5	16	80	3	1	5.5
	10.	39.0	+++	++	+	4,2	14,0	10	89	1	—	12.0
Normal values		38.5	—	—	—	6—8	9—12	26	69	3	2	3—5
		39.0	—	—	—	—	—	—	—	—	—	—

and laboratory examinations

SGPT	SGOT	Serum bilirubin (mg%)		Blood sugar (mg%)	Alkaline phosphatase (I.U./ml)	Urine			
I. U.						Protein	Bilirubin	Blood	Sediment
30	10	0.3	0.1	107	—	+	++	—	Negative
49	39	0.9	0.4	62	—	+	++	—	Negative
51	23	1.1	0.8	—	—	++	++	—	Negative
27	30	0.6	0.1	—	—	+++	+	—	Negative
76	75	19.5	10.3	—	171	+++	+++	—	Few renal and bladder epithelial cells
109	102	1.9	1.3	137	—	+++	+++	+	Many RBC, few leukocytes, 1—3 renal cells
90	29	8.7	5.1	151	—	+++	+++	—	Negative
52	36	2.6	1.5	107	—	+++	+++	++	Many RBC and leukocytes, renal cells
60	70	2.7	1.1	—	—	++	++	—	Negative
53.5	45	0.9	0.6	—	240	+++	+++	—	Renal cells, bacteria
83.0	20.5	1.8	1.3	128	—	+++	++	++	Many RBC, renal and bladder cells
69.0	71.0	4.8	1.2	130	190	++	++	—	Few renal cells and leukocytes
51	31	1.2	0.9	—	—	+++	+++	—	Negative
450!	380!	7.1	3.8	—	—	+++	+++	+++	Many RBC and renal cells
53.5	47.5	25.2	22.0	135	168	+++	+++	++	Many leukocytes and RBC, tubular casts and renal cells
105	87	6.8	5.7	120	160	+++	+++	+	4—5 leukocytes, 4—5 RBC, bacteria
50	18	9.4	5.6	—	120	++	+	—	2—3 RBC, few renal cells
11	22	0.7	0.1	—	—	++	++	—	Negative
160	152	9.2	5.8	132	164	+++	+++	+	Leukocytes, RBC, renal cells
3.7±	9.4±	0.5	0.1	70—80	20—50	—	—	—	
2.7	5.9	—	—	—	—	—	—	—	

epithelial cells may be present in the urine sediment. The red blood cell count is usually indicative of anaemia (4—5 million cells). The leukocyte count is as a rule low, with the differential count suggesting neutrophilia. The clotting time is slightly prolonged (5—8 minutes). After 1—5 days the serum bilirubin rises to moderately or extremely high levels (19 mg%), owing chiefly to an increase of direct bilirubin. SGPT and SGOT activities also reach levels considerably above normal. Alkaline phosphatase activity rises to about 100 I.U./ml. All dogs but one had a blood sugar level above 100 mg%.

The subacute course is characterized by a gradual progression of the symptoms. It lasts 6—14 days. The sick dogs become listless and lose appetite, their temperature at first rises slightly, if at all, but during the moribund stage falls slightly below normal. There is as a rule a severe icterus, but the degree of anaemia is varied. Both cutaneous and mucosal haemorrhages are distinct and extensive (Fig. 2). Liver enlargement is initially considerable but later regresses; occasionally the liver may have returned to its normal size by the end of the disease. The sick animals are stuporous, uncontrollable vomiting and severe diarrhoea are frequent, and ataxia is often observed. The red blood cell count falls to a greater or lesser extent. There was an extreme elevation of leukocyte count in three cases, but in the rest it rose only moderately or not at all. The differential count indicates neutrophilia. Clotting time is prolonged markedly.

Serum bilirubin, particularly the direct bilirubin, rose to a very high level, in some cases up to 10 mg%. Elevation of SGTP and SGOT levels was of similar degree in the acute course. Blood sugar level rose above normal. The urine contained a large amount of protein, blood and bile, the latter imparting a yellowish green colour. Red blood cells were often found in the urine sediment. In its acute and subacute forms, canine virus hepatitis also causes distinct ophthalmological symptoms. The diseased dogs may develop iridocyclitis, with photophobia, suffusion into the anterior chamber of the eye, or inner ophthalmy. The unilateral or bilateral parenchymatous keratitis generally associated with milder cases and responsible for the so-called "hepatitis blue eye" could be cured by appropriate treatment within 1—2 weeks (Fig. 3).

The characteristic symptoms stem from the fundamental pathophysiological changes associated with the condition. The main changes are the impairment of vascular endothelium and liver cells (LARIN, SKULSKI and ORBELL, 1958). Liver hypertrophy, mucosal haemorrhages and prolongation of the clotting time form a trial of constant symptoms. KIRK (1968) regards the prolongation of clotting time as a typical coagulopathy brought about by heparin release from the impaired liver cells; nevertheless disturbances in the synthesis of hepatic clotting factors (prothrombin, etc.) may also play a role. The cutaneous and mucosal haemorrhages correspond with vascular purpura.

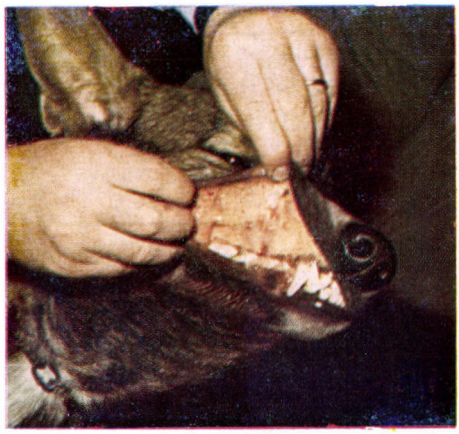

Fig. 1. Jaundice and haemorrhages on oral mucosa

Fig. 2. Jaundice and cutaneous haemorrhages

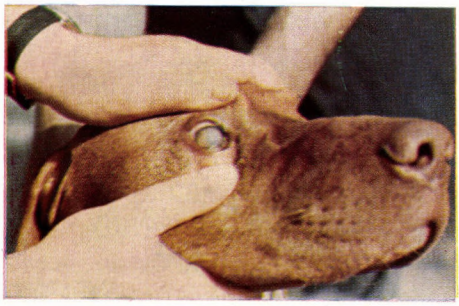

Fig. 3. The so-called "hepatitis blue eye"

Laboratory tests show changes typical of liver dystrophy and the prolongation of the clotting time results in the elevation of plasma GOT, GPT, alkaline phosphatase and bilirubin levels. Rise of the blood sugar level indicates increased glycogenolysis in the damaged liver. Of the abnormal urine components, bilirubin increases in consequence of hepatocellular icterus; proteinuria, haematuria and abnormality of the urinary sediment are initially due to kidney degeneration, later to nephritis.

On the basis of the above symptoms and laboratory tests, Rubarth's disease can easily be recognized, especially if it takes an acute or subacute course.

We regard liver biopsy as a dangerous method of early diagnosis. In the first 2—3 days of the disease the liver is in a state of serous inflammation, with its capsule taut and liable to rupture and exsanguination on even slight trauma. The risk is much less in the semi-acute stage but then there are fewer inclusion bodies so that biopsy is unlikely to be worthwhile. At this stage the clinical symptoms are already obvious enough to serve as a basis of diagnosis.

As to the diagnostic differentiation of Rubarth's disease from other conditions, canine distemper is initially manifested by catarrhal symptoms, later by nervous signs, but there is no liver involvement. Clinical differentiation from toxoplasmosis is more difficult; indeed, the pictures are sometimes so similar that only additional catarrhal changes suggest the protozoan causation. Serological evidence is as a rule necessary to arrive at unequivocal conclusions. Differentiation from non-infectious diseases may be made primarily on the basis of the high fever occurring during the acute stage, later by haemorrhages, icterus, clinical course and laboratory tests.

Specific antiserum treatment is indicated during the acute stage. Repeated blood transfusions are absolutely necessary because recovery can scarcely be expected without them. Complementary treatment with roborants such as B-vitamins (above all B_{12}), pantothenic acid, vitamin K, glycocorticoids, calcium preparations and cardiactive compounds, as well as prevention of secondary bacterial infection(s), and application of broad-spectrum antibiotics are all important. Weakened animals should receive an easily digestible diet, rich in carbohydrates and lipotropic substances. A liver-sparing diet is necessary during illness, convalescence and for an period after recovery.

Recovery depends on the nature of the disease and on the speed of veterinary intervention, but the prognosis is usually doubtful. Hyperacute cases are hopeless and even with very careful treatment 60% of the dogs showing an acute or subacute disease died in our clinic.

Treatment with a modified live viral vaccine seems to be the best prophylactic measure. In immunization experiments, the Rubarth disease vaccine conferred a lasting protection on 77—95% of the vaccinees.

SUMMARY

Clinical studies and laboratory tests of Rubarth's disease are reported. Thirty-six naturally infected dogs, most of which were ½—1½ years old, were observed, and detailed laboratory examinations were carried out in 19 cases. Hyperacute, acute and subacute forms of the disease were distinguished. Hyperacute cases are rarely encountered by the veterinarian because of their extremely rapid course. The characteristic symptoms are marked liver enlargement, listlessness, vomiting and high fever; blood tests reveal leucopenia. The acute course takes 3—5 days during which icterus, mucosal haemorrhages and liver enlargement develop and the liver region displays sensitivity to palpation. The serum GPT, GOT, alkaline phosphatase and bilirubin as well as the blood sugar level rise, while the red blood cell count falls. The urine contains protein. The subacute course takes 6—14 days. There are usually severe icterus and extensive haemorrhagic diathesis of mucosa and skin. The clotting time is markedly prolonged but otherwise the laboratory findings resemble those of the acute form. Blood appears in the urine and certain components of the urine sediment are indicative of nephritis. Characteristic ophthalmological symptoms (iridocyclitis, parenchymatous keratitis) may already appear in the acute stage, but they are commonest in the subacute form.

The prognosis of the disease is doubtful, even with complex therapy (specific immune serum, hepatoprotective agents, glycocorticoids, blood transfusion, etc.); of the 36 dogs treated in this clinic, 22 died. Chances of survival are poor if the dog is less than 6 months or more than 4 years old.

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THE EFFECT OF IRRADIATION ON THE EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT OF *AMIDOSTOMUM ANSERIS* (ZEDER, 1800)

By

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The effect of ionizing radiation has already been studied on several species of parasitic organisms, including some nematode parasites of the poultry, such as *Ascaridia galli* (BABERO, 1952; SHIKHOBALOVA et al., 1958; VARGA, 1964a; RUFF et al., 1965; RUFF and HANSEN, 1967), *Ascaridia columbae* (CASAROSA et al., 1966), *Heterakis gallinarum* (OSTLIND and HANSEN, 1966), *Syngamus skrjabinomorpha* and/or *S. trachea* (SHIKHOBALOVA and PARUZHINSKA'YA, 1962; VARGA, 1964b; ZIEGLER, 1966).

Since similar work relative to *Amidostomum anseris* (Zeder, 1800) has not been encountered in the literature, an experiment was designed to investigate the effect of irradiation on the eggs and infective stage larvae of this gizzard-dwelling parasite of geese. The primary objective of the study was to determine what dose level of irradiation would suitably reduce the pathogenic effect of the larvae so that they might be used for immunization experiments without endangering the health of the host animals.

Material and methods

Eggs of *A. anseris* were obtained from the faeces of artificially infected geese. Fresh faecal samples were well mixed with about five volumes of a solution (made of equal volumes of saturated magnesium sulphate and sodium thiosulphate solutions mixed with tap water to give a specific gravity of 1300), strained through a sieve of 300 μ mesh, and the suspension was centrifuged for 3 minutes at approx. 1200 rev. per minute. The supernatant fluid was poured off into a 1 litre beaker, diluted with approx. 10 parts of tap water, and allowed to sediment for 15 minutes. The supernatant fluid was slowly poured into another beaker and replenished with tap water while the sediment was poured into a Petri dish. This sedimentation procedure was repeated three times, and the whole lot of eggs collected in the Petri dish was further cleaned by repeated sedimentation and removal of the supernatant with the aid of a pipette, using a dissecting microscope, until the egg suspension became free of debris.

Since the eggs of *A. anseris* reach the first larval stage as soon as in 12 hours at 30 °C (KOBULEJ, 1956), they were immediately irradiated (within 3 hours after being passed with the faeces) or kept at room temperature over 4 days to reach the infective (third larval) stage. Petri dishes containing

the third-stage larvae swimming about in the water were cooled to +1 °C and the temporarily immobile, sedimented larvae were quickly collected with the aid of a Pasteur pipette under a dissecting microscope to obtain adequate larval concentrations.

A Gamma 3500 Irradiation Unit (Noratom) with a dose rate of 0.338 kilorad (krad) per sec. was used as the source of gamma rays. The eggs (approx. 500 eggs per 1 ml of water) or infective stage larvae (approx. 4000 larvae per 1 ml of water) were kept in 8 × 50 mm glass tubes placed in a vacuum flask containing tap water during the irradiation.

After irradiation the eggs were transferred into small Petri dishes and allowed to embryonate at room temperature. The rate of development of eggs and larvae was recorded by examining 200 eggs and/or larvae in each culture every 24 hours over 5 days. Arbitrarily, three stages of in vitro development were distinguished: 1. eggs containing a small blastomere morula or "tadpole" form; 2. larvae inside the eggs; 3. hatched larvae.

Development of the larvae irradiated at the infective stage was followed in the host. On the first post-hatching day, groups of four Rajna breed goslings were each inoculated into the crop with 500 irradiated or normal larvae. From day 13 of the infection, droppings of the birds were examined for the presence of *Amidostomum* eggs, using both the common concentration method and the standard McMaster egg-counting technique. Embryonic development of eggs collected from faeces of these goslings was also followed. The animals were killed four weeks after infection and the gross pathological changes in the gizzard of birds from the various groups were compared. The worms recovered by teasing the mucosa of gizzard into small pieces under a dissecting microscope were sexed and the fertility of the females examined. The length of worms was determined by measuring 30 male (if possible) and 30 females selected at random from each gosling.

For statistical analysis Student's t-test was applied.

Results

Exposure of *A. anseris* eggs to various doses of irradiation caused a dose-dependent deleterious effect on their development. This resulted in a smaller percentage of eggs capable of completing segmentation and reaching the infective larval stage. Final developmental stages obtained five days after the exposure are shown in Fig. 1. Although cleavage proceeded for a while in nearly all irradiated eggs, it ceased before the tadpole stage was reached in most affected eggs; the bulk of eggs with such an arrested development exhibited irregular differentiation, the abnormal morula frequently containing one or more vacuoles (Fig. 2). Variable numbers of eggs treated with different

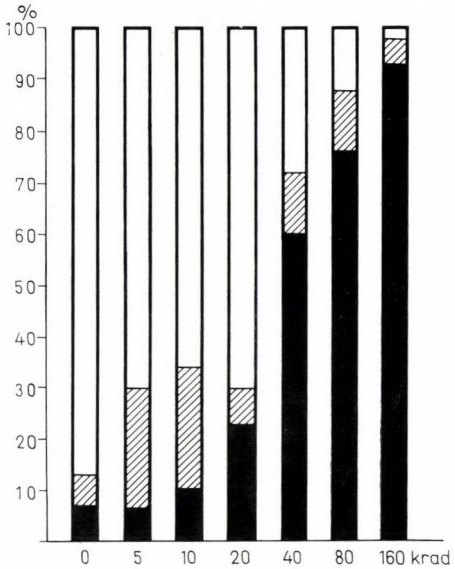

Fig. 1. Development of eggs of *Amidostomum anseris* irradiated at various dose levels (■ morula, ▨ larvae inside the eggs, □ hatched larvae)

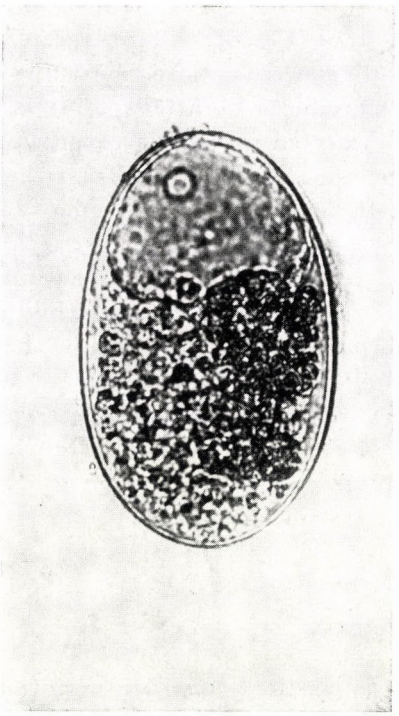

Fig. 2. Egg of *A. anseris* showing abnormal cleavage and large vacuole (× 630)

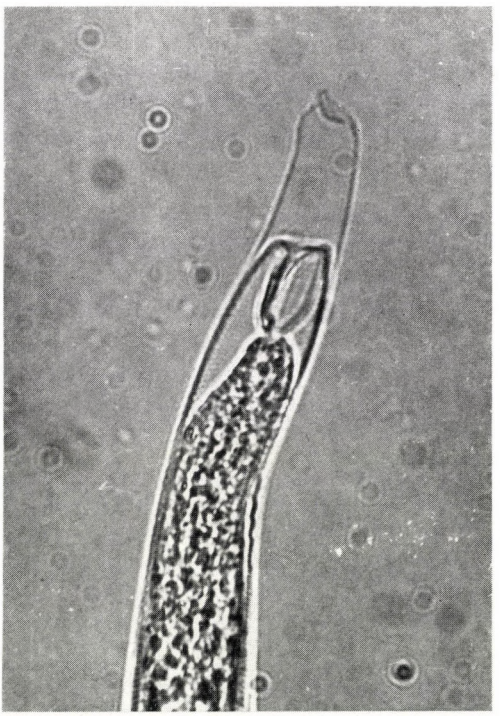

Fig. 3. Hatched larva unable to cast off the cuticle of the second moulting at the anterior end (× 1000)

doses df radiation reached the larval stage, but many of the larvae were unable to hatch and soon disintegrated inside the eggs. Some larvae managed to leave the egg, were normal in appearance or showed somewhat reduced mobility as compared to the lively movement very characteristic of normal third-stage larvae of *Amidostomum*, while others could not complete the first and/or second moulting (Fig. 3).

Amidostomum eggs could not be detected in the faeces of goslings which were infected with larvae previously exposed to a dose of 20 krad or more at the infective stage. Some eggs were, however, demonstrated in the faeces of goslings from the 10 krad group on days 23, 26 and 27 after infection, and egg-counts comparable to those in the control group (range: 75—675 eggs per gram of faeces) were regularly found in the faeces of goslings of the 5 krad group from day 19 onwards. Eggs collected from faeces of the latter group of goslings showed a reduced capacity for segmentation: 98 per cent of these eggs failed to complete segmentation, as compared to 11 per cent in the control group.

Gross patho-morphological changes characteristic of *Amidostomum* infection were found in the gizzard of every bird infected with normal larvae or with larvae which had been exposed to radiation dosages of 5 to 40 krad. Although the changes were most prominent in animals infected with normal larvae, serious damage to the gizzard mucosa was also seen in goslings which were given larvae irradiated with 5, 10 or 20 krad (Fig. 4). In birds receiving larvae treated with 40 krad, only slight alterations were noticed, consisting of a few haemorrhagic patches and curly tracts produced by a few parasites in the mucosa. None of the goslings which were infected with larvae exposed to a dose of 80 krad of radiation contained any lesion in the gizzard. A gizzard representing lesions of medium severity for the specified group is shown in Fig. 4.

Infectivity of the larvae, as measured by worm burdens at autopsy, decreased as the dose of irradiation increased (Table I). The males exhibited a higher radiosensitivity than the females. Apart from the females in the 5 krad group, and individual worms in 20 and 40 krad group, respectively, the mean length of worms developing from the irradiated larvae decreased as compared to the controls. Roughly one out of four females arising from the 20 krad group had only a rudimentary uterus and complete lack of eggs (Fig. 5), while sterile eggs were found in the rest of the worms.

Discussion

The effect of irradiation on the embryogenesis of *A. anseris* is essentially similar to that reported for other nematode parasites of poultry: the higher the dose of radiation the smaller the percentage of eggs reaching the larval

Table I

The effect of various levels of larval irradiation upon the take (worm conuts expressed as percentage of larvae inoculated), sex ratio and length of worms recovered from goslings which were each infected with 500 *Amidostomum anseris* larvae at the age of 2 days and killed 4 weeks later

No. of goslings	Irradiation (krad)	Mean take ± S.D.	Worm sex ratio M : F	Length of worms in mm (mean + S.D.)	
				♂♂	♀♀
4	0	77.7 ± 5.9	1 : 1.172	13.5±0.8	18.9±1.7
4	5	64.2 ±30.0	1 : 1.442	13.2±0.9[a]	19.4±1.2
4	10	43.7 ± 6.1	1 : 2.203	11.0±1.1[b]	17.3±1.4[b]
4	20	25.2 ± 4.4	1 : 471.0	15 (1 ♂)	14.9±1.6[b]
4	40	0.05± 0.0	1 ♀	0	16 (1 ♀)
3	80	0	—	—	—

Level of significance: a, $p < 0.05$; b, $p < 0.001$

stage. Although a certain number of irradiated eggs developed into larval stage at each of the dose levels applied in the experiment, many of them failed to hatch. Apparently less affected larvae, however, hatched even after an exposure to as high a dose as 160 krad, and were morphologically indis-

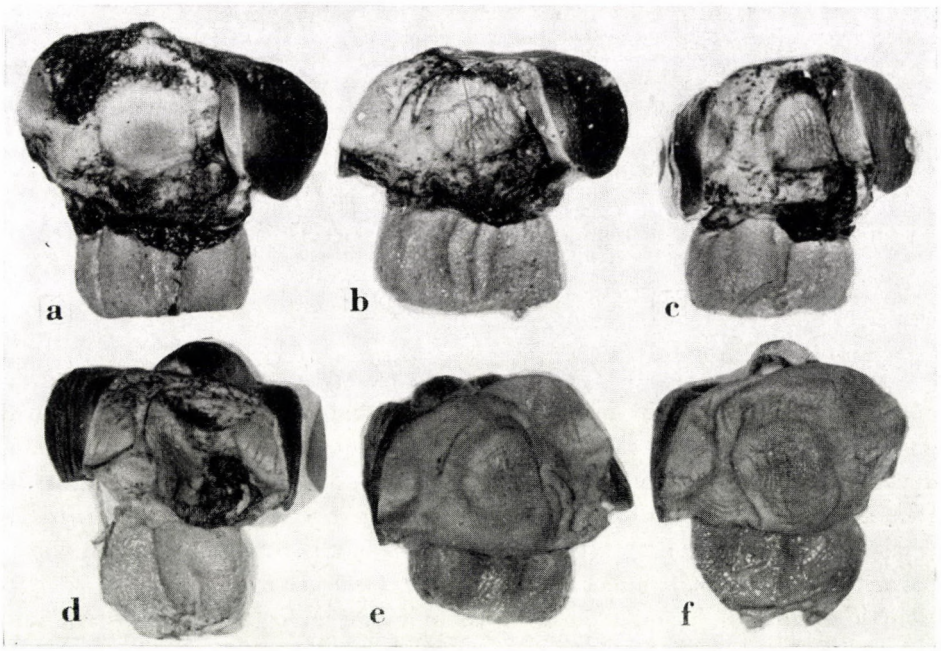

Fig. 4a—f. Gross pathological changes in the gizzard of goslings infected with larvae of *A. anseris* after exposure to various levels of irradiation: a, unirradiated; b, 5 krad; c, 10 krad; d, 20 krad; e, 40 krad; f, 80 krad

tinguishable from normal infective stage larvae of *Amidostomum*. It was only some of the free larvae that could not cast off the cuticle of first and/or second moultings and therefore two additional larval sheets were readily recognised. Unfortunately, the infectivity of such larvae was not investigated.

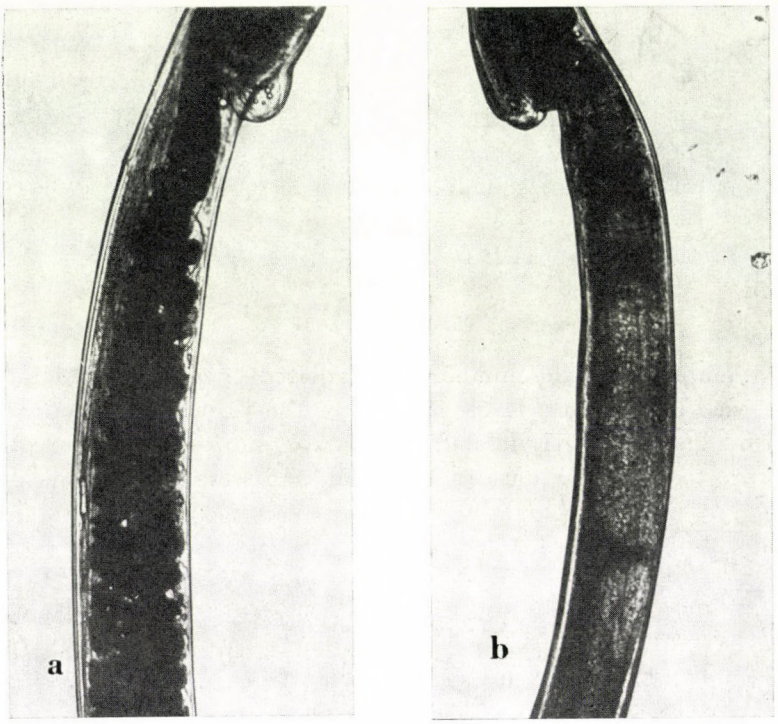

Fig. 5. Posterior end of a normal mature female of *A. anseris* (a) compared with a sterile female, (b) developing from a larva irradiated at 20 krad (×125)

Irradiation of third-stage larvae at 80 krad renders them non-infective, as neither signs of earlier lesions nor parasites could be detected in the gizzard of goslings inoculated with such larvae. Lower irradiation levels resulted in a dose-dependent reduction of infectivity of larvae and growth inhibition of worms. The remarkable shift in the sex ratio indicates that, as with males of *Ascaridia galli* (SHIKHOBALOVA and PARUZHINSKA'YA, 1958; etc.) and some other nematode parasites of mammals, male larvae of *A. anseris* are more sensitive to radiation than females. Though no alterations in the males were discovered under light microscopic examination, some damage to the male gonads at low dose of irradiation (5 krad) may perhaps account for the high proportion of infertile eggs produced by normal-looking females. The complete lack of eggs in

females is not regarded as a unique feature of post-irradiation effect on this parasite, since similar changes were invariably observed also with other nematode species.

The extension of lesions in the mucosa of gizzard is in close relationship with the number of burrowed worms. Thus, it is inferred from the present radiation effect titration study that an exposure amounting to about 40 krad of irradiation may be necessary to achieve suitable attenuation of *A. anseris* larvae for use in immunization experiments. This is approximately four times the dose required for attenuation of *Ascaridia galli* larvae (VARGA, 1964a; DEO et al., 1971) or those of *Syngamus trachea* (VARGA, 1964b; ZIEGLER, 1966).

*

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SUMMARY

Eggs of *Amidostomum anseris* exposed to various doses of gamma rays, ranging from 5 to 160 kilorad, were arrested at different stages of development. In proportion to the dose increase, more and more eggs remained at the stage of an irregular morula showing abnormal cleavage and containing vacuoles. A considerable number of larvae were unable to hatch, whereas others could not cast off completely the cuticle of first and/or second moulting. Irradiation of third stage larvae reduced their infectivity for goslings, resulted in development of sterile females or ones producing infertile eggs, and caused a remarkable shift in worm sex ratio. A significant decrease in the size of worms was also noted. The pathogenic effect of *A. anseris* can be greatly reduced by exposing the infective larvae to a dose of approx. 40 krad of gamma irradiation.

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EFFECT OF SOME AGROINDUSTRIAL BYPRODUCTS ON COCK SEMEN

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Introduction

An adequate plane of nutrition is recognized as an essential factor for reproduction of poultry. Unfortunately, nutritional requirements for semen production have been far less intensively studied than those for egg production and hatchability, even though the fertility of the individual cock is undoubtedly more important than that of the hen.

The need for research on this subject is especially topical in countries such as India, where nutritionists — in the attempt to minimize competition with man for certain cereal grains — are striving hard to exploit newer sources of feed for poultry, as it is equally imperative on the nutritionists' part to consider what effects new feed ingredients will have on breeding stock. Two agroindustrial byproducts which seem to have great potential application in India are *sal seed cake*, which is the residue remaining from seeds of the tree *Shorea robusta* after the fat content has been removed to make butter; and *damaged wheat*, that is, grain that has deteriorated due to fungal and insect infestation and is no longer suitable for human consumption but still acceptable for animal nutrition.

Utilization of sal seed (PANDA, 1969) and sal seed cake (VERMA and PANDA, 1970) has been found to give satisfactory results in White Leghorn cockerels, although the studies were conducted only up to 8 weeks of age. Feeding of damaged wheat, however, has only been investigated in broilers up to 11 weeks of age (THAKUR and PANDA, 1971). With both products there is no information whatsoever about their effects on semen characteristics. It was therefore felt that if feeding of these ingredients to breeding cockerels is to be advocated, it would be necessary to obtain pertinent data on any possible disadvantageous effects they might have on performance.

Review of literature

Even disregarding the lack of data about the effect of feeding sal seed cake and damaged wheat, the literature dealing with the relationship between nutrition and semen characteristics of cocks is very limited.

Breneman (1941) observed that restriction of the diet of young male chicks causes a retardation of testicular growth, but whether this was related to lower fertility was not studied. Parker (1943) and Lorenz (1959) further observed that the body weight of the cocks was reduced and showed that inanition decreases the level of fertility. Semen volume, number of spermatozoa per collection and fertilising capacity of the males were all adversely influenced.

Lack of protein or essential amino acids in the diet of laboratory animals may lead to inhibition of the development of the male reproductive organs and lowered fertility. Experiments with rats conducted by Moustgaard (1959) have shown that pronounced calcium deficiency may cause infertility in both sexes. Feeding of an extremely low phosphorus diet to prepubertal rats caused inhibition of the development of testes and arrested spermatogenesis.

Perek (1966) demonstrated a relationship between thyroid activity and spermatogenesis in birds by feeding thyroprotein. He assumed that the process of sperm production and fertility are closely related to the body's general metabolism.

Cooper (1968) established that breast width and body weight of turkeys could be correlated with fertility in males. Extremely heavy toms may be responsible for some of the problems of fertility. He pointed out that lighter weight males may be more agile and hence able to complete more matings. He conducted experiments to find means of reducing the body weight of turkey males and to determine if fertility could be improved when lower weight males were used in natural matings.

Kuhns and Arscott (1969) showed that vitamin E restored fertility and sperm concentration in White Leghorn males fed a diet high in linoleic acid but low in vitamin E. The fertility of the adult male chicken is a sensitive measure of deficiency of this vitamin, but neither its nutrient nor its antioxidant effects on male fertility have been clarified. In absence of vitamin E and ethoxyquin, fertility and sperm concentration were reduced.

Materials and methods

Mixing the ration

The ingredients were ground to a granular consistency and thoroughly mixed to produce a uniform mash. Micro-ingredients were first mixed in a little quantity of mash and this premix then mixed in the bulk mash. All the experimental mashes were prepared in accordance with their gross composition. Representative samples were drawn from every mash and stored in clean, dry, tightly stoppered plastic bottles for analysis.

Experimental birds

Thirty White Leghorn cocks, 8 months of age and of uniform size, vigour and body weight were obtained from the Institute's Experimental Poultry Farm. The cocks were wingbanded, weighed individually and distributed randomly into three sets of 10 cocks, each composed of two replicate groups. The replicate groups were randomly allotted to commercial finishing battery compartments in which five cocks could be comfortably housed. The three groups were put on different experimental mashes with the gross compositions shown in Table I.

Table I

Gross composition of the experimental diets
(on wt. % basis)

Ingredients	Diet 1	Diet 2	Diet 3
Maize	60	50	—
Whate bran	20	20	20
G. N. Cake	10	10	10
Corn gluten meal	4	4	4
Sal seed cake	—	10	—
Damaged wheat	—	—	60
Fish meal	2	2	2
Meat meal	1	1	1
Mineral mixture	3	3	3

Vitamins added/100 kg of diet: Vit. A 1.5 g; Vit. B_2 0.5 g; Vit. D_3 0.4 g

Feeding

The three groups were fed the different diets during the entire period of experiment. The dry mash system was followed. The quantity of mash offered was adjusted according to consumption to avoid wastage.

Watering

Clean, fresh drinking water was available to the birds at all time, the water containers were thoroughly cleaned daily.

Experimental period

The experiment was started on 3rd June 1971 and terminated on 7th August 1971. The first period of 6 weeks was assigned only for feeding of the birds. After this period was over, collection of semen from individual cocks was started from 14th July 1971 and was continued every week till 7thAugust 1971. The cockerels were weighed individually at the start and at the termination of the experiment.

Collection of semen

The semen was collected from the cock by the abdominal massage technique suggested by BURROWS and QUINN (1937). The bird was stimulated to

ejaculatory responses by massaging the soft sides of its abdomen, between the gizzard and the pelvic bones. During these responses, the copulatory organ erects and is rapidly thrust out and withdrawn. The semen was caught in a small beaker held under the vent with the left hand. The semen was taken to the laboratory for examination immediately after collection.

Semen samples were restricted to those as free as possible from contaminating fluids such as "the clear watery fluid", while urates, faeces and visible foreign material were avoided.

Counting of spermatozoa

The semen was diluted to 1 : 200 with 0.85% normal saline solution containing a few drops of formaldehyde, employing the standard procedure used for counting of red blood cells (TANEJA and GOWE, 1961).

Percentage of living spermatozoa

Immediately after collection, one drop of semen from each sample was mixed with 5 drops of stain (0.6 g nigrosin and 0.16 g eosin in 10 ml 3.55% sodium citrate dihydrate) and 15 minutes later two smears were made on glass slides. The air-dried smears were examined under oil immersion lens (1000 × magnification). The live sperms were differentiated from dead sperms by the presence of a clear nonstaining sperm head, dead sperms having a violet-stained head (MUKHERJEE and SINGH, 1968).

Metabolic activity of sperms

A reliable and quick method is the "Methylene blue reduction time" (MBRT). Equal quantities of 0.885% sodium chloride diluent and fresh egg yolk were mixed. To 13.5 ml of this mixture, 1.5 ml of methylene blue was added from a stock solution containing 50 mg methylene blue per 100 ml distilled water and thoroughly mixed. The MBRT determinations were made by mixing 0.05 ml undiluted fresh semen with 0.45 ml of the yolk diluent–methylene blue solution (pH 6.5, measured by Philips pH meter). After stirring, several drops of liquid paraffin were added to the surface of each mixture. All the test tubes were immersed simultaneously in a water bath at 40 °C and the time required for the appearance of a definite yellow colour was recorded.

Methods of analysis

For proximate analysis of the feed samples, the procedures were adopted according to ISI recommendations.

Gross energy

The gross energy of the feeds offered during the experimental period was determined by measuring the heat of combustion of the material in an adiabatic Toshniwal Bomb Calorimeter, following the procedure recommended by ISI (1968). The water equivalent of the apparatus was determined with pure benzoic acid.

Results and discussion

The results of chemical analysis of the experimental mashes used in this experiment are presented in Table II, and the summarized findings of the examinations of the birds in Table III.

Table II

Chemical composition of the experimental diets
(Expressed on D. M. basis in %)

Ingredients	Diet 1	Diet 2	Diet 3
Dry matter	87.84	87.85	87.64
Total ash	7.0	8.3	8.9
Insoluble ash	0.817	1.120	2.227
Calcium	1.12	1.14	1.10
Phosphorus	0.94	0.93	0.93
Ether extract	3.9	4.3	3.1
Crude fibre	5.3	6.1	5.8
N.F.E.	66.17	64.73	64.42
Uric acid	0.092	0.170	0.091
Crude protein	17.63	16.57	17.78
Gross energy	4096	4016	3887

Table III

Summary of results

	Control	Sal seed cake	Damaged wheat	No. of observations in each group
No. of spermatozoa[1]	1.20±0.11	1.18±0.10	1.16±0.10	30
Live%	93.96±1.10	94.43±1.10	93.56±0.98	30
MBRT[2]	7.10±0.57	7.00±0.49	7.13±0.61	30
Testes weight[3]	0.675±0.087	0.830±0.078	0.660±0.090	10
Initial body weight[4]	1634.3±22.4	1616.0±30.8	1569.3±106.3	10
Final body weight[4]	1695.0±45.6	1742.0±34.0	1596.0±73.7	10

Note: 1, million/μl; 2, methylene blue reduction time in min.; 3, % of final body weight; 4, in gram

It may be seen from the data that the diets contained recommended levels of protein and crude fibre. The nitrogen-free extracts of all the mashes indicate that they were well certified with energy. The percentage of calcium is on the ISI recommended level and phosphorus is well above the recommended level. The diets were reasonably balanced to meet the nutrient requirements of the breeding cockerels. No reduction in body weight of the cockerels was noted under any treatment, indicating that there was no inadequacy in the diets offered. The cockerels fed on sal seed cake and damaged wheat diets exhibited no deficiency symptoms. The results show that the inclusion of ingredients viz. sal seed cake and damaged wheat in no way lowered the nutritive value of the rations. This experiment agrees with those of VERMA and PANDA (1970), who observed satisfactory growth in White Leghorn cockerels by feeding sal seed meal at 10% level up to 8 weeks of age.

Recently in some experiments conducted on broilers, THAKUR and PANDA (1971) have shown that damaged wheat up to 60% level could be used in broiler diets by replacing completely the normal maize and the growth observed in broilers was also quite satisfactory. In the present investigation also, damaged wheat proved quite promising as a substitute for normal maize in the diets of breeding cocks.

Semen quality

Semen quality was assessed on the basis of the sperm concentration, metabolic activity of sperms and percentage of live sperms, which are closely related to fertility (LORENZ, 1959; CHERMS, 1968). Semen volume was not included because it shows no relationship to fertility (CRAWFORD, 1964).

Sperm concentration

There was no marked difference in the sperm concentration of cocks fed sal seed cake (1.18 million/μl) or damaged wheat (1.16 million/μl) from the average concentration of 1.20 million sperms/μl found in the control group (60% maize). These values are somewhat lower than the values cited in the literature (WHEELER, 1943). However, the latter are averages for the whole year, and if the variation due to seasonal effects is considered the values reported here are in close agreement with the figures reported by PARKER (1943), who observed a decline in sperm concentration during July, when the present examinations were made. Similar observations have been reported in turkey by CARSON (1955).

Testicular weight

The results obtained in the present studies are in agreement with the analysis of data on testes weight and sperm concentration carried out by BURROWS (1939), who showed that there was a significant correlation between these two characters (correlation coefficient 0.78), indicating that about 61 percent of the variation in semen production was due to difference in testes size.

Percentage of live sperms

The percentages of live sperms were 93.96, 94.43 and 93.56 in groups fed diets 1, 2 and 3, respectively. There was thus practically no difference in the live sperm percentage due to feeding the cocks on damaged wheat and sal seed cake diets. The results are quite satisfactory when compared with the results obtained by BAJPAI (1963) whose findings are about the same as our control diet values. The spermatozoa exhibited a completely normal morphological picture, showing none of the deformations, viz. head "bent" in loop or in a "C" form, broken heads, swelling at base of head, coiled or broken tails, that are correlated with the sterility in cocks (SAMPSON, 1939).

Methylene blue reduction time

There was no noteworthy difference in the MBRT of the different groups. The results of the present investigations are quite in agreement with those reported by BOGDONOFF (1954).

*

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SUMMARY

Practically no differences in weight gain or semen characteristics (sperm concentration and viability, and sperm metabolism measured by the methylene blue reduction time) were noted on the basis of the values for a control group in two groups of 10 eight-month-old White Leghorn cockerels kept for three months on diets containing 10% sal seed cake or 60% damaged wheat partially or wholly in place of the 60% maize of the control diet. In view of the absence of adverse effects and their cheapness relative to maize, the utilization of both foodstuffs in the diet of breeding cocks can be advocated at the indicated levels, and these new feed replacers help keeping the cost of feeding at considerably lower level.

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PLASMA CORTISOL CONCENTRATION AS AN INDEX OF ADRENAL CORTEX FUNCTION IN PIGLETS WITH ENTERIC COLIBACILLOSIS

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Several workers have shown that massive doses of corticosteroids can be beneficial when given to animals or man in *E. coli* endotoxin shock. The successful administration of hormones can be explained by the well known physiological and pharmacological effects of glucocorticoids, such as corticosterone (11-dehydrocorticosterone) and cortisol (17-hydroxycorticosterone, hydrocortisone). In large doses, these hormones have anti-inflammatory and antiallergic influences and block the systemic effects of bacterial toxins.

The literature contains several observations on the effect of glucocorticoids in the treatment of diseases associated with *E. coli* infection. The lethal effect of lipopolysaccharide endotoxins can be reduced or prevented with prednisolone (BERTÓK and TÓTH, 1963). Resistance of 2 to 3-week-old ratlings to *E. coli* endotoxin fell by a factor of 27 following adrenalectomye Administration of cortisol and adrenalin led to a significant rise of their resistance (BELOKRYLOV and OSTROVSKY, 1968). As NAMIOKA et al. (1968) have reported, glucocorticoids are effective for the treatment of piglet scours (3-week enteritis). The hormone therapy seems to be reliable if administered in the earlier stages of the illness. The lethal effect of *E. coli* endotoxin on 10 to 11-day-old chick embryos was prevented by the administration of cortisol (GRUNINGER and HOWE, 1969). Adrenal corticosteroids have also been used to help neutralize the cellular action of endotoxin in calves with colibacillosis (LARSON and BULL, 1970).

According to the general opinion, stress factors may contribute to an increase in the intestinal haemolytic *E. coli* population, consequently they have a negative role in the pathogenesis of enteric colibacillosis (MINIATS and ROE, 1968; THOMLINSON, 1969). On the basis of data reported in the recent literature, a further and perhaps more interesting consideration arises concerning the relationship of stress and colibacillosis. In the experiments of GROSS and SIEGEL (1965), subjection of cockerels to various degrees of social interaction markedly affected their resistance against infectious agents. A high degree of social stress induced increased resistance to *E. coli* infection. The resistance seemed to be correlated with relatively high levels of plasma corticosteroids in socially mixed cockerels (GROSS and COLMANO, 1967). When ACTH was administered, the relative plasma values of corticosteroids were greatly increased, and the birds were also more resistant to *E. coli* infection than the controls. These experiments clearly demonstrate that the host response to *E. coli* infection is conditioned by prior exposure of the host to certain kinds of stress. The mechanism by which previous stress affects resistance to endotoxin, however, is not completely understood. RAVIN and FINE (1962) pointed out that the reticuloendothelial system is the common pathway between *E. coli* endotoxin and stress. Animals conditioned to certain forms of stress develop some tolerance to endotoxin, because their reticuloendothelial cells more efficiently take up endotoxin and detoxify it.

Several workers have shown that the pituitary-adrenocortical system is not fully developed during the early stage of postnatal life (JAILER, 1950; KEMÉNY et al., 1964). As compared with that of the adult, the response of neonatal animals to stressors is inadequate. SCHAPIRO et al. (1962) termed this early stage of development, the "stress non-responsive period".

The present study was intended to answer the question of whether the hormonal immaturity of young animals has any role as a predisposing factor in the pathogenesis of colibacillary diarrhoea. Have the moribund piglets some degree of adrenal insufficiency or are their adrenal glands still functioning in the advanced stages of the disease? The functional state of adrenal cortex was characterized by the determination of the circulating glucocorticoid level.

Materials and methods

Experimental animals and procedure

A total of 27 crossbred pigs (Large White × Danish Landrace) from a closed herd was used in this study. The 3—5 days old animals were housed and managed in the same way. With the exception of the moribund piglets, they sucked their dam until the time of examination.

Among the animals studied, 16 piglets from four litters were affected with naturally occurring enteric colibacillosis. At the time of examination, they had showed profuse diarrhoea for 12—24 hours. None of them had been treated by veterinarians during their illness. At the same time, 11 healthy piglets from three litters served as controls.

In view of the diurnal rhythm of cortisol secretion, the examinations were carried out similarly in case of each animal. The piglets were removed from their dam and transported to the laboratory in the late morning hours. The blood samples were drawn anaerobically from the anterior vena cava for the determination of pH, standard bicarbonate and plasma protein values. The relevant laboratory methods were published in an earlier paper (KUTAS and SZABÓ, 1971). After this, the animals were sacrificed by severance of the cervical blood vessels. The heparinized blood samples collected in this way were centrifuged and the plasma was used for determination of glucocorticoid level.

Determination of corticoids in blood plasma

The corticoids were determined by the method of BUSH (1961). One part of 0.2 N NaOH solution was added to 20 parts of heparinized blood plasma. The corticoids were then extracted three times with about 3 volumes of chloroform. After filtration, the chloroform was evaporated in a water bath at 55—60 °C. The dry extract was dissolved in 1.5 ml of chloroform and chromatographed on Whatman No. 1 paper. At the same time, standard amounts (2, 5 and 10 μg) of cortisol were run similarly.

The descending paper chromatograms were developed in the A system of BUSH (100 ml light petroleum, 80 ml methanol, 20 ml water). After running, the chromatograms were removed from the tank and dried. For the separation of steroid fractions, the strips were chromatographed at 37 °C in the B_5 system of BUSH (100 ml benzene, 50 ml methanol, 50 ml water).

The dried paper strips were sprayed with an alkaline solution of blue tetrazolium and the formazans were eluted with 7 : 3 mixture of ethylacetate : methanol. The optical density was read in Spectromom 360 photometer at 560 mμ.

Post-mortem findings

All diarrhoeic and control piglets were necropsied, and bacteriological examinations were carried out for confirmation of the diagnosis established *in vivo*. The diarrhoeic piglets showed catarrhal inflammation of the stomach and intestine. Haemolytic strains of *E. coli* were isolated in pure, or almost pure culture from the small intestine of all piglets in the diarrhoeic group. In contrast, no pathological changes were observed at necropsy of the control animals. The bacteriological examination of small intestine was negative for haemolytic *E. coli* in these piglets.

The data were analyzed statistically employing Student's *t* test.

Results

At the time of examination, each animal belonging to the diarrhoeic group showed the well known clinical symptoms of baby piglet enteritis. The faecal material from the affected pigs was semi-fluid in consistency and yellowish in colour, having a characteristic fetid odour. A loss of turgor and elasticity was noted, the extremities were cool and cyanotic. The most severe form of colibacillosis was observed in piglets No. 1, 4, 5 and 15. These animals were unable to walk and their comatose condition indicated the terminal stage of illness.

The clinical status of the piglets is described with the same laboratory parameters used in our earlier work (KUTAS and SZABÓ, 1971). For the assessment of the acid–base status we used the pH and standard bicarbonate values in blood. The water balance of the animals was characterized by the plasma protein level. Table I presents the data obtained for venous blood of the diarrhoeic and control piglets. The elevation of plasma protein level in piglets with enteric colibacillosis indicates a decline of plasma volume caused by rapid dehydration. The parameters of acid–base balance showed severe metabolic acidosis in each diarrhoeic animal.

The chromatographed samples yielded two ultraviolet-absorbing and tetrazolium-reducing fractions. Since its mobility was the same as that of standard cortisol chromatographed simultaneously (R_F 0.40), Fraction I was considered as cortisol concentration. The plasma of piglets with enteric colibacillosis contained over ten times as much cortisol as did the plasma samples from control animals (see Table I).

Attempts failed to identify Fraction II with the following standards: cortisone, corticosterone, DOC, 6β-hydroxycortisol, 17-hydroxy-11-deoxycorticosterone. The unidentified fraction was especially high in the blood plasma of diarrhoeic piglets.

Table I

Concentrations of blood constituents in diarrhoeic and normal piglets

Piglet No.	Sex	Age	Body weight	Plasma	Blood		Plasma
				Total protein	pH	Standard HCO_3^-	cortisol
		days	g	g/100 ml		mEq/l	μg/100 ml
1	♂	3	830	9.5	7.08	10.0	16.6
2	♀	3	910	—	6.83	6.1	28.5
3	♀	3	1050	9.0	6.73	6.8	25.0
4	♂	3	920	10.5	7.04	7.3	27.7
5	♂	3	900	8.4	7.07	9.4	62.5
6	♀	3	1105	7.5	6.99	8.5	42.0
7	♀	3	1090	7.8	7.15	11.8	20.8
8	♀	3	1185	7.5	7.19	13.2	23.0
9	♀	3	1070	8.3	—	13.2	25.0
10	♀	3	1130	7.3	7.00	8.6	17.0
11	♂	5	1030	6.8	7.09	9.6	14.0
12	♀	5	1750	7.9	6.99	9.1	50.0
13	♂	5	1460	9.0	6.99	7.4	42.5
14	♂	5	1440	6.7	7.03	9.0	15.3
15	♂	2	1125	10.8	6.98	8.8	46.1
16	♀	2	1140	7.2	7.17	13.5	16.0
Mean and S.E. in diarrhoeic piglets				8.28 ±0.33	7.02 ±0.03	9.52 ±0.58	29.50 ±3.66
Mean and S.E. in controls (n = 11)				6.36 ±0.34	7.30 ±0.02	22.86 ±0.55	2.81 ±0.27
P				<0.001	<0.001	<0.001	<0.001

Discussion

The studies reported here demonstrate a significant rise in the cortisol level of piglets with severe enteric colibacillosis when compared with values of control animals. Although the haemoconcentration of the dehydrated animals may have some role in this phenomenon, the relatively smaller change in plasma volume cannot be responsible for the tenfold rise of circulating hormone level. The high cortisol concentration is an index of increased adrenocortical activity and characteristic of a stress condition. It would be of interest to examine the nature of the stressors responsible for stimulation of the pituitary-adrenocortical system. The findings of the present study and the literary data referred here would seem to indicate a pathomechanism of stress condition along the following lines:

It has been suggested that the primary events in the pathogenesis of diarrhoea are caused by "enterotoxin", a toxin or group of toxins produced by enteropathogenic strains of *E. coli* (SMITH and HALLS, 1967). Enterotoxin appears to be distinct from classical lipopolysaccharide endotoxin, which is involved more in the terminal events of disease. It is just possible that enterotoxin makes the intestine more permeable to the passage of endotoxin. It is known that endotoxin derived from *E. coli* strains is absorbed continuously into the blood stream in shocked animals (RAVIN and FINE, 1962). Yet, the organism is not defenceless against endotoxin; this can be removed from the blood by the cells of the reticuloendothelial system. However, if the reticuloendothelial system is injured by hypoxia, deficient blood flow, etc., as in piglets with enteric colibacillosis, its ability to detoxify endotoxin is reduced. Consequently, a significant part of the endotoxin absorbed remains free in the circulation to exert its effects on other host tissues. Among these is the very characteristic response of adrenal cortex to endotoxin. Recent studies (DAVIS and COLMANO, 1971) have demonstrated that plasma corticosterone increases in weanling pigs after injection of *E. coli* endotoxin. Since hypophysectomy abolishes the stressing effect of endotoxin, the endotoxin does not appear to act directly upon the adrenal cortex. It is most likely that endotoxin causes an increase of plasma corticosteroids by acting on the hypothalamic-pituitary-adrenal axis at the level of the nervous system (MOBERG, 1971).

In addition, it should be emphasized that change in the "internal environment" of the cells (hypoxia, acidosis, hypovolemia, etc.) may also act as stressing agent, stimulating the secretion of corticosteroid hormone. Thus, it seems possible that the increased cortisol concentration in diarrhoeic piglets is a consequence of both endotoxin absorption and imbalance of homeostasis.

Judging from the results of the present investigation, it appears that there is no reason to suspect that the rapid and fatal course of illness is connect-

ed with adrenal insufficiency of the affected piglets. The adrenal cortex of the neonatal pig secretes substantial quantities of cortisol, even at the terminal stage of illness, indicating that the adrenals are still functioning at normal level. Most recently a similar observation has been made on diarrhoeic calves: the significantly elevated plasma 17-hydroxycorticosteroid level was suggestive of raised adrenocortical activity (DVOŘÁK, 1971). It is hardly to be expected that exogenous hormone administration would be very effective in the treatment of the severe form of baby piglet enteritis.

SUMMARY

The level of circulating cortisol hormone was measured in plasma of 16 piglets, 3—5 days of age, affected with a severe form of naturally occurring enteric colibacillosis. At the same time, 11 clinically healthy piglets of the same age served as controls. Tenfold higher cortisol levels were noted in the plasma of diarrhoeic animals than in that of controls. This rise is an index of increased adrenocortical activity at the terminal stage of illness.

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EFFICACY OF TERENOL IN THE TREATMENT OF CATTLE WITH NATURAL RUMINAL PARAMPHISTOME INFESTATIONS

By

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Introduction

The observation that 2,6-dihydroxybenzoic acid-4'-bromanilide (Terenol, Farbwerke Hoechst AG, Frankfurt/M) possesses vigorous paramphistomicidal properties is of fairly recent origin. Preliminary critical tests conducted by the manufacturer on sheep and calves as well as later experiments by LÄMMLER et al. (1969) on kids have demonstrated that in a dose of 65 mg/kg body weight the drug can almost completely eliminate both juvenile paramphistomes living in the small intestine and adults established in the rumen.

The present paper is a report on the efficiency of Terenol in the treatment of cattle with natural ruminal paramphistome infestations.

Material and methods

The experimental group of 80 infested animals was set up on the basis of single individual coprological examinations carried out during December 1970 in a large herd of 250—400 kg heifers and adult cattle located in an endemic area of paramphistomosis. In January 1971, a randomly selected half of the group received 65 mg/kg Terenol, the remaining half being left untreated for control purposes. The drug was administered individually to the chosen animals as a drench, in the form of an aqueous suspension.

The efficiency of treatment was judged by three successive coprological examinations performed on each animal on February 18—19, March 16—17 and April 7—8. The procedure employed was as follows: On the days in question, the morning faeces excreted by each of the experimental animals was collected and mixed with a little water to an even consistency. From this mixture two 5 g samples were taken and examined parallelly in different laboratories by sedimentation technique (straining across a fine silk screen

Table I

Trial of the efficacy of Terenol in ruminal paramphistomosis

Treated group					Untreated group				
Animal	Result of coprological examination				Animal	Result of coprological examination			
	1st (before treatment)	2nd (after treatment)	3rd (after treatment)	4th (after treatment)		1st	2nd	3rd	4th
1	+	−	−	−	41	+	+	+	+
2	+	−	−	−	42	+	−	+	+
3*	+	−	+	−	43	+	+	+	+
4	+	−	−	−	44	+	+	+	+
5	+	−	−	−	45*	+	−	−	+
6	+	−	−	−	46	+	+	+	−
7	+	−	−	−	47	+	+	+	+
8	+	−	−	−	48	+	+	+	+
9	+	−	−	−	49	+	+	+	+
10	+	−	−	−	50	+	+	+	+
11*	+	−	−	−	51	+	−	+	+
12	+	−	−	−	52	+	−	−	+
13	+	−	−	−	53*	+	+	+	+
14	+	−	−	−	54	+	+	+	+
15	+	−	−	−	55	+	+	−	+
16	+	−	−	−	56	+	+	+	+
17	+	−	−	−	57	+	+	+	+
18*	+	−	−	−	58	+	+	+	−
19	+	−	−	−	59*	+	+	+	+
20	+	−	−	−	60	+	+	−	+

* Animals later inspected at slaughterhouse

into a conical flask under a thin water-jet, sedimentation, then spreading on a microscope slide of 1 ml sediment taken by pipette from the bottom of the flask). A treatment was taken to be successful if in none of the three coprological control examinations a single *Paramphistomum* egg was found.

During the summer of 1971, 10 of the animals from the experimental group (6 treated and 4 untreated) were sent for slaughter, thereby providing an opportunity to check on the efficacy of treatment by counting the worm burdens.

Results

The outcome of the investigations is summarized in the two tables. The data presented there attest to the exceptionally powerful paramphisto-

of cattle: results of coprological examinations

Treated group					Untreated group				
Animal	Result of coprological examination				Animal	Result of coprological examination			
	1st (before treatment)	2nd	3rd	4th		1st	2nd	3rd	4th
		(after treatment)							
21	+	−	−	−	61	+	+	+	+
22	+	−	−	−	62	+	+	+	+
23	+	−	−	−	63	+	+	+	+
24	+	−	−	−	64	+	+	+	+
25	+	−	−	−	65	+	−	+	+
26*	+	+	−	−	66*	+	+	−	−
27	+	−	−	−	67	+	+	+	+
28	+	−	−	−	68	+	+	+	−
29	+	−	−	−	69	+	+	+	+
30	+	−	−	−	70	+	+	+	+
31	+	−	−	−	71	+	−	−	+
32	+	−	−	−	72	+	+	−	+
33	+	−	−	−	73	+	−	+	+
34	+	−	−	−	74	+	−	+	+
35	+	−	−	−	75	+	+	+	+
36	+	−	−	−	76	+	+	−	+
37	+	−	−	−	77	+	+	+	−
38*	+	+	+	+	78	+	+	+	+
39	+	−	−	−	79	+	+	+	+
40*	+	+	+	−	80	+	+	+	+

micidal action of Terenol in 65 mg/kg dose.* From the results of the coprological examinations it may be concluded that a single therapeutic intervention rid 90.0% of the medicated animals of their worm burdens. Even the figures for the four animals (Nos 3, 26, 38 and 40) found to be still infected at coprological examination do not spoil this excellent result, for inspection at the slaughterhouse demonstrated that in these, too, the ruminal worm burden had been substantially reduced. Thus whereas an average of 45.2 worms was recovered from these four animals at slaughter, the average number found in four untreated animals at much the same time was 288.5 (see Table II). It seems

* Species determination was carried out on worms deriving from a few randomly selected hosts only, but in all cases the parasites proved to be *Paramphistomum microbothrium*. As all the cattle used in the experiment originated from the same area, it is presumed that this was the only species involved.

Table II

Trial of the efficacy of Terenol in ruminal paramphistomosis of cattle: results of P.M. investigation

Animal	Time of slaughter	No. of worms found in rumen
	Treated group	
3	5. 5. 1971	30
11	5. 5. 1971	0
18	10. 6. 1971	0
26	10. 6. 1971	121
38	23. 7. 1971	96
40	18. 6. 1971	41
	Untreated group	
45	29. 4. 1971	56
53	7. 7. 1971	632
59	2. 6. 1971	406
66	30. 6. 1971	60

that in these cases the treated animals did not receive a dose of Terenol corresponding to their body weight.

No undesirable side-effects were noted among the medicated animals, and appetite and milk yield were undisturbed.

Discussion

The results summarized in the tables confirm the observations made in the manufacturer's preliminary trials (DÜWEL, personal communication, 1970), and by LÄMMLER et al. (1969) that Terenol has a powerful action against both young and adult paramphistomes. The preparation therefore promises to be an effective instrument in the hands of the practising veterinarian for combating a helminthosis that in practice has proved resistant to hitherto known anthelmintics.

Various drugs have been recommended in the past for use against paramphistomes, but the number of those which could claim to have led to more or less successful cures is relatively small. GRETILLAT and DAUMAS (1955) applied dispersed Na alkyl sulphate against adult flukes, while BOSMAN et al. (1961) reported on obtaining some success with hexachlorophene. In investigations with Freon, Freon BU (du Pont De Nemours), hexachlorophene, Promintic [2-(β-methoxyethyl)pyridine] and Lintex [N-(2'-chlor-4-nitrophenyl)-5-chlor-

salicylamide] against bovine and ovine paramphistomosis, HORAK (1962, 1964) found that although all the preparations, with the exception of Freon, had a paramphistomicidal effect, this action was in no case at a level sufficient to meet the demands put on a modern anthelmintic.

Freon BU acts on adult paramphistomes alone, while a single application of hexachlorophene or Promintic can be expected to have only 43—45% efficacy, and Lintex has a satisfactory effect only on paramphistomes in the small intestine and abomasum.

SUMMARY

2,6-dihydroxybenzoic acid-4'-bromanilide (Terenol, Farbwerke Hoechst AG , Frankfurt/M.) proved an effective treatment for ruminal paramphistomosis of cattle. Applied orally in a dose of 65 mg/kg body weight, the drug completely eliminated the paramphistome burdens out 36 of of 40 (90.0%) naturally infested animals.

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РЕЗЮМЕ

ЗАРАЖЕНИЕ ЦЕСАРОК ВИРУСОМ ИНФЛЮЭНЦЫ ТИПА-А

Я. ТАНИ

Описаны эндемические вспышки — две в 1969-ом и одна в 1970-ом годах — инфлюэнцы среди цесарок. Первых два изолята показывали антигенное сродство с вирусными штаммами Duck (England) 62 и Quail (Italy) 65, а третий изолят с последним штаммом. В лабораторных пассажах первым изолированный штамм постепенно терял сродство со штаммом Duck (England) 62 и наконец стал неотличимым от третьего изолированного штамма.

Определение вирусных штаммов осуществлялось в течение 14 месяцев на территории одной хозяйственной здиницы радиусом в 8 км. Во время этих поисков за вирусными штаммами регистрированы вспышки заболования в поголовьях несушек и молодняка по 4—6 тысяч штук. Заболевание сопровождалось понижением продукции и падежем животных. У бессимптомно погибших животных при вскрытии обнаруживались гиперимия, кровотечения, воспаление и перерождение внутренних органов, фибринозное воспаление воздухоносных мешков и серозных оболочек. При двух эндемиях пробой торможения гемагглютинации во всех образцах крови обнаружены антитела.

После 4-х пассажей первый изолированный вирусный штамм был патогенен только для дневных цесарчат и индюшат и молодняка цесарки. Но зато в разных группах большинство искусственно и путем контакта зараженных животных, как то, курах, утках, индейках, цесарках, мышах и морских свинках, антитела обнаруживались.

Чувствительность вируса к температуре и другие свойства напоминают свойства родственных вирусов.

Это первый случай в мире изоляции вируса инфлюэнцы из цесарок и первый случай изоляции вируса птичьей инфлюэнцы в Венгрии.

КОЛИЧЕСТВЕННЫЕ ИЗМЕНЕНИЯ СРЕДИ КИШЕЧНЫХ КЛОСТРИДИЙ У РАНО ОТБИТЫХ, БОЛЕЮЩИХ КОЛИБАЦИЛЛЕЗОМ ПОРОСЯТ

Дь. ШИНКОВИЧ

Сравниваются некоторые методы изоляции нескольких анаэробных микробов кишечной флоры поросят. В количестве споровой и вегетативной форм изолированных разными методами клостридий у здоровых поросят имелась разница только в толстом отделе кишечника, тогда как такой разницы в количестве этих двух физиологических фаз в тонком отделе кишечника не обнаружено.

При колибациллезной энтеротоксимии наблюдается мобилизация клостридий тонкого отдела кишечника, доказательством чего является увеличение количества вегетативных форм за счет спор. Данное явление наблюдается только в тонком отделе кишечника и исследованием испражнений этой разницы нельзя выявить. Автор считает возможным, что образующийся при размножении клостридий токсин усугубляет колибациллезную энтеротоксимию.

РЕНТГЕНАНАТОМИЯ КОСТЕЙ ЧЕРЕПА СОБАКИ

Дь. КОВАЧ, Л. ГЛОС и М. ГЕРТНЕР

При фронтобазальном и синистродекстральном направлениях лучей приготовлены рентгеновые снимки с длинным фокусным расстоянием костей черепа долихокефальных, мезокефальных и брахикефальных собак. При обоих направлениях лучей определен пункт падения центральных лучей. Ради лучшего понимания рентгеноанатомического положения костей черепа дается их краткое анатомическое описание с учетом рентгеноанатомической точки зрения. Авторы описывают главным образом те контуры костей черепа, которые особенно важны с клинической точки зрения. Рентгеновыми снимками и рисунками контуров иллюстрируется детальное описание костей черепа: на них описанные в тексте тени и контуры обозначены цифрами.

НАБЛЮДЕНИЯ ПО ВЫЛУПЛЕНИЮ СПОРОЗОИТОВ У КОКЦИДНОГО ВИДА EIMERIA STIEDAI

У. ДЮРР, Г.-Г. ХОЙНЕРТ, Б. МИЛТАЛЕР и Г.-К. ГАЛЛЭ

При помощи киносъемки велись in vitro наблюдения по вылуплению живых спорозоитов из спороцист вида Eimeria stiedai. При инкубации в смеси из трипсина и желчи наблюдается живое движение спорозоитов в спороцистах еще до начала вылупления. Последнее начинается разыскиванием микропилэ спороцисты передним концом спорозоита. Наиярче бросающееся в глаза явление при вылуплении — это высовывающееся из микропилэ утончение апикального конца вылупляющегося спорозоита, достигающего иногда длины 4 μ. Вылупление первого спорозоита всегда длится дольше второго. Наблюдались единичные спорозоиты, вылупляющиеся задним концом и случаи, когда оба спорозоиты пытались вылупиться из спороцисты одновременно.

К КЛИНИКЕ БОЛЕЗНИ РУБАРТА

З. ХОРВАТ, Ф. КАРШАИ и Л. ПАПП

Резюмируются наблюдения авторов по клинике и лабораторным исследованиям болезни Рубарта.

Тщательно изучена клиника болезни на 36 собаках возраста 1/2—1 1/2 года, заразившихся в естественных условиях, на 19 животных среди них проводились и лабораторные исследования. Авторы различают подострую, острую и полуострую формы болезни. Подострая форма из-за очень быстрого течения ветеринарным врачом редко регистрируется. Для клиники этой формы характерны сильное увеличение печени, угнетение, рвота, высокая температура; лабораторным исследованием выявляется лейкопения. Течение острой формы болезни длится 3—5 дней при наличии желтухи, кровотечений на слизистых оболочках, увеличении печени и чувства болезненности в области печени. Среди существенных показателей лебораторных исследований имеется повышение активности SGPT, SGOT, щелочной фосфатазы, количества билирубина и сахара в сыворотке и уменьшение количества эритроцитов. В моче появляется белок. Течение полуострой формы длится 6—14 дней. В большинстве случаев имеется сильная желтуха, интенсивные кровотечения на слизистых оболочках и в коже (кровяной диатез). В кровяной картине преобладает нейтрофилия и лейкоцитоз; впрочем результат лабораторных исследований таков же как и при острой форме. В моче появляется и кровь, состав же осадка напоминает нефрит. Характерная клиника со стороны глаз (иридоциклит и паренхиматозный кератит) имеется уже в острой форме, но чаще в подострой таковой.

Вопреки комплексной терапии (антисыворотка, печень защищающие средства, глюкокортикоиды, переливание крови и т. п.) прогноз болезни сомнителен. Среди 36 собак, больных болезню Рубарта, погибло 22. Особенно чувствительны к болезни собаки моложе пол года и старые таковые.

ЭФФЕКТ ОБЛУЧЕНИЯ НА ЭМБРИОНАЛЬНОЕ И ПОСТЭМБРИОНАЛЬНОЕ РАЗВИТИЕ AMIDOSTOMUM ANSERIS (ZEDER, 1800)

Д. В. ФУК и И. ВАРГА

Развитие яиц Amidostomum anseris было приостановлено на разных стадиях облучением разными дозами лучей гамма — от 5 до 160 килорад. Пропорционально с повышением дозы все больше яиц останавливалось на стадии неправильной морулы, показывающей неправильное дробление и содержащей вакуоли. Значительное количество личинок не вылупилось, остальные же не были способными полностью совершить вторую линьку (См. сн. 3). Облучение лишало инвазионных личинок инвазирующей способности, из совершавших инвазию вырастали стерильные самки и в популяции червей наблюдался резкий сдвиг в пользу самок. Наблюдалось тоже заметное уменьшение размеров червей. Патогенный эффект A. anseris можно существенно снизить облучением инвазионных личинок дозой прибл. 40 крад гамма лучей.

ВЛИЯНИЕ НЕКОТОРЫХ ПОБОЧНЫХ ПРОДУКТОВ С—Х ПРОМЫШЛЕННОСТИ НА СЕМЯ ПЕТУХОВ

Дь. ЛЕНЧЕШ

Практически не было разницы в привесах и характера семени (концентрация и жизненность живчиков, обмен живчиков на основании времени редукции метиленовой синьи) петушков контрольной и двух экспериментальных групп, содержавшихся в течение 10 месяцев на рационах, содержавших 10% жмыха семени индийского растения Shorea robusta и 60% частично или полностью испорченной пшеницы вместо 60% кукурузы контрольного рациона, соответственно. На основании полученных данных автор приходит к заключению рекомендовать применение упомянутые дешевые корма в показанной концентрации вместо дорогой кукурузы в кормлении племенных петухов. Это снизит себестоимость продукции в птицеводстве.

КОНЦЕНТРАЦИЯ КОРТИЗОЛА В СЫВОРОТКЕ КАК ПОКАЗАТЕЛЬ ФУНКЦИИ КОРЫ НАДПОЧЕЧНИКОВ У ПОРОСЯТ С КИШЕЧНЫМ КОЛИБАЦИЛЛЕЗОМ

Ф. КУТАШ, МАРИЯ КОЗМА, Й. САБО и ЛИДИЯ КОВАТШ

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EIMERIA MRIGAI N. SP. (EIMERIIDAE: SPOROZOA) IN BLACK BUCK (ANTELOPE CERVICAPRA)

By

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(Received May 15, 1971)

The first eimerian species described from an antelope is *Eimeria antelocaprae* Huizinga 1942 (from *Antilocapra americana*). Subsequently, *E. antelocervi* Ray and Mandal, 1960 was recorded in the black buck *(Antelocervus caprae/Antelope cervicapra)*. Oocysts identical to *E. cheetali* Bhatia, 1968 in *Axis (Cervus) axis* were recovered from this host (BHATIA, 1968) and have subsequently been found in *Gazella gazella*. In these three species, the oocysts, though possessing a micropyle, lack a polar cap.

Of the species described from wild cervidae and bovidae, the oocysts in twelve lack a micropyle, in twenty-four others a micropyle is present and in two cases a micropylar cap is also present (PELLÉRDY, 1965). To this list *E. tragocamelis* has to be added. This species has recently been described from nilgai *(Boselaphus tragocamelus)* in which the thin narrower end of the oocystic wall was suggestive of a micropyle (BHATIA, 1968).

The rectal sample available from an antelope (black buck), shot on 28. 10. 1968, yielded numerous oocysts which, after study, proved to belong to a new species described as *Eimeria mrigai n. sp.* The nematodes that were available, included 23 specimens of *Haemonchus contortus*, 36 of *Bunostomum trigonocephalum* and 12 of *Trichostrongylus colubriformis*.

Materials and methods

The alimentary tract of the black buck, examined some hours after the hunt, exhibited signs of postmortem changes though the thickened and severely congested patches yielded the oocysts and gametocytic stages. The smears from the rectal contents were examined directly and after centrifugation. A small quantity was, after straining, left in 2.5% potassium dichromate solution. Examination of scrapings from the lesioned patches in the jejunum yielded oocysts and some of the other endogenous stages. A few pieces were fixed in 10% formalin for histologic study. The 5—6 μ thick serial sections were stained with Erhlich's haematoxylin and eosin. Drawings were made with the help of a Spencer's camera lucida under oil-immersion objective of an Olympus phase-contrast microscope with 15 $\times$ eyepiece. Morphological details of the sporocysts and the sporozoites, liberated after treatment of the sporulated oocysts with 5% trypsin (pH adjusted between 7—8) followed by gentle pressure with the needle on the coverslip preparations, were observed.

Constant examination of the sporulating oocysts enabled an appraisal of the sequence in the sporulation process of the sporont.

Observations

Eimeria mrigai n. sp.

The ellipsoidal/ovoidal oocysts (50 measured), with a double-contoured wall, measured 39—55 μ in length (mean 48.5 μ) and 26—32 μ in width (mean 29.6 μ). The length-width ratio was 1.4—1.8 (mean 1.6) (Figs 1 to 7). The smooth oocyst wall, of 1.6—2.3 μ (mean 1.6 μ) thickness, had a light yellowish-green outer layer of 1.2—1.6 μ thickness and a thin inner yellowish-brown layer

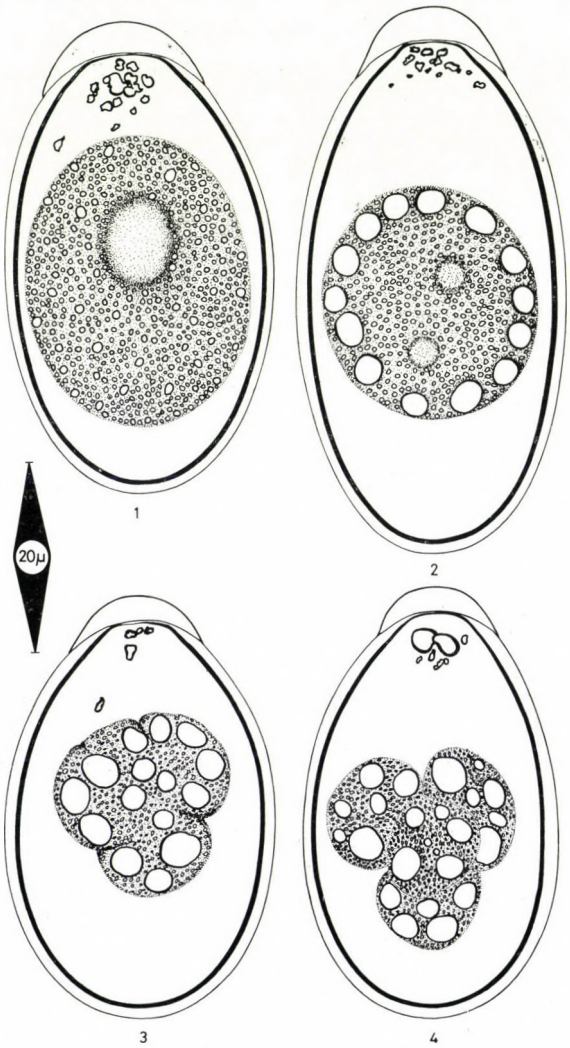

Fig. 1—4. 1, Unsporulated oocyst. *2*, Sporulating oocyst with large refractile globules at the periphery and the two daughter nuclei. *3*, Sporulating oocyst showing the beginning of cleavage lines. *4*, Sporulating oocyst with four round protoplasmic masses

of 0.4—0.7 μ thickness. A micropyle of 5—8 μ in width (mean 6.5 μ), carried a prominent but transparent and a somewhat helmet-shaped cap of 13—18 $\mu \times 2$—3 μ size (mean 15.6 $\mu \times 2.6$ μ). A tenue body present as an irregularly-shaped clump of dark but large refractile granules lying just beneath the micropyle, both in the unsporulated and sporulated oocysts. Oocyst residuum absent. Sporulation completed in seven days under room temperature. Sporocysts ellipsoidal, with the narrower end capped by a thin dark Stieda body measured 19—23 μ in length (mean 21 μ), 9—10 μ in width (mean 9.8 μ) and with the length–width ratio of 2.0—2.4 (mean 2.2) (Figs 7 and 8). Sporocyst residuum present as dark granules dispersed around the sporozoites or occurring in small clumps at the poles and/or in the centre. Sporozoites elongate, of 16—18 $\mu \times 3.9$ μ (mean 17 $\mu \times 3.9$ μ) size, with one end pointed and the other broad — the broad-

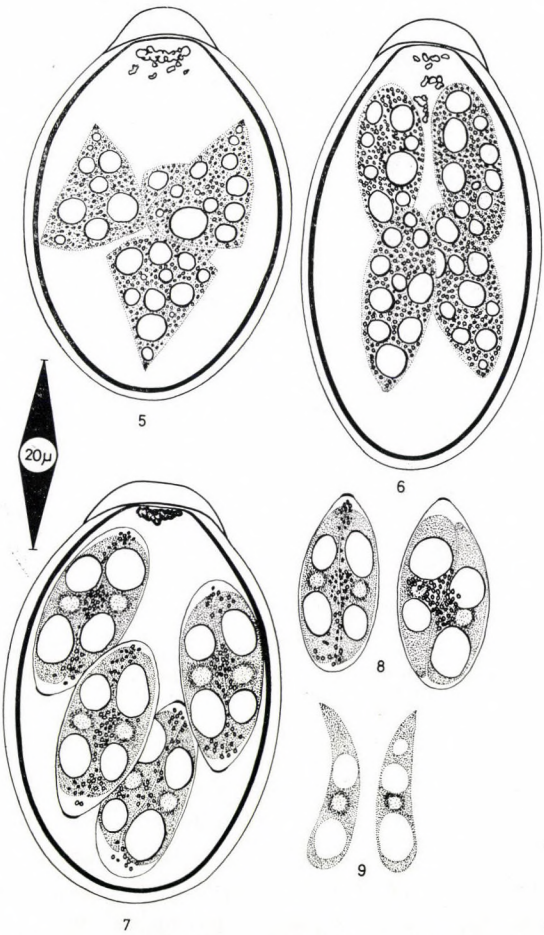

Fig. 5—9. 5, Sporulating oocyst with the pyramidal shape of the four developing sporoblasts. *6*, Sporulating oocyst with four ellipsoidal sporoblasts. *7*, Sporulated oocyst with four sporocysts — each with two sporozoites and a sporocystic residuum. *8*, Sporocysts. *9*, Sporozoites

er end carrying a large refractile globule, with one or two smaller refractile globules present towards the other end, and the nucleus situated nearly in the centre between the two globules (Fig. 9).

In the division of sporont, the initial stage consisted of a large central and coarsely granular mass of an almost spherical or ovoid shape and with a prominent vesicular nucleus occupying a lighter area in the middle (Fig. 1).

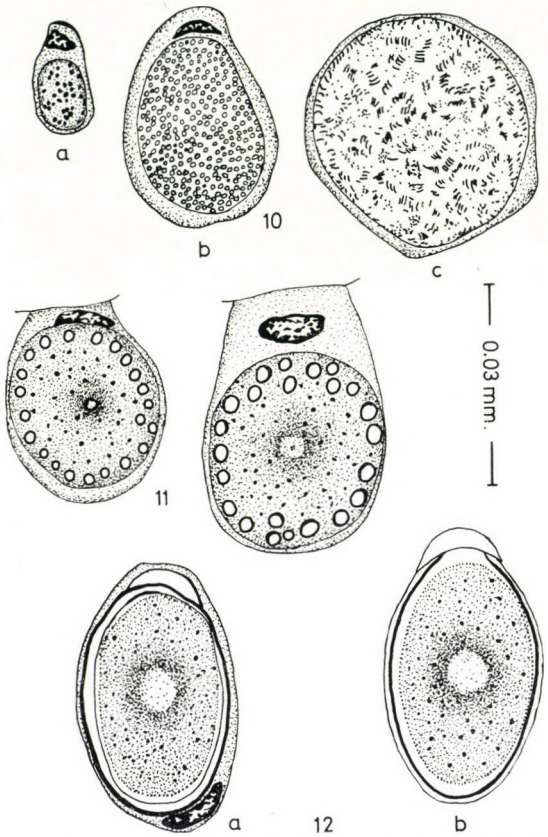

Fig. 10—12. 10, Microgametocytes: *a,* youngest; *b,* immature — both with host-cell nucleus; *c,* mature with rod-like microgametes. *11,* Macrogametes. *12a,* Capped oocyst inside an epithelial cell with its pushed nucleus; *b,* Capped oocyst detached from the lining

With its subsequent contraction, large refractile globules appeared along the periphery and the nucleus had divided into two daughter nuclei (Fig. 2). Following the appearance of the lines of cleavage, the sporont produced four protoplasmic masses — each carrying a number of refractile globules (Fig. 3). Later, four rounded sporoblasts, with many globules of different sizes (Fig. 4), developed and subsequently assumed a pyramidal form with the narrower angle carrying a dark point — the precursor of the Stieda body (the body of Schneider, according to VETTERLING, 1968; Fig. 5). Further differentiation of the

sporoblasts resulted in their ellipsoidal shape and carrying numerous globules (Fig. 6). With the development of a membrane around these sporoblasts, two sporozoites and a granular residuum had appeared in each sporocyst (Fig. 7). Two or three large refractile globules and a small nucleus in the centre became distinct in each sporozoite.

In addition to the characteristically capped oocysts, microgametocytes and macrogametes occurred in different developmental stages inside the villar epithelial cells of the jejunal region of the small intestine. In numerous sections studied, these include: a mature microgametocyte of 33×31 μ and full of numerous rod-like and 2.0—2.5 μ long microgametes (Fig. 10c) — many immature microgametocytes ranging between 25—30 $\mu \times 20$—30 μ (Fig. 10b) including the youngest of 10.9×7.3 μ in dimensions (Fig. 10a); macrogametes of 24—38 μ $\times 18$—27 μ (mean 27.5 $\mu \times 22.2$ μ) size and with prominent plastic granules and a central nucleus (Fig. 11); and oocysts of 30—50 $\mu \times 17$—30 μ in size (mean 37.5 $\mu \times 21.0$ μ) (Fig. 12). Hypertrophied nuclei of the attacked host cells were observed in all these stages.

Discussion

On account of the shape and the general colour of the oocysts and the presence of a micropyle, a polar cap and the sporocyst residuum, our oocystic material shows some resemblance to the three eimerian species (known from wild cervidae and bovidae as also domestic ruminants); *E. sordida* Supperer and Kutzer, 1961 (from *Cervus elaphus*, red deer); *E. yakimoffmatschoulskyi* Supperer and Kutzer, 1961 [from *Rupicapra (= Capella) rupicapra*, chamois]; and *E. brasiliensis* Torres and Ramos, 1939 (from *Bos taurus* and *Bubalus bubalis*, cattle and buffalo). The present oocysts are, however, distinguishable from all the three species. Though the elliptical oocysts are smaller in size in *E. sordida* and *E. yakimoffmatschoulskyi* and have a shorter sporulation time, the description of these two species appears incomplete as particulars about the size of the sporozoites, the presence or absence of a Stieda body and a tenue body are lacking. From the available accounts, *E. sordida* appears distinct because the outer oocystic wall is rough 'sordid'; a polar granule is present, and the sporozoites are smaller. In *E. yakimoffmatschoulskyi*, the differences relate to a comparatively thinner character of its oocystic wall, the easily detachable nature of the polar cap and a much smaller size of the sporocysts. The oocysts and its contained structures, in *E. brasiliensis*, have slightly smaller sizes, the oocystic wall is thinner and the tenue body consists of a homogeneous and granular protoplasmic mass. Accordingly, the present material is assigned to a new species, *Eimeria mrigai* which, because of the capped character of its oocysts, is distinguishable from *E. antelocervi* and *E. cheetali*, described from the Indian antelope. The other distinctive features in *E. antelocervi*, are: the

cylindrical shape and the smaller size of the oocysts (28—34 $\mu \times 12$—16 μ), the piriform shape and the smaller size of the sporocysts (10.9 $\mu \times 6.6$ μ) (the measurements of the sprozoites have not been given) and a shorter sporulation time. *E. cheetali*, described from *Axis (Cervus) axis*, was not differentiated from *E. antelocervi*. The former, with the smaller dimensions of the oocysts, was also encountered in the black buck. The oocysts in this species, though within the same size-range, are ellipsoidal in shape, have a thinner oocystic wall, a wider micropyle and are with ovoidal sporocysts. Thus, the three species in our black buck are: *E. antelocervi*, *E. cheetali* and *E. mrigai*.

SUMMARY

The sample of rectal contents from a black buck *(Antelope cervicapra)*, during the post-mortem examination, yielded characteristically capped eimerian oocysts which, as studied on unsporulated and sporulated characteristics and in the serial sections of invaded jejunum including the earlier gametocytes, have been described and determined as belonging to a new species. *Eimeria mrigai* which has been differentiated from the allied forms hitherto known from the wild cervidae and bovidae and the domestic ruminants. To the two eimerian species so far described from *Antelope cervicapra*, a third species has been added.

*

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A NEW GENUS AND SPECIES OF CRYPTOSPORIDIID COCCIDIA FROM INDIA

By

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(Received May 15, 1971)

The asporous and tetrazoic coccidian oocysts are known in the genus *Cryptosporidium* Tyzzer, 1910 (*Cryptosporidiidae* Poche, 1913) [WENYON (1965-facsimile of 1926 edition; LEVINE, 1961)]. HOARE (1956) has, however, reduced *Cryptosporidiidae* to a subfamily status under *Eimeriidae* (Minchin, 1912) Poche, 1913. This classification has been followed by DAVIES *et al.* (1963) and PELLÉRDY (1965). Five species so far described under this genus are included in the important compilations of WENYON, LEVINE, DAVIES *et al.* and PELLÉRDY. Besides, oocysts assignable to this genus have also been recorded in *Canis dingo* (Bearup, 1954) and *Felis chaus* (Dubey and Pande, 1963). In these reports, measurements of the oocysts and the contained sporozoites have alone been appended.

Four 13—25 day pups, maintained for administering helminthic infection, were found to harbour, on autopsy, an interesting cryptosporidiid infection which proved to be distinct from *Cryptosporidium* and is described below under a new genus designated as *Hoareosporidium* after Dr. Cecil A. HOARE F. R. S., with *H. pellerdyi* as its type species.

Material and methods

The rectal contents of the four pups slaughtered at intervals and, in two cases, the scrapings from jejunum which had revealed a slight degree of congestion were examined directly and after centrifugation. Typical cryptosporidial oocysts were encountered during these examinations. Small pieces of the suspected lesions were immediately fixed in 10% formalin and the 5—6 μ thick serial sections stained with Ehrlich's haematoxylin and eosin. The oocysts, from different locations, were studied and sketched with the aid of Spencer's camera lucida under the oil-immersion objective of an Olympus phase-contrast microscope with 15× eyepiece.

Observations

Hoareosporidium pellerdyi g. n. et sp. n.

The ellipsoidal oocysts (50 measured), in the faeces and fresh scrapings, measured 12—17 $\mu \times 8.5$—10.5 μ (mean 14.8 $\mu \times 9.14$ μ) with the length-width ratio of 1.2—1.8 (mean 1.55) (Figs 1 and 2). The 0.5 μ thick oocystic wall was double-contoured, homogeneous, colourless and transparent but with a light

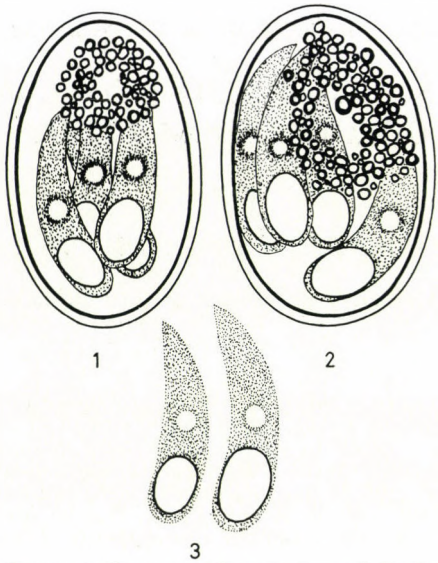

Fig. 1—3. 1, 2, Sporulated oocysts from rectal contents and fresh smears from scrapings of jejunum. 3, Sporozoites

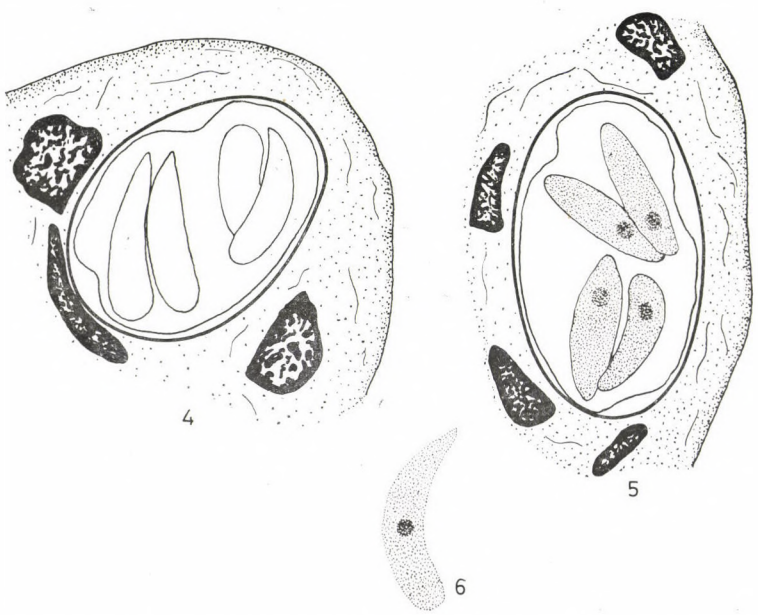

Fig. 4—6. 4, An entire sporulated oocyst in a section. Observe the four sporozoites and the pushed out host-cell nucleus. (This stage has not taken up the stain). *5,* Sporulated oocyst cut in a section showing the contained parts. Observe the nucleus in the sprozoites. *6,* A complete sporozoite from an oocyst in section

yellowish-green hue. A micropyle or a knob-like attachment was absent. An oocyst residuum consisted of a prominent mass of large-sized granules. The four naked sausage-shaped sporozoites, with one end pointed and the other broader — the broad end carrying a large refractile globule and with a small nucleus towards the centre, were 9—10.5 $\mu \times 2.6$—3 μ in size (mean 9.3 $\mu \times 2.7$ μ) (Fig. 3). In fresh smear preparations of the intestinal scrapings, some of the oocysts occurred inside the attacked cells.

In the numerous sections, oocysts were detected inside the epithelial and subepithelial cells of the villi — an attacked cell harbouring a single sporulated oocyst of 12—17 $\mu \times 7$—10 μ in size (Figs 4 and 5) (mean 14.2 $\mu \times 8.8$ μ, of 25 oocysts) and with the contained sporozoites, of 6—10.5 $\mu \times 2$—2.5 μ (mean 8.2 $\mu \times 2.4$ μ), revealing the central nucleus (Fig. 6).

From the presence of the oocysts, a mild pathological picture was evident in the sections. Aggregations of red blood corpuscles inside the core of the villi and at the tips were suggestive of congestion of the mucosa in the attacked region. A few eosinophils were observed at different places. In most of the sections, the villar tips had exhibited a denudation of the surface epithelium.

Discussion

Besides its oocystic characteristics, *Cryptosporidium* is distinguishable on account of the extra-cellular habitat of its endogenous stages, the presence of an attachment organ on the oocystic and schizontal surfaces and in the non-flagellate character of its microgametes.

On account of intra-cellular position of the oocysts (as revealed in the smears and sections), the present oocystic material cannot be included under *Cryptosporidium* which has thrice been recorded from wild carnivores. Though the dimensions given for the oocysts in *C. vulpis* Wetzel, 1938 (from common red fox) are well within the range encountered in our material, the oocysts in this species, according to PELLÉRDY, could possibly be the freshly-shed sporocysts of some known or as yet unknown isosporan species. PELLÉRDY has also quoted HOARE that the complete life cycle must be known before a final classification was possible.

The finding of typically sporulated cryptosporidian oocysts inside cells of the jejunal lining establishes beyond any doubt the intra-cellular localisation of the mature stage — a character fundamentally different from that known for *Cryptosposidium* and *Isospora*. We have to await work on the morphology of all the endogenous stages recovered in cases of natural infections or studied after infecting laboratory-raised clean pups with the infective oocysts of this new species for an accurate appraisal of the details of its life history. This will enable a fuller concept of the new genus. Possibly, the oocysts from the three wild carnivores included under *Cryptosporidium* might prove to belong to this new genus, *Hoareosporidium*.

SUMMARY

During autopsical work with four of the 13—25 day old pups, the rectal contents and smears of the jejunal scrapings and stained serial sections in two cases with evidence of slight congestion, revealed asporous and tetrazoic oocysts resembling those of *Cryptosporidium* which, however, is extra-cellular in habitat. On account of the detection of the typical oocysts inside attacked cells, in some of the smear preparations and in numerous sections of the invaded intestinal region, the present oocystic material warrants the establishment of a separate genus, herein named as *Hoareosporidium*, which, on this character, is distinguishable from *Cryptosporidium*. Further studies on the various endogenous stages of this new species (designated as *H. pellerdyi sp. n.*), presently based on its oocysts, is emphasised.

*

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INVESTIGATIONS INTO THE MAGNESIUM SUPPLY OF CATTLE HERDS IN HUNGARY

By

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Mineral feed additives are seldom required under conditions of extensive management and feeding. The raising of plant and animal yields, however, often results in short supply of one or another important feed component. The increased requirements of highly productive animals are usually satisfied by supplementation, although it would probably be a more correct approach to arrange for alteration of the fodder plants and agricultural technology (TÖLGYESI, 1971). The mineral supplements available in Hungary need improvement in several respects. Routine determinations of feed compositions are still limited to only a few components, the few specialized laboratories cannot cope with the task, and management failures are often responsible for wide fluctuations of the levels of certain vitally important elements in rations.

Many authors have dealt with magnesium tetany, to mention only the comprehensive revieny of ALLCROFT and BURNS (1968) and BREIREM and HVIDSTEN (1966). The incidence of the disease is 0.5—2% in more rainy countries of the moderate climatic zone. In Hungary, it was important to ascertain whether or not cattle need a regular magnesium supplementation under the conditions of present feeding technologies.

Information on the extent of magnesium depletion can be obtained from the frequency of occurrence of magnesium tetany and from the magnesium contents of feeds and blood, urine and hair of the animal. For physiological and metabolic considerations and for the sake of simplicity, the urine test was chosen about the magnesium supply of cattle herds in Hungary.

It has often been shown that the urinary magnesium level is proportional to the amount of ingested and absorbed magnesium (FIELD, 1964; ENDER et al., 1957; MEYER and GRAND, 1963; SIMESEN, 1963; MÁRKUS and TÖLGYESI, 1972, etc.). As in the case of magnesium depletion the urinary level may fall 20—100 times compared to normal, this seems to be more conclusive than the serum levels. With an appropriate analytic method, atomic absorption photometry (TRUDEAU and FREIER, 1967), and fresh urine samples, several hundred tests can be carried out daily in an economic manner.

Preliminary examinations on groups of 8—10 cows from each of 10 herds suggested that individual deviation from group averages varies between 10—46% (average: 26%). This value is low compared to the difference between the urinary magnesium levels of appropriately supplied and magnesium-deficient cows.

A general survey of the magnesium supply of Hungarian cattle herds was performed during the summer season of 1971, the PHYLAXIA Co. and the Department of Animal Nutrition, University of Veterinary Science, Budapest.

Material and methods

Samples were collected from large herds with the assistance of the regional Veterinary Institutes and Veterinary Services. Spontaneously excreted urine from 8—10 cows per herd was pooled in a bottle sealed with paraffin coated cork and immediately sent to the laboratory along with an accompanying letter specifying the quantity and quality of diet fed during the preceding week. The fresh urine samples, if free of sediment, were appropriately diluted and examined with a type 290 B Perkin-Elmer atomic absorption spectrophotometer. Samples which arrived after some delay or contained a sediment were acidified prior to examination, to dissolve any magnesium present in the sediment.

Urine, milk, blood and faecal samples were additionally procured from 10 large herds. Blood magnesium content was determined directly after dilution of the sample, milk and faecal samples were treated with perchloric and nitric acid. Statistical evaluation of the results was carried out according to that of SVÁB (1967).

Results

Regional (county) averages of urinary magnesium levels are shown in Table I. The national average for the total of 775 samples received was 491 ppm compared to the regional average of 452 ppm. Evaluation was made for a dual purpose: first of all, to establish the frequency of herds with suspected magnesium depletion and, secondly, their regional distribution. In clinically manifest cases of magnesium deficiency the urinary level falls to 5—10 ppm. On the other hand, the magnesium content of the serum does not fall to the level of subclinical depletion (2.0 mg%) as long as the urinary concentration persists at 100 ppm. Yet, instead of the figures, the more reliable value of 200 ppm was taken as minimal limit. According to our experience, group averages above 200 ppm indicate absence of magnesium depletion in the herd. Fifty-nine samples (7.6%) were segregated from the total on this basis, but even these did not meet the criteria of depletion. To put it more correctly, 719 samples (93.4%) could be judged as definitely not originating from a magnesium-deficient herd. To check the reliability of our procedure, second samples were asked for from those herds in which group averages were less than 200 ppm. The fact that this time 27% of the samples had magnesium contents below this level proved the effectiveness of our screening. Seasonal fluctuations were, of course, frequent: in

some herds, urinary magnesium was low during summer, but it rose above the critical level in the autumn. Thus relatively low magnesium excretion was measured at both screenings in few herds only (0.9%).

Geochemical correlations were also sought in the evaluation. Dixon's test was used to sort out extreme values, but none such were found among the 20 county averages. The latter were uniformly distributed in relation to the national average. A relationship was then sought between county averages of urinary Mg and acreage of acid soil. It is known that soils regarded as having a low magnesium content from the agricultural point of view are usually acid. As can be seen from Table I, no such tendency emerges from the comparison

Table I

County averages of urinary magnesium concentration of cattle

County	No of samples examined	County av. (ppm)	Acid soil (%)	No. of samples containing less than 200 ppm
1. Baranya	61	605	27.7	1
2. Vas	7	604	85.5	0
3. Somogy	34	552	31.1	6
4. Zala	23	539	69.1	2
5. Fejér	73	531	6.3	6
6. Győr-Sopron	67	518	37.2	3
7. Heves	91	512	74.8	4
8. Szabolcs-Szatmár	28	505	60.8	0
9. Bács-Kiskun	40	505	0.8	2
10. Borsod-Abauj-Zemplén	42	503	72.1	1
11. Veszprém	39	502	37.0	2
12. Tolna	67	487	0.8	0
13. Pest	8	454	9.1	1
14. Nógrád	15	442	66.4	4
15. Békés	69	412	41.6	12
16. Komárom	43	403	12,4	9
17. Budapest	1	400	11.3	0
18. Hajdu-Bihar	6	342	64.2	0
19. Csongrád	23	358	12.6	3
20. Szolnok	38	349	54.4	3
National average for the 775 samples			491 ppm	
County average			452 ppm	

Rank correlation coefficients for relationship between acreage of acid soil and urinary Mg levels:
Percentage of highly acid soil — Mg less than 200 ppm : $r = -0.180$
Acreage of highly acid soil (kh) — county average Mg: $r = +0.094$
Acreage of acid soil (kh) — county average Mg: $r = +0.003$

of either absolute values or frequencies. Surprisingly, the urinary Mg levels were found even to exceed the national average in certain counties with large acreages of acid soil (Borsod-Abauj-Zemplén, Heves, Somogy, Szabolcs-Szatmár, Vas, Zala). Later feed analyses revealed that this apparent contradiction is due to a lesser leaching of magnesium from acid Hungarian soils than from similar areas in the more humid Western European Countries.

The result of a complex analysis also performed on sets of urine, blood serum, milk and faeces from 10 herds are shown in Table II. In conformity with earlier observations of MÁRKUS and TÖLGYESI, herd differences in magnesium supply, as expressed in term of variation coefficients, decreased in the sequence urine (53.0%) milk (24.7%) serum (7.1%) faeces (6.8%). Thus differences can be best assessed from urinary Mg levels. No correlation was found between the Mg contents of the four test materials; even the comparison of milk and faecal levels suggests no trend ($r = 0.38$).

Table II

Average magnesium contents in faeces, urine, milk and serum of cattle from herds in September, 1971

Cattle farm	Faeces (g/kg TM)	Urine (mg/l)	Milk (mg/l)	Serum (mg/l)
Alsópéli State Farm	9.4	860	50	42.4
Balatonnagyberek State Farm	9.4	480	58	43.2
Lajtahanság State Farm I.	10.6	740	52	45.8
Rákóczi Cooperative, R-falva	7.0	680	50	40.0
Magyarnándor State Farm	8.8	300	57	41.7
Kiskunság State Farm	11.8	260	61	39.2
Komárom State Farm	14.0	158	51	39.6
Hajdunánás State Farm	9.4	480	62	40.2
Lajtahanság State Farm II.	12.4	1000	94	38.9
Bácsalmás State Farm	12.8	580	82	34.9
Average	10.5	514	60	40.6
St. deviation	0.7	272	14.8	2.9
Variation coeff.	6.8	53.0	24.7	7.1

Rank correlation coefficients according to SPEARMAN:
Faeces — Urine: —0.15 Urine — Milk: 0.03
Urine — Serum: 0.09 Faeces — Milk: 0.38
none of these correlations is significant

Discussion

The reconsideration of Hungarian premix formulas required a decision as to the amount of magnesium that should be included in mineral supplements for cattle. General information on the magnesium supply of herds was obtained

from measurements of magnesium content in 775 pooled urinary samples, each representative of a herd average. The number of samples and the regional distribution of their sources permitted general conclusions. Taking the lower limit of urinary Mg level very rigorously as 200 ppm, only few herds (about 0.9%) can be said to have regularly low levels. On this basis, magnesium supplementation seems to be at most a regional rather than a general problem of cattle feeding in this country. In the case of a clinically manifest Mg depletion (magnesium tetany), the urinary Mg falls to as little as 10—20 ppm, or 10—20 times less than the value taken by us as the minimal limit. In this light our general conclusions are also regarded as valid for the early spring season, when dietary Mg levels were indeed lowered but still reached about half of the summer maximum concentration. Given the known correlation between dietary and urinary Mg concentrations, the national average of urinary Mg is unlikely to fall below 200 ppm, even in the early spring. In some of the herds coming under the low-level category (0.9%), magnesium deficiency may occur in the early spring owing to grazing on pastures heavily treated with fertilizers containing potassium and nitrogen. Such problems, however, should be resolved locally, because sporadic local incidence is not sufficient to justify a policy of preventive Mg-supplementation on the national level. Excess magnesium affects the utilization of calcium and phosphorus (GÜNTHER, 1966) and interferes with metabolic processes in general owing to increased earth alkaline alkalinity.

SUMMARY

A total of 775 pooled urine samples, each collected from a representative group of 8—10 cows per herd, were examined for magnesium content. The national average was found to be 491 ppm, the regional (county) average 452 ppm. No extreme values were found with Dixon's test. There was no correlation between the county acreage of acid soil and the urinary Mg levels. Only 0.9% of the 775 herds had urinary Mg concentration less than 200 ppm, which was regarded as the rigorous established lower limit. From this it was concluded that magnesium supplementation of cattle feed is not necessary on a national level, and the rare occurrence of clinical magnesium deficiency during early spring could be prevented by purely local measures.

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ROLE OF PSEUDOMONAS AERUGINOSA IN ACTINOMYCOSIS-LIKE BOVINE MASTITIS

By

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(Received November 30, 1971)

Tanner reported in 1952 that out of 98 bovine udders discovered at a Stockholm abattoir to be showing actinomycosis-like microscopic lesions, 84 contained *Staphylococcus aureus* or *Staph.albus*, 12 mixed saprophytic microflora and 2 *Pseudomonas aeruginosa*. Later Mora and Cavrini (1959) isolated pathogenic staphylococci from 15 and *Ps. aeruginosa* from one of 16 similar cases in Italy. Chronic mastitis morphologically resembling actinomycosis, was observed by Fuchs, Hornich and Walter (1969) in six cows of a large German herd and *Ps. aeruginosa* was isolated from each case.

In the abattoir and emergency slaghterhouse of Pécs, 28 cases of actinomycosis-like bovine mastitis were identified histologically over the period 1968—71. Bacteriological examinations performed in duplicate *Ps. aeruginosa* was isolated from 19 udders, *Staph. aureus* from five, a mixed saprophytic microflora from one, while the remaining one udder proved bacteriologically sterile.

History and clinical observations

Fourteen of the 19 cows with *Ps. aeruginosa*-mastitis had been emergency slaughtered while the other five cases (?) been detected at normal slaughter. Eight of the 14 cows had been emergency slaughtered for mastitis and six through an erroneous diagnosis. Emong the 14 emergency slaughtered cows originating from large herds there were 5 animals from the same herd during two years.

On the basis information obtained from the veterinarians attending the cattle farms, the course and symptoms of the disease can be characterized as follows: The udder abruptly became swollen and painful and the body temperature rose. A dilute serum-like substance was discharged from the affected quarters at milking, and within a few days many nodules, varying in size from that of a hazelnut to a walnut, appeared. The nodules were easily palpable and in some cases even visible. The healthy quarters dried off with the progression of the nodular change, the animals lost flesh and their general condition worsened as the condition gradually become chronic. All therapeutic attempts failed once the nodules had established. Occasionally fistulae ap-

peared on the skin of the affected quarters and decubitus wounds developed on bony areas of the body surface. Emergency slaughter is usually necessary several weeks or months after onset.

Macroscopic lesions

Generally two quarters of the udder were affected, either both hindquarters or the fore- and hind quarter on one side; involvement of a single or more than two quarters was rare. The diseased quarters enlarged up to twice original size. Occasionally the surface appeared uneven, carrying protrusions or, less often, fistular openings, other cutaneous changes were noticed; the subcutaneous connective tissue was slightly oedematous.

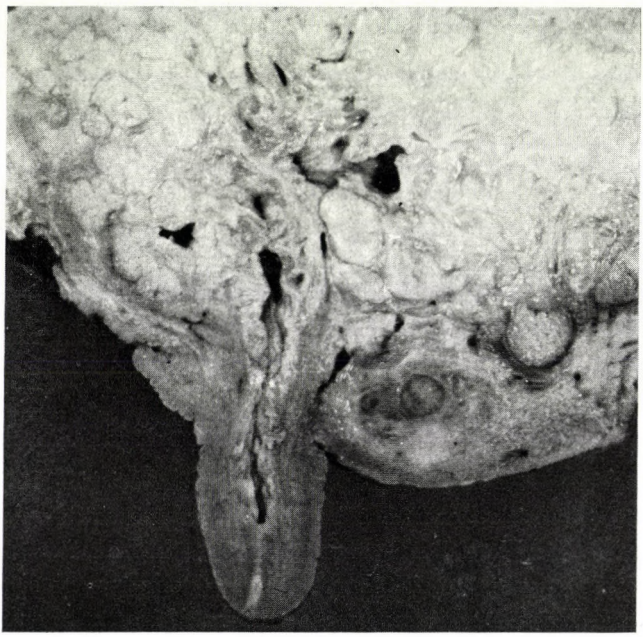

Fig. 1. Gross appearance of actinomycosis-like chronic mastitis caused by *Pseudomonas aeruginosa*. Foci of proliferative tissue are surrounded by connective tissue capsules of varying thickness

The affected quarters were compact and resisted cutting. The teat channels, milk sinus and larger lactiferous ducts contained either retained milk or a thick pus-like exudation. This change usually involved the entire quarter, but small intact areas were sometimes present. Many growths, consisting of a soft, yellowish-white tissue and varying from rice-grain to hazelnut size, appeared in the udder parenchyma. The growths were surrounded by a greyish-white fibrous capsule of variable thickness, which extended process into the surrouding tissues (Fig. 1): they bulged above the plane of the udder's cross-

section and in places penetrated the lumina of lactiferous ducts (Fig. 2). Larger growth were often suppurative, transforming to "actinomycotic" abscesses, with thick yellowish or yellowish-green, pus-like contents. The abscess walls were lined inside by a soft layer, outside by a tough layer of greyish-white connective tissue (Fig. 3).

The mammary lymph node was usually swollen, sometimes to two or

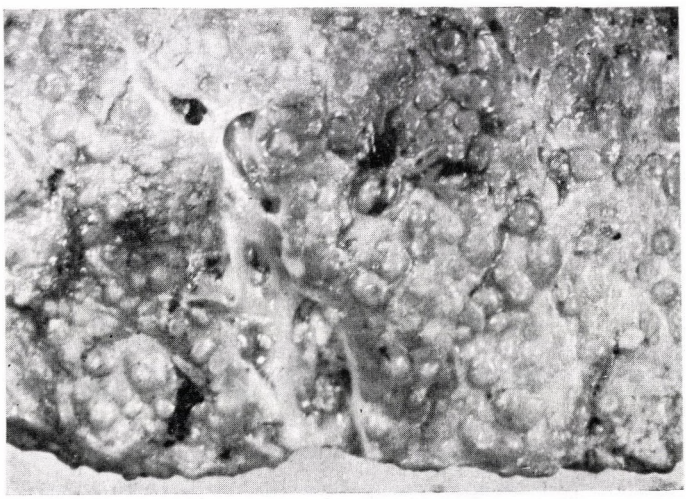

Fig. 2. Gross-section of an affected udder. The actinomycosis-like growths bulge above the plane of section and penetrate a minor lactiferous duct

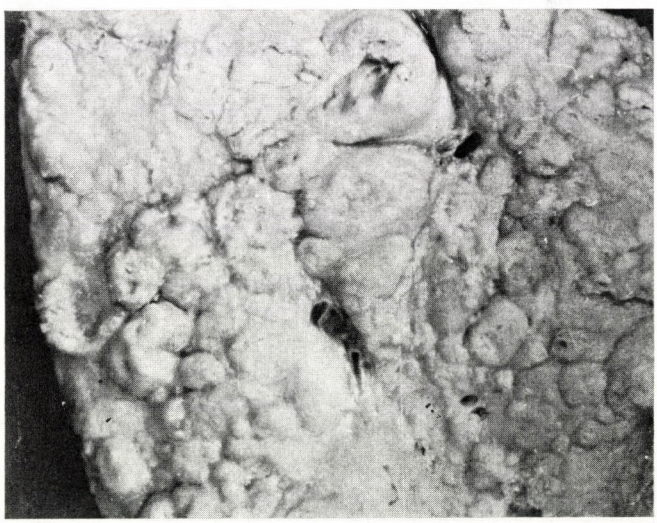

Fig. 3. Larger, partly suppurated actinomycosis-like abscesses in chronic mastitis caused by *Pseudomonas aeruginosa*

three times its original size and become soft and medullary on the affected side. Some animals had a swollen, pale and friable liver.

Seven cows also showed lesions indicative of a concurrent disease, such as lamellar adhesions between the second stomach and mural peritoneum, due to injury by a foreign body, serous inflammation of the fetlock and knee joint, focal interstitial nephritis and renal amyloidosis.

Microscopic lesions

The udder, mammary lymph node and occasionally also changed parts of other organs were fixed in formalin, embedded in paraffin, and sections were stained with haematoxylin and eosin or PAS-haematoxylin.

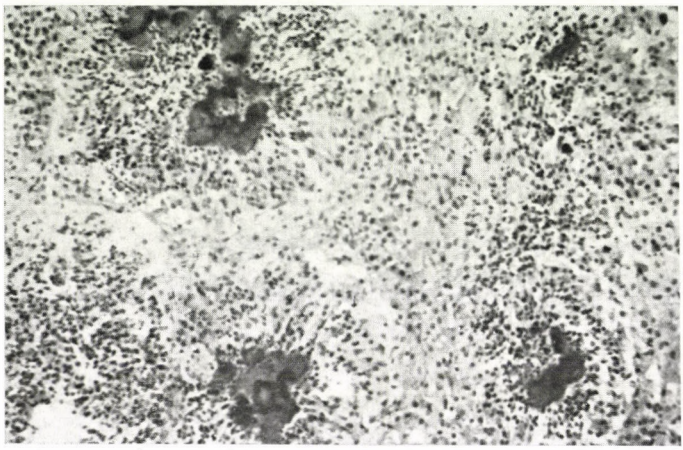

Fig. 4. Micro-abscess comprising actinomycosis-like flocculi (pus stops) inside a proliferative tissue in *Pseudomonas aeruginosa*-mastitis. (Haematoxylin and eosin, 80×)

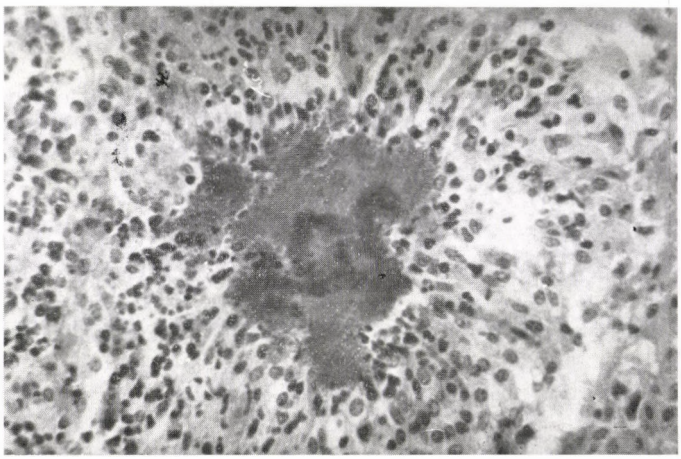

Fig. 5. Pus stops from Fig. 4 at a higher magnification. (Haematoxylin and eosin, 200×)

The microscopic appearance of the lesions corresponded with that of an extensive granulomatous mastitis (Figs 4, 5 and 6). The granulomas caused by *Ps. aeruginosa* differed from staphylococcal *(Staph. aureus)* granulomas in certain fine histological details (TANNER, 1952; FUCHS et al., 1969); the latter contained Gram-positive cocci, the former Gram-negative flocculi or "pus spots". The aim of this study was to seek similarities rather than dissimilarities between the two kinds of granulomatous process: pictures of staphylococcal *(Staph. aureus)* mycosis-like mastitic lesions from our material are therefore presented for comparison (Figs 7, 8 and 9).

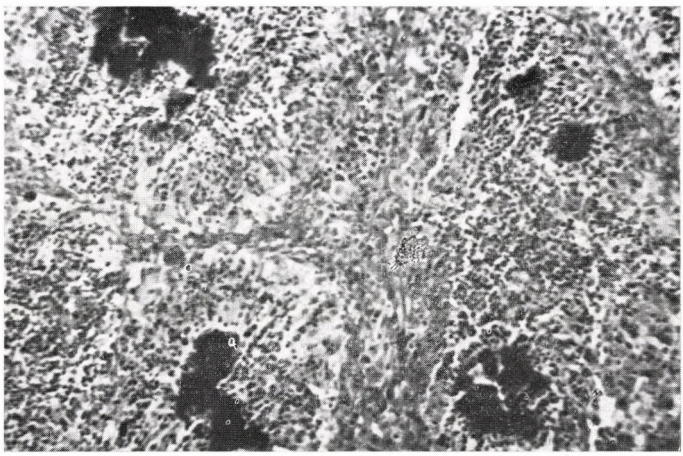

Fig. 6. Pseudomonas aeruginosa-mastitis. Note the distinct PAS-reaction of pus stops. (PAS-haematoxylin, 80×)

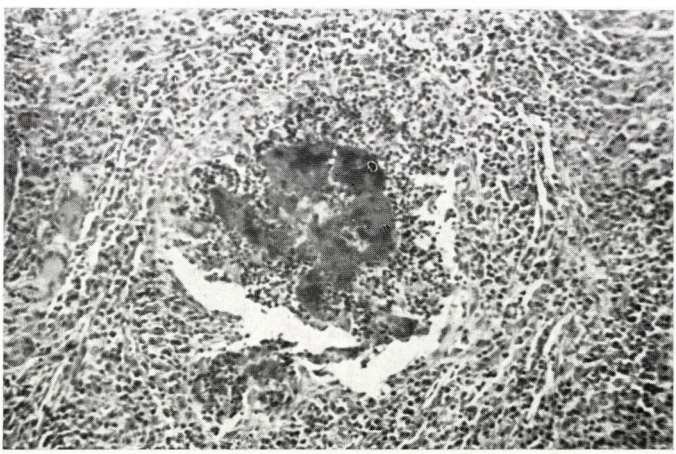

Fig. 7. Actinomycosis-like granuloma of the udder caused by *Staphylococcus aureus:* small abscess with a typical pus centre and surrounding polymorphous proliferative cell reaction. (Haematoxylin and eosin, 80×)

The diffuse or confluent granulomas contained a pus centre in which focal pus spots (flocculi) of various sizes and with typical morphological and staining properties could be seen. In certain cases the pus spots had already become necrotic or sclerotic. The abscesses containing the pus spots were encased by fibrous tissue. The proliferative tissue consisted of hystiocytes, pseudoexanthoma cells, multinucleated giant cells (Langhans or foreign-body giant cells), plasma cells and lymphocytes.

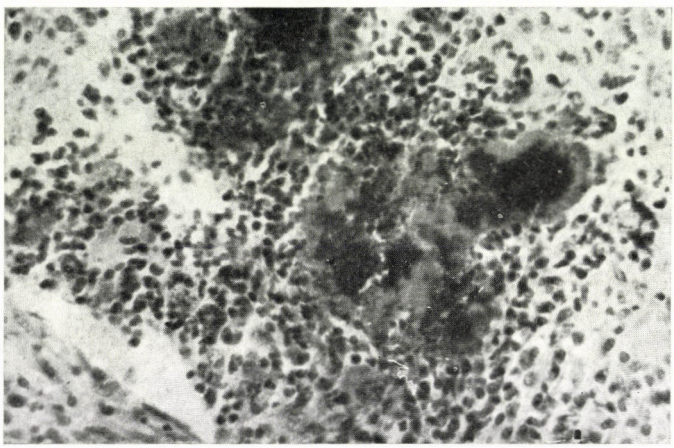

Fig. 8. Actinomycosis-like granuloma of the udder caused by *Staphylococcus aureus:* pus centre of the abscessus shown in Fig. 7 at a higher magnification. (Haematoxylin and eosin, 200×)

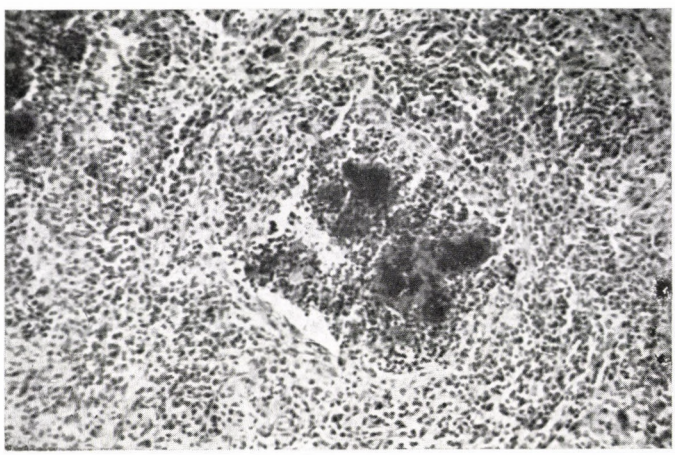

Fig. 9. Actinomycosis-like granuloma of the udder caused by *Staphylococcus* aureus: PAS-reaction. (PAS-haematoxylin, 90×)

Bacteriological examinations

Bacterial isolations were attempted from spleen, liver, kidney, two muscle samples, praescapular and contralateral popliteal lymph node, udder and mammary lymph node. The udder examinations usually included separate inocula from abscess wall, abscess contents, connective tissue separating the abscesses and preserved udder parenchyma.

The applied media were: common broth (pH 7.4) agar containing 10% bovine blood, 5% serum agar, Drigalszky agar with bromthymol blue; Preuss' potassium tetrathionate agar for salmonellae; Kitt-Tarozzi's liver broth for isolation of anaerobic organisms.

Gram-negative rods were isolated from all udder samples and from several mammary lymph nodes as well. Flat colonies with characteristic aromatic odour grew on the serum and blood agar; colonies growing on serum agar caused a greenish discoloration of the surrounding medium, while those growing on blood agar were haemolytic. Broth cultures assumed a green colour extending downward from the surface, and membranaceous fragments floated on their surface. Fresh cultures exhibited a blue colour change on shaking with chloroform. The isolated organisms moved actively, did not form hydrogen sulphide or indole, did not decompose urea, showed positive catalase and oxydase reactions, and caused the coagulation and peptonization of milk.

On the basis of colony morphology, staining properties, biochemical and serological tests, the isolates were identified as *Ps. aeruginosa*. Serological examination showed that three randomly picted strains belonged to type 04 and one to type 01.

Discussion

In human medicine, the fungus *Actinomyces bovis* is still regarded as the exclusive causative agent of actinomycosis (ANDERSON, 1966), whereas in veterinary pathology various agents (*Corynebacterium israeli*, *Actinobacillus ligniéresi*, *Staph. aureus*, etc.) are known to cause actinomycosis-like granulomas in domestic animals (JUBB and KENNEDY, 1970).

According to the veterinary terminology, the disease caused by *C. israeli* is called actinomycosis, that by *Act. ligniéresi* actinobacillosis while the granulomatous process due to *Staph. aureus* a staphylococcal granuloma.

As a rule *Staph. aureus* is responsible for the so-called mammary actinomycosis of cows. Lesions of this kind very seldom contain *Act. ligniéresi* and never *C. israeli* (HEIDRICH and RENK, 1963); previous reports on isolation of *Ps. aeruginosa* from such cases were cited in the introduction of this paper.

The observations of TANNER (1952), MORA and CAVRINI (1959), FUCHS, HORNICH and WALTER (1969) as well as our own experience suggest an aetio-

logical relationship between certain cases of bovine mammary actinomycosis and *Ps. aeruginosa*.

It should be noted in this context that *Ps. aeruginosa* is a well known causative agent of various forms of bovine mastitis; according to HEIDRICH and RENK, apart from actinomycosis-like mastitis, it may cause mild catarrhal, severe acute and chronic purulent inflammations of the udder.

In contrast to the findings of TANNER (1952), MORA and CAVRINI (1959), in our hands isolations of *Ps. aeruginosa* were much more frequent than those of *Staph. aureus* (19 : 5) and the general incidence of *Ps. aeruginosa* mastitis was particulary notable compared to that of other forms of udder inflammation in the same period. Formerly, isolations of *Ps. aeruginosa* were chiefly made from cases emergency slaughtered for severe acute mastitis (FORRAY and SZÁZADOS, 1968), but since 1968 the incidence of this form has fallen markedly. During the last two years, not a single case has been observed *Ps. aeruginosa* is exclusively responsible for actinomycosis-like chronic forms of mastitis.

It seems remarkable that we have not encountered the chronic purulent form of *Ps. aeruginosa*-mastitis described by GARDINER and CRAIG (1961) several years ago either by histological or by parallel bacteriological examinations. However, since GARDINER and CRAIG did not histologically examine the udder itself but only other organs (kidney, adrenals), it does not seem improbable that the cases they observed in fact corresponded to an actinomycosis-like rather than a purulent mastitis. In our view, the actinomycosis-like form might well be the only manifestation of chronic *Ps. aeruginosa*-mastitis.

As to the aetiological relationships between the different forms of actinomycosis-like mastitis, FUCHS et al. concluded that in view of the microbiological antagonism between *Staph. aureus* and *Act. ligniéresi*, a cross-infection seems improbable, but they did not suggest which might have the primary causative role.

In our hands, *Ps. aeruginosa* was regularly isolated in pure culture not only from the purulent contents of the abscesses, but also from their walls, from the connective tissue between the abscesses, and from normal appearing glandular tissue as well. No other bacteria were isolated in any case. We agree, however, with FUCHS et al. that further detailed examinations are required to affirm the causative role of *Ps. aeruginosa* in actinomycosis-like bovine mastitis.

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SUMMARY

Over a four-year period (1968—1971) pure cultures of *Pseudomonas aeruginosa* were isolated from the udders — walls and purulent contents of abscesses, connective tissue between abscess and normal appearing of glandular tissue — of 19 cows with histologically verified actinomycosis-like mastitis after normal or emergency slaughter.

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COMPARATIVE STUDY ON SPECIAL SEROTYPES OF ERYSIPELOTHRIX RHUSIOPATHIAE STRAINS ISOLATED IN HUNGARY AND ABROAD

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Since first WATTS (1940), ATKINSON (1941), then GLEDHILL (1945) divided *Erysipelothrix rhusiopathiae* strains into two serological types by means of the agglutination test, many authors have dealt with the serological properties of this organism. The research workers were lead in their investigation by different respects. It was of cardinal importance to find a suitable method for the isolation of highly antigenic strains suited for swine erysipelas vaccine production. Thus the attention was directed to the hemagglutination-capacity of the organism, because the investigators (DINTER, 1949) came to the conclusion, that swine erysipelas strains preserved their good antigen character even in a killed state can be found among those which are able to hemagglutinate chicken erythrocytes at high dilutions.

After DEDIÉ's (1949) observation that good vaccine strains belonged to only one of the two formerly identified serotypes, interest was centered again on the serological behaviour of *E. rhusiopathiae*. By means of the precipitation test DEDIÉ set up a third type, designated N for those strains which showed no reaction with either A or B specific sera.

In the meantime, a relationship was searched between the serological type of erysipelas strains and the development of different pathological lesions, and attempts were made to classify the serotypes of bacteria isolated from different animal species and man. HEUNER (1958), using different methods for antigen extraction, was the first to identify new serotypes (A_2, B_2, C, D) within the group of strains not belonging to serotypes A or B. Subsequently MURASE et al. (1959, 1960) determined new special serotypes (C, D, E, F) by agglutination and precipitation tests.

The success of the initial typing efforts initiated further investigations along this line and additional types have been described under the designations E (KUCSERA, 1962, 1963), P (TRUSZCZYNSKI, 1963), G (KUCSERA, 1964), H (EWALD, 1967), I., II., III., resp. I, J, K (CASTRO, 1969, 1970), and I, J, K, L, M and O (KUCSERA, 1971). Unfortunately by these descriptions the researchers failed to compare their strains to be suspected new serotypes with other ones described so far. The main reason being that the identification of many of the new types was carried out practically simultaneously in distant parts of the world, which hindered exchange of information among investigators.

MURASE et al. (1959, 1960), for example, did not refer to the findings of HEUNER (1958) and very probably did not compare his C and D types with the similarly named types of HEUNER. I myself was unaware of MURASE's work when I first used the designation E for an *E. rhusiopathiae* strain isolated from fish (KUCSERA, 1962); at that time I had only HEUNER's type strains for comparison. TRUSZCZYNSKI (1963) performed his examinations in possession of MURASE's strains (C, D, E, F) but for the lack of HEUNER's C and D and KUCSERA's E strains. When later I determined a new serotype I designated it G (KUCSERA, 1964) knowing about MURASE's work and choosing the letter symbol as successive in alphabetic order to MURASE's types. EWALD (1967) segregated the new serotype H in possession of HEUNER's (C, D) and KUCSERA's (E, G) type strains, but without comparing it to MURASE's types. In 1968 CASTRO sent me for typing three *E. rhusiopathiae* strains isolated in Brazil and I typed them along with other strains isolated in Hungary. The outcome of this work was the description of six serotypes, designated in alphabetic order as I, J, K, L, M, O, the letter N being omitted so as to leave the original designation for *E. rhusiopathiae* strains not possessing an acid-soluble, thermostable antigenic component (KUCSERA, 1971). These examinations were performed without MURASE's and TRUSZCZYNSKI's type strains, then not available. In the meantime CASTRO had himself

typed his three isolates and described them first in his thesis (1969) as types I., II., III., and the following year (CASTRO et al., 1970) as types I, J, K; whereas my own designations for the corresponding strains had been M, L and I.

These circumstances have resulted in confusion of the serological type designations of *E. rhusiopathiae* strains. Evidently, a precondition for comparison of the incidence and importance of various serotypes isolated in different parts of the world, is the standardisation of typing methods and nomenclature. This entails a comparative study of all serotypes described at different times in different parts of the world, investigated partially by different methods and described by various designations, by a uniform method. I have undertaken this task in possession of type strains collected for years and others not formerly available (MURASE's and TRUSZCZYNSKI's types) and I hoped that the complementary examinations will have clarified the misunderstandings and justify the nomenclature proposed for the existing types.

Materials and methods

1. Strains

The original culture was always obtained by inoculating into broth a separate S-type colony from a 48-hour agar-plate culture. This ensured the uniform serological behaviour of the cultures. *E. rhusiopathiae* strains isolated both here and abroad were freeze-dried after culturing in horse meat broth containing 20% horse serum for 24 hours at 37 °C. All but four strains of the home isolates have been described in previous papers (KUCSERA, 1962, 1963, 1964, 1971). The strains Pécs 4586 and Pécs 4587, isolated by FORRAY in 1971 from tonsils of healthy pigs, were found to belong to serotype L, one of the six new types reported previously (KUCSERA, 1971), while strains Pécs 3596 and Pécs 3597, from the same source, did not react with precipitating antisera to any other *E. rhusiopathiae* strain and antisera prepared with them in rabbits reacted exclusively with the homologous antigen.

The strains used in the examinations and the data of their origin are summarized in Table I.

Type strains from abroad were kindly supplied by Dr. WELLMANN, Dr. HEUNER, Dr. EWALD, Dr. TRUSZCZYNSKI, Dr. CASTRO and Dr. MURASE and the author wishes to take this opportunity to express his appreciation for their courtesy.

2. Preparation of antigen

a) Autoclaved antigen for precipitation tests was prepared from cultures grown in horse meat broth containing 10% horse serum for 48 hours at 37 °C. After centrifugation at 5000 r.p.m. for 30 minutes the cultures were washed

three times in an equal volume of saline, then the bacterial sediment was resuspended in 1/30 of the original volume of distilled water. The bacterial suspension was heat-treated in autoclave for 1 hour at 120 °C, and after adding merthiolate in 1 : 10.000 endconcentration, for preservation, stored in the refrigerator until used.

It should be noted that the broth medium was always prepared from horse meat; experiences of many years have shown that this is important for obtaining a good antigen. Addition of 10% horse serum to broth prepared from other species, never has the same effect, although recently WHITE and VERWEY (1970) have presented convincing proof of the stimulatory action of horse serum on synthesis of the protective antigen of *E. rhusiopathiae*. Use of a basal medium other than horse meat broth can easily frustrate the typing of certain weak antigen producing strains.

b) Two types of antigen, formol-killed and live bacteria, both were used for the preparation of precipitating sera. The formol-killed antigen was prepared as follows: the culture washed three times as described in the foregoing was made up to a density of Brown degree 4 in saline containing 0.2% formalin, placed in the incubator for 24 hours and, after sterility tests in solid and liquid media, stored in the refrigerator until use. The live antigen was prepared similarly, except that the washed bacteria were suspended in plain saline.

3. Preparation of precipitating sera

Formalin-inactivated antigen was administered intravenously to rabbits weighing 2.5—3 kg on four occasions at 4-day intervals, using doses rising from 1 to 4 ml. Subsequently the animals were treated with live antigen on the same schedule. The rabbits were killed by bleeding eight days following the last antigen administration, after starvation for the last 24 hours. Sera collected after clotting were preserved with 1% merthiolate in saline to an endconcentration of 1 : 10.000 and stored in the refrigerator. Sera no longer reacting with the homologous antigen at dilutions of 1 : 32—1 : 64 were excluded from the examinations.

4. Precipitation tests were carried out by the double agar gel diffusion method of OUCHTERLONY (1949). Fifteen ml melted agar was poured into each 10 cm diameter Petri dish and after solidification 7 mm diameter wells, spaced equidistantly 6 mm from one another, were cut into the agar plate to held serum and antigen. Plates were read after incubation for 48 hours at 37 °C and again after being allowed to stand at room temperature for three further days.

Table I

Data of strains examined

Designation of strain	Origin of strain					
	Isolated by	Country	Species	Described by	Year	Type
422/1	Unknown	USA	unknown	HEUNER	1958	A_2
NF 4	Unknown	USA	unknown	HEUNER	1958	B_2
EW 2	GLADHILL	U.K.	unknown	HEUNER	1958	A_2
EW 24	GLADHILL	U.K.	unknown	HEUNER	1958	A_2
MEW 22	GLADHILL	U.K.	unknown	HEUNER	1958	N
Seelachs	WELLMANN	GFR	fish	HEUNER	1958	B_2
Wittling	WELLMANN	GFR	fish	HEUNER	1958	C
Doggerschabe	WELLMANN	GFR	fish	HEUNER	1958	D
Heilbutt	WELLMANN	GFR	fish	HEUNER	1958	D
Kuniyasu	MURASE	Japan	pig	MURASE	1959	A
A-1	MURASE	Japan	pig	MURASE	1959	B
P-190	MURASE	Japan	fish	MURASE	1959	C
P-32	MURASE	Japan	fish	MURASE	1959	D
P-43	MURASE	Japan	fish	MURASE	1960	E
P-92	MURASE	Japan	fish	MURASE	1960	F
P	TRUSZCZYNSKI	Poland	ground squirrel	TRUSZCZYNSKI	1963	P
G-4 (S-52)	CASTRO	Brazil	pig	CASTRO	1969—70	I (I)
JAC-10 (S-66)	CASTRO	Brazil	pig	CASTRO	1969—70	II (J)
4-PR (S-4)	CASTRO	Brazil	pig	CASTRO	1969—70	III (K)
T 131	EWALD	GFR	pig	EWALD	1967	H
A 360	Unknown	unknown	unknown	DEDIÉ	1949	A

B_2	Unknown	unknown	unknown	DEDIÉ	1949	B_4
Szentes-2	KEMENES	Hungary	goose	KUCSERA	1971	A_2
Kiskunlacháza	KEMENES	Hungary	goose	KUCSERA	1971	A_2
Tótkomlós	KEMENES	Hungary	goose	KUCSERA	1971	A_2
Kaparék	CSONTOS	Hungary	fish	KUCSERA	1962	E
IV.12/8	KUCSERA	Hungary	pig	KUCSERA	1964	G
Pécs 52	FORRAY	Hungary	pig	KUCSERA	1970	G
Pécs 100	FORRAY	Hungary	pig	KUCSERA	1970	G
Pécs 9	FORRAY	Hungary	pig	KUCSERA	1970	I
M 2	FORRAY	Hungary	cow	KUCSERA	1970	I
Pécs 18	FORRAY	Hungary	pig	KUCSERA	1970	J
Pécs 56	FORRAY	Hungary	pig	KUCSERA	1970	J
Iszap-4	KEMENES	Hungary	from mud of Zoo pond	KUCSERA	1970	K
Pécs 67	FORRAY	Hungary	pig	KUCSERA	1970	L
Pécs 4586	FORRAY	Hungary	pig	KUCSERA	1971	L
Pécs 4587	FORRAY	Hungary	pig	KUCSERA	1971	L
Tuzok	KEMENES	Hungary	Zoo great bustard (*Otis tarda*)	KUCSERA	1970	M
Vadkacsa	KEMENES	Hungary	Zoo mallard *(Anas platyrhynchos)*	KUCSERA	1970	M
M 1	FORRAY	Hungary	cow	KUCSERA	1970	M
Pécs 110	FORRAY	Hungary	pig	KUCSERA	1970	N
Pécs 112	FORRAY	Hungary	pig	KUCSERA	1970	O
Goda	KEMENES	Hungary	Zoo black-tailed godwit *(Limosa limosa)*	KUCSERA	1970	O
Pécs 3596	FORRAY	Hungary	pig	KUCSERA	1971	—
Pécs 3597	FORRAY	Hungary	pig	KUCSERA	1971	—

Table II

Results of agar gel diffu-

Antigens				Precipitating								
Designation of strain	Described by	Year	Type	A 360	Kuniyasu	EW 2	Szentes—2	B_4	A—1	NF 412	Wittling	Dogger
A 360	DEDIÉ	1949	A	+	+	—	—	—	—	—	—	—
Kuniyasu	MURASE	1959	A	+	+	—	—	—	—	—	—	—
EW 2	HEUNER	1958	A_2	+	—	+	+	—	—	—	—	—
Szentes—2	KUCSERA	1970	A_2	+	—	+	+	—	—	—	—	—
B_4	DEDIÉ	1949	B	—	—	—	—	+	+	+	—	—
A—1	MURASE	1959	B	—	—	—	—	+	+	+	—	—
NF 412	HEUNER	1958	B_2	—	—	—	—	+	+	+	—	—
Wittling	HEUNER	1958	C	—	—	—	—	—	—	—	+	—
Doggerschabe	HEUNER	1958	D	—	—	—	—	—	—	—	—	+
MEW 22	HEUNER	1958	N	—	—	—	—	—	—	—	—	—
P—190	MURASE	1959	C	—	—	—	—	—	—	—	—	—
JAC 10(S-66)	CASTRO	1970	II.(J)	—	—	—	—	—	—	—	—	—
Pécs—67	KUCSERA	1970	L	—	—	—	—	—	—	—	—	—
P—32	MURASE	1959	D	—	—	—	—	—	—	—	—	—
G—4 (S—4)	CASTRO	1970	I. (I)	—	—	—	—	—	—	—	—	—
Tuzok	KUCSERA	1970	M	—	—	—	—	—	—	—	—	—
P—43	MURASE	1960	E	—	—	—	—	—	—	—	—	—
T 131	EWALD	1967	H	—	—	—	—	—	—	—	—	—
P—92	MURASE	1960	F	—	—	—	—	—	—	—	—	—
Goda	KUCSERA	1970	O	—	—	—	—	—	—	—	—	—
Kaparék	KUCSERA	1962	E	—	—	—	—	—	—	—	—	—
P	TRUSZCZYNSKI	1963	P	—	—	—	—	—	—	—	—	—
IV. 12/8	KUCSERA	1964	G	—	—	—	—	—	—	—	—	—
4 PR (S—4)	CASTRO	1970	III. (K)	—	—	—	—	—	—	—	—	—
Pécs—9	KUCSERA	1970	I	—	—	—	—	—	—	—	—	—
Pécs—56	KUCSERA	1970	J	—	—	—	—	—	—	—	—	—
Iszap—4	KUCSERA	1970	K	—	—	—	—	—	—	—	—	—
Pécs 3596	KUCSERA	1971	—	—	—	—	—	—	—	—	—	—

sion precipitation tests

antisera

MEW 22	P–190	JAC 10	Pécs–67	P–32	G–4	Tuzok	P–43	T 131	P–92	Goda	Kaparék	P	IV. 12/8	4 PR	Pécs–9	Pécs–56	Iszap–4	Pécs 3596
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
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–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
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–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+

Table III

System and type designation proposed for serologica

Type 1				Type 2			
Designation of strain	Described by	Year	Type	Designation of strain	Described by	Year	Type
A 360	DEDIÉ	1949	A	B_4	DEDIÉ	1949	B
EW 2	HEUNER	1958	A_2	NF 4	HEUNER	1958	B_2
Kuniyasu	MURASE	1959	A	A-1	MURASE	1959	B
	Type 5				Type 6		
P-190	MURASE	1959	C	P-32	MURASE	1959	D
Jac-10 (S-66)	CASTRO	1969 (1970)	II (J)	G-4 (S-52)	CASTRO	1969 (1970)	I (I)
Pécs-67	KUCSERA	1970	L	Tuzok	KUCSERA	1970	M
	Type 9				Type 10		
Kaparék	KUCSERA	1962	E	P	TRUSZCZYNSKI	1963	P
	Type 13				Type 14		
Pécs-56	KUCSERA	1970	J	Iszap-4	KUCSERA	1970	K

Results

The precipitation tests were set up in a checkerboard-like system, using antigens and precipitating sera produced by means of home and foreign strains. Each test was carried out in at least two replicas. The results are summarized in Table 2. To facilitate orientation, only one representative strain has been included for each serotype described by the different authors and identical results obtained with other strains of the same type have been omitted.

As can be seen from Table II, the types described by MURASE et al. (1959) as C and D do not correspond with HEUNER's (1958) identically denoted types established earlier, but do correspond with KUCSERA's L and M (1971) as well as CASTRO's II(J) and I(I) types. MURASE's type E strain (1960) is not identical with KUCSERA's type E, but it shows a cross-reaction with EWALD's type H (1967). MURASE's type F corresponds with KUCSERA's type O (1971), while CASTRO (1969, 1970) used the designation III (K) for the type described by KUCSERA (1971) as serotype I. The differentiation of the remaining serotypes is reasonable, because their specific antisera reacted exclusively with the homologous antigen.

classification of *E. rhusiopathiae* strains

Type 3				Type 4			
Designation of strain	Described by	Year	Type	Designation of strain	Described by	Year	Type
Wittling	HEUNER	1958	C	Dogger-schabe	HEUNER	1958	D
	Type 7				Type 8		
T-34	MURASE	1960	E	P-92	MURASE	1960	F
P-131	EWALD	1967	H	Goda	KUCSERA	1970	O
	Type 11				Type 12		
IV.12/8	KUCSERA	1964	G	4 PR (S 4)	CASTRO	1969 (1970)	III (K)
				Pécs-9	KUCSERA	1970	J
	Type 15						
Pécs-3596	KUCSERA	1971					

The irregular serological inactivity of strain G-4 (S-52) from Brazil was already described in a previous paper (KUCSERA, 1971).

To facilitate understanding, type identities and differences are tabulated in Table III. From this it can be easily seen which strains belong to one and the same type, despite their dissimilar designations, and which represent different types, despite their identical designations.

Discussion

With autoclaved, merthiolate-preserved antigens and high-titre precipitating sera, the agar gel diffusion precipitation test proved to be a reproducible, simple and reliable method for the standardization of serological typing of *E. rhusiopathiae* strains. The use of this method for comparative studies of *E. rhusiopathiae* serotypes deriving from different parts of the world and described by various authors, permitted a revision of type differentiation, e.g. revealed the discrepancy of strains originally designated similarly and the identity of strains previously designated differently. Thus it was possible to fit *E. rhusiopathiae* strains of different origin in a uniform serological system.

The decision now remains to be taken as to what final system of symbols should be adopted for type designation, with special regard to avoid difficulties in the identification of existing and forthcoming erysipelas types.

Although the use of successive letters of the alphabet has been the accepted practice for almost a quarter of a century, insistence on this scheme does not seem fortunate because on the one hand it would be very difficult to establish within it the correct chronology of type descriptions, while on the other hand the alphabet might become exhausted sooner than types still to be identified. The serial numbering of the serotypes used in Table III seems to be a more reasonable approach, allowing, as it does, not only an unlimited extension of the system, but also chronological sequence of type-descriptions.

To designate variations of antigenic pattern within a given serotype, as in A and B postfixion of a small letter to the type numbering is proposed (e.g., 1a, 1b, 2a, 2b for types A_1, A_2, B_1, B_2 respectively). Frequent variations within certain serotypes should also be taken into consideration — but this will be the subject of a subsequent paper.

Apart from the proposed serial numbering, the letter N should be retained for the designation of those strains which either have no type-specific antigen at all or possess one in such a low amount for it to be undefinable by the usual methods. A further criterion of classification into type N should be the absence of antibody stimulation after inoculation into rabbit, viz. the absence of a precipitation reaction even with the homologous antigen.

SUMMARY

Comparative serological studies by means of the agar gel precipitation test using autoclaved antigens of 45 *Erysipelothrix rhusiopathiae* type strains isolated in Hungary and abroad are reported.

It was found that certain types designated with an identical letter are serologically unrelated, whereas several types having different designations belong to one and the same serological group. Among others, the strains described by MURASE et al. as types C and D differ from HEUNER's C and D types, but correspond with CASTRO's II (J) and I (I) and KUCSERA's L and M strains. MURASE's strain E is not identical with KUCSERA's similarly designated type, but it gives a crossreaction with EWALD's type strain H. MURASE's type F and KUCSERA's type O represent a single serological group, as do CASTRO's type III (K) and KUCSERA's type I. Differentiation of the other examined serotypes [A, B (DEDIÉ); A_2, B_2, C, D (HEUNER); P (TRUSZCZYNSKI); E, G, J, K, without designation (KUCSERA)] still seems justified. A new serotype, isolated from the tonsils of a healthy swine, was also established.

In a view of the present nomenclatural confusion of *E. rhusiopathiae* serotypes, described by different authors, a uniform scheme of type designation by serial numbering is proposed. The known serotypes would be numbered from 1 to 15, with as much consideration as possible being given to the chronological sequence of type descriptions, and intratypic variations of antigenic pattern would be designated by a small letter postfixed to the serial number. The designation N should be retained from the alphabetic system for those strains which do not possess a type-specific antigen and consequently do not even stimulate homologous antibody production. Using the proposed form of terminology, designation of forthcoming serotypes would fail any difficulty.

The proposed serological classification of the *E. rhusiopathiae* strains is, accordingly, as follows: Type 1a = A360 (A, DEDIÉ), Type 1b = EW2 (A_2 HEUNER), Type 2a = B4 (B, DEDIÉ), Type 2b = NF4 (B_2 HEUNER), Type 3 = Wittling (C, HEUNER), Type 4 = Doggerschabe

(D, Heuner), Type 5 = P-190 (C, Murase), Type 6 = P-32 (D, Murase), Type 7 = P-43 (E, Murase), Type 8 = P-92 (F, Murase), Type 9 = Kaparék (E, Kucsera), Type 10 = P (P, Truszczynski), Type 11 = IV 12/8 (G, Kucsera), Type 12 = 4 PR (S4) (III, viz. K, Castro), Type 13 = Pécs-56 (J, Kucsera), Type 14 = Iszap-4 (K, Kucsera), Type 15 = Pécs-3596 (undesignated, Kucsera).

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A SIMPLE MICRO-METHOD FOR VIRUS NEUTRALIZING ANTIBODY ASSAY AND VIRUS TITRE MEASUREMENT BY TISSUE CULTURE TECHNIQUE

By

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The intensification of livestock farming and above all the specialization of herds and flocks has imposed new tasks on veterinary laboratories. Among others, the frequent outbreaks of viral disease — and their often serious consequences — require fast and reliable routine diagnostic methods to facilitate detection and prevention of latent infections in seemingly healthy stock and, in the long run, the establishment of "clean" herds and flocks. Owing to the speedy expansion of intensive farming, however, veterinary institutions can rarely cope with more than a fraction of diagnostics, through the lack of trained personnel and facilities. The traditional diagnostic methods of virus isolation and demonstration of specific antibodies in convalescent sera are laborious and time-consuming procedures as long as tissue cultures grown in tubes are employed for the purpose.

SCHMIDT (1966) has described a technique with which he was able to demonstrate neutralizing antibodies to parainfluenza viruses by means of monolayers grown in the U-shaped wells of Takátsy's microtitrator plates (TAKÁTSY, 1955). A similar method was elaborated for titration of swine adenovirus strains by KWON and SPRADBROW (1971), while BIBRACK and MCKERCHER (1971) has determined neutralizing antibodies to adenoviruses in cattle sera by means of a microtest procedure.

In this institute a simple, reliable microtechnique has been elaborated for the qualitative and quantitative determination of specific antibodies to cytopathic viruses. This method not only enables the diagnostic determination of manifest and latent viral infections but can also be used for titration of viruses treated with desinfectants or virocidal agents. Its principal advantage is that it increases working efficiency 10—20 times over that for tube culture assays, while the material requirements (cells, tissue culture medium, glassware) are 20—40 times less.

Apparatus and material:

a) Plastic titrating plates of Takátsy's Microtitrator Set*, each plate containing 96 (8 × 12) U-shaped wells of 0.2 ml useful volume.

* Supplied by Labor Műszeripari Művek, Budapest.

b) Dropper pipettes of 0.025 ml volume;

c) Takátsy's diluting cups of 0.025 ml volume

d) Primary tissue cultures or cell lines plus media required for their maintenance;

e) Virus strains of appropriately high titre and sera of known antibody content;

f) Other equipment: incubator (37 °C), pipettes, flasks, plastic boxes with tightly fitting lids or a suitable incubator to preserve a CO_2 enriched atmosphere, inverted microscope for readings.

Apparatus and materials are prepared as follows:

The titrating plates are thoroughly in distilled water and sterilized with UV radiation from 30 cm distance for 30—60 minutes. Plates prepared by injection moulding and packed at the factory need no additional sterilization. Droppers must not be heat-sterilized; they should be soaked in a disinfectant easily removed by washing (e.g. 2% Iosan solution) and left to stand in distilled water for 1—2 days after disinfection. Dilution cups are flamed prior to use. Washing and sterilization of the glassware are carried out according to the procedures commonly employed in virology.

Cells grown on the plates require the same media as tube cultures. They are prepared by the usual procedure; though cells from primary cultures or cell lines should be used for preference, because freshly trypsinized suspensions may contain many injured elements.

Virus inocula and aliquots of serum are both diluted with Hanks' solution containing 0.5% lactalbumin hydrolysate.

As the tissue cultures grown in the wells are not airtight, it must be arranged for the surrounding air to contain 5—10% CO_2. For this purpose, the plates should either be placed in boxes with airtight lids or in a special incubator automatically supplying the required concentration of CO_2.

Virus neutralization tests can be performed in the wells containing the cell culture to determine the antibody titres in terms of either the neutralization index, or serum dilution.

For serum dilution tests the wells of the plates should have an U-shaped cross section. First 0.025 ml diluting fluid is placed in each well by means of the dropper pipette. The serum samples to be tested, along with positive and negative control sera, are then diluted in twofold serial steps, each in a separate row of wells, by means of the diluting cups. One or more rows on each plate should be left clear for virus controls. Dilution ranges are 1 : 2—1 : 256 in short rows (8 wells) and 1 : 2—1 : 4096 in long rows (12 wells). After dilution of the serum, 20—100 $TCID_{50}$ virus in 0.025 ml volume is added to each dilution step and the plates are incubated for one hour at 37 °C, to allow neutralization to take place.

For the virus dilution (neutralization index) procedure the serum sample

is prepared as usual and 0.025 ml, diluted 1 : 2 or 1 : 5, as required, is placed in each well of a horizontal row. Virus dilutions in the range of $10^0 - 10^7$ are then dropped in the wells of a vertical row, likewise 0.025 ml volume per well. The serum-virus mixture is incubated for one hour at 37 °C, during which the plates should preferably be kept in a moist airtight chamber to prevent evaporation.

After incubation, two drops (0.05 ml) of cell suspension are placed in each well; cell counts vary between 15—50 thousand depending on the type of cell.

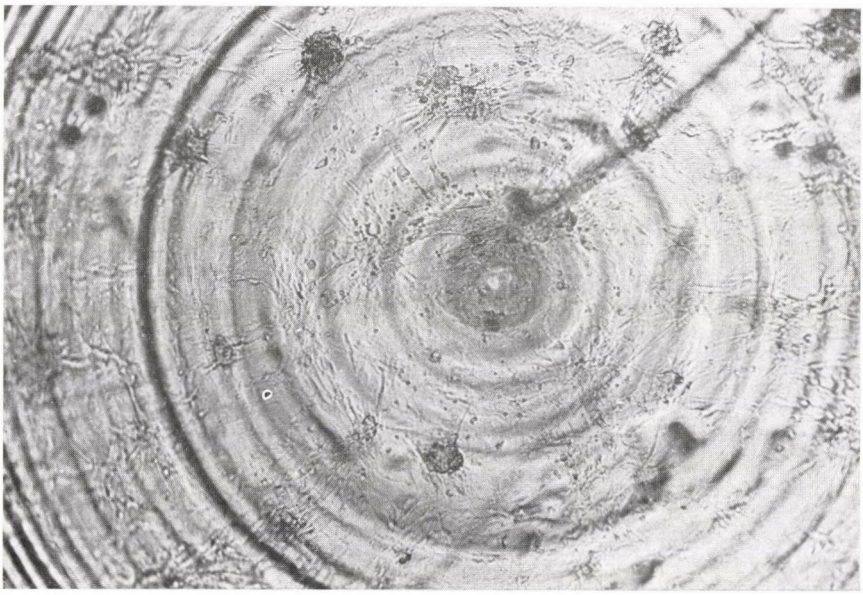

Fig. 1. Uninoculated Secondary bovine testicle tissue culture, 36 hours 35×

At this stage of the test each well contains 0.025 ml test serum, 0.025 ml virus and 0.05 ml cell suspension, making a total volume of 0.1 ml. To control wells a corresponding amount of diluent is added for whatever ingredient was omitted.

The plates are then piled on top of one another, the topmost one is covered with an empty plate, and the pile is placed in a box with an airtight lid. Prior to closing the lid, wet cottonwool should be placed in the box to prevent evaporation and about 5—10 per cent by volume CO_2 added to the atmosphere. During subsequent incubation at 37 °C, the cells multiply, and where active virus is present the wells will show cytopathic changes (Figs 1, 2, 3). The cytopathic effect (CPE) is read with an inverted microscope at low (25×) magnification. Reading times are the same as for neutralization tests in tube cultures.

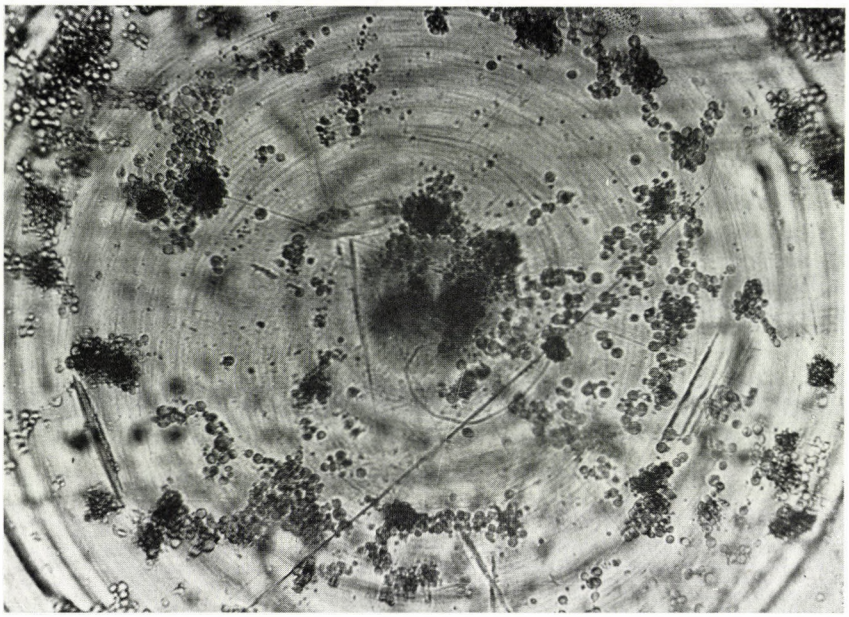

Fig. 2. Secondary bovine testicle tissue culture inoculated with Aujeszky virus. Early cytopathological changes. 35×

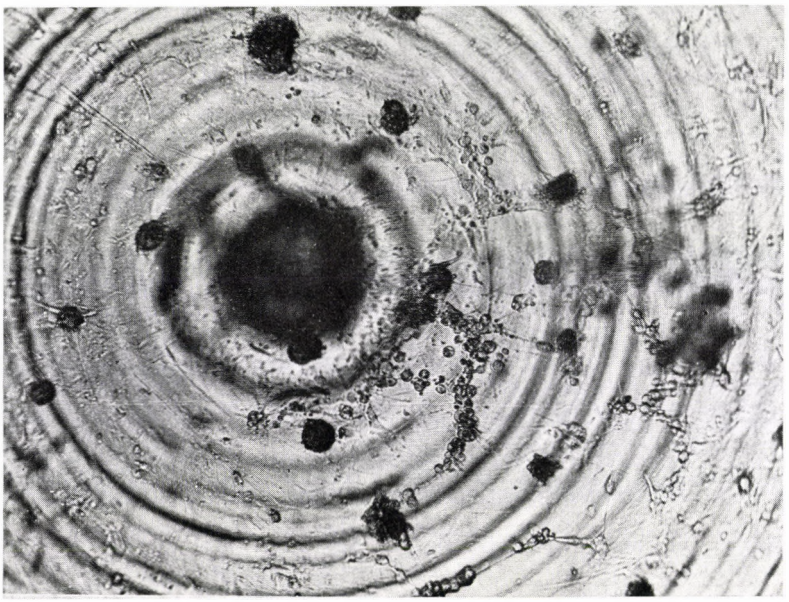

Fig. 3. Secondary bovine testicle tissue culture inoculated with Aujeszky virus. Total destruction. 35×

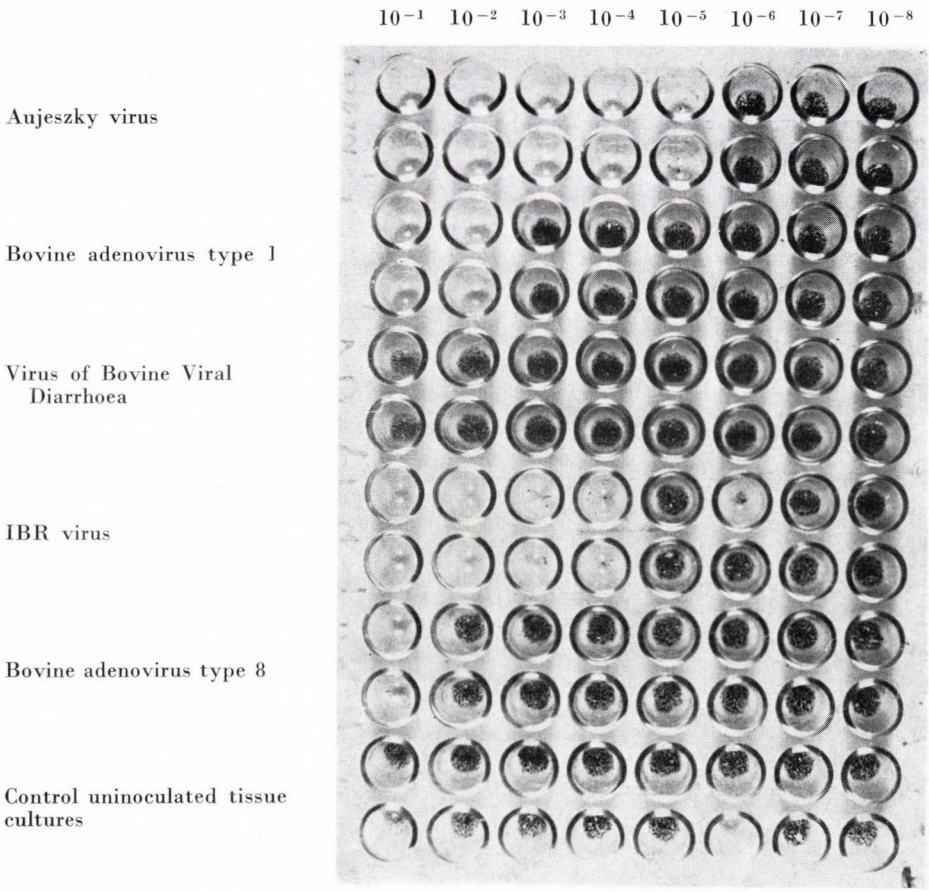

Fig. 4. Estimating the titre of different viruses

Cell cultures can be fixed for reading by the usual methods (e.g. by placing the plates in 10% formalin solution, which has the advantage of killing the active virus). Fixed cultures can be stained either by the common techniques or with simple aniline dyes. Changes can be read with the naked eye, because cells not affected by the virus form a coherent layer, whereas unneutralized virus causes the cell layer to detach or break up (Figs 4, 5). Fixed and stained plates can be stored and used to document the results at a later date.

Another way of carrying out the micro-neutralization test is first to place the cell suspension in the wells and, after the cell sheet has grown 1—6 days later to remove the growth medium by a powerful knock of the plate against blotting paper and then replace it with preincubated serum-virus mixture. Pre-cultured cell sheets can also be used for virus titration in the wells.

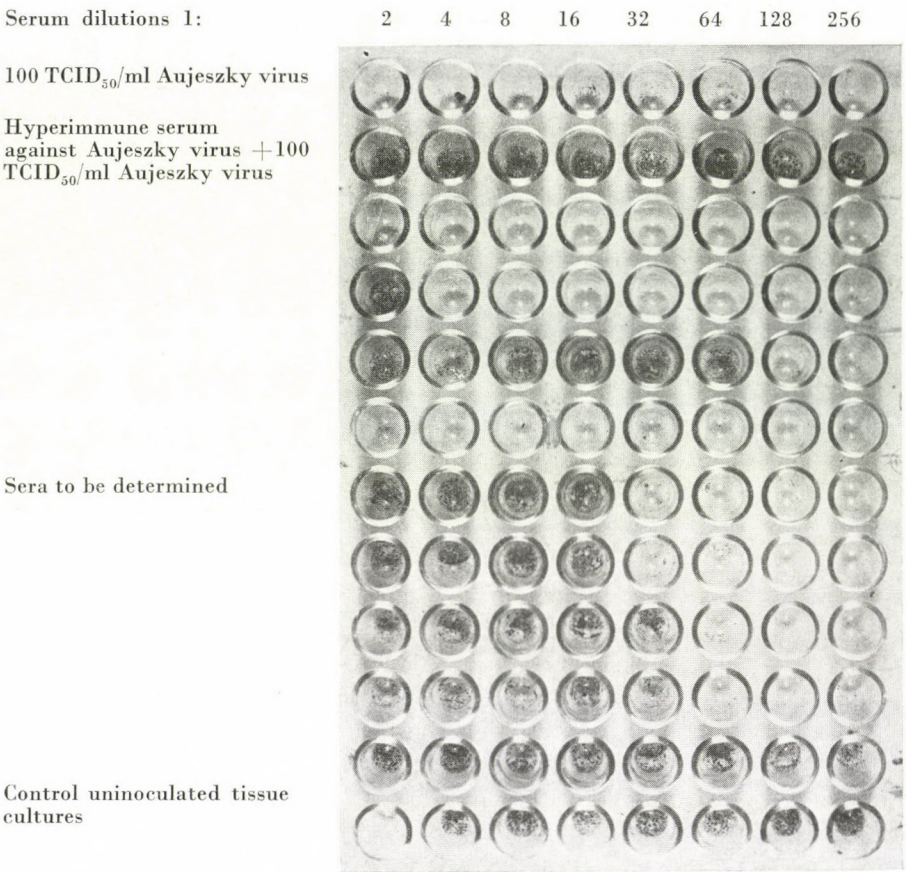

Fig. 5. Estimating the neutralizing antibodies against Aujeszky virus in swine sera

The plate-titration method is suitable for detecting of the viruses survival in resistance tests to various agents (heat, disinfectant, antiviral substances, cations, pH, etc.). For this purpose the virus material is diluted in tenfold serial steps. Dilutions can be prepared directly on the titrating plate, as follows: Two plates are used. Four drops (0.1 ml) of diluent are placed in each well of the first (auxiliary) plate and one drop (0.025 ml) in the wells of the second (working) plate. Undiluted virus is then taken up with the titrating cup (0.025 ml) and swirled with the contents of the first well of the auxiliary plate to dilute the virus 1 : 5. The cup is then flamed to remove the adherent virus, allowed to cool and used to take up 0.025 ml of 1 : 5 diluted virus from the auxiliary plate. The contents of filled cup are then transferred to the second well in the first row of the working plate and rotated to dilute the virus 1 : 10. This dilution is transferred to the second well of the auxiliary plate in which a 1 : 50 dilution is obtained by swirling the cup. The latter is again flamed, cool-

ed, and filled with 1 : 50 diluted virus for transfer to the third well of the working plate, in which the 1 : 100 dilution is prepared. The procedure is continued until the required series of dilutions is obtained. Flaming of the cup after auxiliary dilutions is important, because it corresponds to a pipette change. Errors can be prevented by colouring the diluent used in the working plate with phenol red and leaving the fluid auxiliary dilutions colourless. Several cups can of course be utilized simultaneously, if required.

The method is suitable for the examination of all viruses causing easily recognizable cytopathic changes. The unprecedented economy of materials and work offered by the microtitrator set permits very rapid, but precise, performance of mass examinations. The technique is so simple that even laboratories not equipped for virological investigations can join in mass diagnostic work provided they receive adequate support from a specialized laboratory.

SUMMARY

A very rapid, exceptionally economic and reliable micro-method has been elaborated for the diagnostic demonstration of virus-neutralizing antibodies. The method uses monolayer cell cultures established in the U-shaped wells of titrating plates of Takátsy's Microtitrator Set in place of tube cultures, the results of the neutralization tests performed with micro-cell cultures being read with an inverted microscope. The procedure is suitable for neutralization tests of all viruses which cause easily recognizable cytopathic changes and is especially valuable for the screening of herds or flocks for latent viral infection.

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TYPE A INFLUENZA VIRUS INFECTION OF DUCK FLOCKS IN HUNGARY

(TYPE 3, 4 AND 6 AVIAN INFLUENZA VIRUSES)

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Nearly one hundred avian influenza virus strains of eight serological types have up to now been isolated from more than ten bird species. About 20 of these belonging to 6 serotypes, have been isolated from respiratory diseases of several weeks old ducklings since 1953.

Five influenza virus strains, representing three different serotypes, were isolated from growing and adult duck flocks in Hungary in 1970—71. This paper is a report of the clinical flock histories and of the identification and typing of the strains.

Materials and methods

Virus isolation, agar-gel diffusion precipitation test, haemagglutination (HA) and haemagglutination inhibition (HI) tests as well as the preparation of sera for the examinations were carried out according to the usual techniques (BEARD, 1970; PEREIRA et al., 1966, 1967). Reference antigens and antisera were obtained from the World Influenza Centre (London), through the competent department of the World Health Organization (Geneva).

Specific antisera to the new isolates were prepared by immunization of experimental animals.

Epizootology, clinical picture and gross lesions of the natural disease

Outbreak 1 (Farm H). A duckling flock, consisting of 20,000 birds, the age of 3—14 days a virologically verified duckling hepatitis accompanied by disorders resulting from a feeding error, pulmonary mycosis and mild slight salmonellosis. More than 20% of the birds died in this outbreak. Losses fell off for a few days at two weeks of age, but still remained unusually high. Six to eight days later the mortality rate rose again, then it dropped to 0.5—1% daily and persisted at that level for several weeks. By 5—6 weeks of age scarcely more than half of the flock was surviving and development was uneven. A minor part of the birds showed general (listlessness) and respiratory symptoms (dyspnoea, cyanosis). Most affected birds died shortly after the onset, usually without showing other signs; sick, cachectic ducklings were seldom seen. Flock therapy with sulphaquinoxaline, Erra-6 and Neo-te-sol

in succession decreased the losses temporarily by about 20%, but they rose almost to the same extent after medication was discontinued. At post mortem examination of birds above two weeks old, partial or general airsacculitis and changes indicative of fowl cholera were found. The two pictures were usually manifested simultaneously, either of them alone was rare. In bacteriological and mycological examinations, pasteurellas were isolated from many of the carcases and aspergillus from some changed air sacs.

Outbreak 2 (Farm P). A respiratory disease affecting ducklings 10 days to 6 weeks old was observed in a large flock consisting of about 60,000 birds. The outbreak started with general sneezing and shaking of the head followed by a distinct rise of losses in some groups of the about 10-day-old birds. Some ducklings died shortly after the onset of the upper respiratory symptoms, but 3—6 days after the beginning of the outbreak, the course became slower and the birds sinusitis and cachexia for several days before they died. The cases tended to decrease in number at about 4—5 weeks of age, but sudden deaths occurred among well developed, healthy-appearing ducklings. Losses persisted above the usual level throughout the period until marketing at 8 weeks.

Gross lesions found in the carcases of birds that died when 10—12 days old were almost exclusively a delicate fibrinous airsacculitis and serositis; a sporadic occurrence of sinusitis was also noted. Some ducklings dying at 16—21 days of age additionally showed changes indicative of fowl cholera. From the age of 21 days, lesions of the respiratory disease and fowl cholera were usually present simultaneously, and from 4—5 weeks, the latter became predominant, while respiratory lesions tended to diminish until they became quite rare by 6—7 weeks.

Bacteriologically, pasteurellas were isolated from lesions indicative of fowl cholera, whereas bacteriological and mycological isolations from the changed air sacs were both negative. The results of flock therapy for fowl cholera were variable but in general prevented major losses.

There was a history of an abrupt fall of egg production in the breeder flock (5,000 birds) several months prior to the outbreak. The egg yield of the layers, then at the height of the egg producing period, had fallen precipitately to 10% in about one week and, after persisting at that level for 3 weeks, had risen to the expected level again. The hatching rate of the eggs laid during the low-production period had been less than 60%, contrasted to the annual mean of 70%. Of the poorly hatching eggs 21% had been infertile while the numbers of cullings at the first and second candling and stuck embryos likewise surpassed the average. Ducks in the breeder flock had shown no signs of disease either before, or during the fall of egg yield. Scarcely more than average losses occurred among them, and the carcases showed only a general haemorrhagic diathesis, then attributed feed toxicosis. The layers had been marketed as table ducks prior to our examinations.

Outbreak 3 (Farm K). Adult, but not yet laying birds developed three days after re-grouping and transfer to new premises a disease manifested by loss of appetite and listlessness, which killed 3% of the flock, originally consisting of 6,000 birds. The outbreak ceased as suddenly as it appeared. General organic hyperaemia and visceral uric acid disease were found at post mortem examination of the carcases.

Outbreak 4 (Farm Pa). The egg-laying period of this breeder flock had been prolonged beyond the usual time, with the result that moulting coincided with an abrupt chilly turn of weather in early winter. Losses rose slightly for a few days, with 3—5 birds dying daily instead of the usual one. At post mortem examination, a general organic hyperaemia and fibrinous air sac inflammation were found.

Outbreak 5 (Farm P). Half a year after the observation of outbreak 2 in this farm on abrupt, under very variable winter conditions, but slight increase of losses was observed among the by then non-laying breeders for a period of about one week. Grossly the carcases revealed a general hyperaemia and a fibrinous aerosacculitis.

Virological examinations

These were performed with each outbreak as follows: Organ suspensions prepared from the respiratory tract (sinus, trachea, lung, air sacs) of fresh carcases were inoculated into the allantoic cavity of 10-day embryonated hen's eggs. Embryonic death occurred within 2—6 days and the allantoic fluid and chorioallantioc membrane produced a precipitation reaction with known type A influenza antisera in the agar-gel diffusion precipitation test. The allantoic fluids also displayed haemagglutinating activity. Antigenic relations demonstrated by HI test, using positive reference sera and inactivated antigens, are shown in Table I.

1. The isolate from outbreak 1 (Farm H), further on referred to as Duck (Hungary)1/70, was found to be closely related antigenically with the type 6 Turkey (Wisconsin) 66 prototype strain. On re-examining the isolate at our request Dr. G. C. SCHILD of the World Influenza Centre (London), was able to confirm this and also established that the neuraminidase activity of the strain is closely related with those of the Turkey (Wisconsin) 66 and the human influenza virus A_2 strains (natural influenza hybrid).

2. The strains responsible for outbreak 2 among growing ducks and outbreak 5 among adult ducks on Farm P did not differ antigenically from one another and both were closely related with the strains Duck (England) 56 and Duck (Czechoslovakia) 56 (types 3 and 4). These isolates received the designations Duck (Hungary) 2/70 and Duck (Hungary) 71, respectively.

Table I

Examination of avian influenza virus isolated from

Antigens	Immune sera				
	D(H)1/70	D(H)2/70	D(H)3/70	(DH)4/70	D(H)71
Duck(Hungary)1/70	1 : 256	<1 : 8	<1 : 8	<1 : 8	<1 : 8
Duck(Hungary)2/70	<1 : 8	1 : 256	1 : 16	1 : 16	1 : 64
Duck(Hungary)3/70	<1 : 8	1 : 32	1 : 128	1 : 64	1 : 32
Duck(Hungary)4/70	<1 : 8	1 : 32	1 : 64	1 : 128	1 : 32
Duck(Hungary)71	<1 : 8	1 : 64	1 : 16	1 : 16	1 : 128
Fowl plague virus	<1 : 8	<1 : 8	<1 : 8	<1 : 8	<1 : 8
Duck(England)56	<1 : 8	1 : 32	<1 : 8	<1 : 8	1 : 32
Chicken(Scotland)59	<1 : 8	<1 : 8	<1 : 8	<1 : 8	<1 : 8
Duck(England)62	<1 : 8	1 : 32	1 : 32	1 : 64	1 : 32
Quail(Italy)65	<1 : 8	<1 : 8	<1 : 8	<1 : 8	<1 : 8
Turkey(Wisconsin)66	1 : 64	<1 : 8	<1 : 8	<1 : 8	<1 : 8
Newcastle Disease Virus (NDV)	<1 : 8	<1 : 8	<1 : 8	<1 : 8	<1 : 8
Normal allantoic fluid	<1 : 8	<1 : 8	<1 : 8	<1 : 8	<1 : 8

Figures in the Table correspond with the reciprocal values of the highest serum or antigen dilutions still capable of 50% inhibition of haemagglutination. Apart from the new isolates and their specific antisera, all inactivated influenza antigens and reference sera were supplied by the World Influenza Centre (London), through the Veterinary Public Health Department of the World Health Organisation (Geneva).

3. The isolates from outbreak 4 (Farm Pa) and outbreak 3 (Farm K) proved to be antigenically related with the type 4 strain Duck (Czechoslovakia) 56, and have been designated as Duck (Hungary) 3/70 and Duck (Hungary) 4/70, respectively. The strain Duck (England) 62, formerly regarded as a prototype, had to be utilized instead of the approved prototype Duck (Czechoslovakia) 56, because only standard antigen and positive serum of the former were available.

Haemagglutination inhibition tests

Forty individual blood samples each from outbreaks 1 and 2, and 20 such samples from outbreaks 3 and 4 were examined by HI test, using 8 haemagglutinating units of the homologous virus. A substantial part of the samples inhibited haemagglutination in a titre range of 1 : 8—1 : 256.

ducks by haemagglutination inhibition test

Fowl plague	D(E)56	Ch(S)59	D(E)62	Q(I)65	T(W)66	NDV	Norm.
<1: :8	<1 : 8	<1 : 8	<1 : 8	<1 : 8	1 : 128	<1 : 8	<1 : 8
<1 : 8	1 : 128	<1 : 8	1 : 128	<1 : 8	<1 : 8	<1 : 8	<1 : 8
<1 : 8	<1 : 8	<1 : 8	1 : 64	<1 : 8	<1 : 8	<1 : 8	<1 : 8
<1 : 8	<1 : 8	<1 : 8	1 : 64	<1 : 8	<1 : 8	<1 : 8	<1 : 8
<1 : 8	1 : 64	<1 : 8	1 : 64	<1 : 8	<1 : 8	<1 : 8	<1 : 8

Infection experiments

Twenty one-day-old chicks, 20 guinea-fowls about 3—6 weeks old, 20 ducklings about 4—14 days old and several turkey poults 2—4 months old were infected intranasally and intraconjunctivally with 0.2—0.5 ml undiluted allantoic from embryonated hen's eggs infected with strains Duck (Hungary) 1/70 or Duck (Hungary) 2/70; the virus content of the fluids was $10^{6}ELD_{50}$/0.1 ml and $10^{6\cdot5}ELD_{50}$/0.1 ml, respectively.

1. On infection with Duck (Hungary) 1/70, the chicks showed no symptoms, while birds of the other three species temporarily lost appetite.

Two chickens each killed at intervals of 3 days had no gross lesions, ducklings and guinea-fowls a slight fibrinous airsacculitis at 3—6 days after infection displayed but not later. Chicks, ducklings, guineafowls and turkey poults not exterminated within 3 weeks of the infection scarcely differed from the non-infected controls in respect of development and general condition. HI antibodies appeared in the serum of all four species 9 days after infection. Titre values soon (after 15—18 days) rose to a high level in most guinea-fowls and turkeys, with considerable individual variations, but remained low throughout in the ducklings.

2. Infection with the strain Duck (Hungary) 2/70 caused loss of appetite and decrease of weight gain only in the ducklings, but after ailing for 8—10

Table II

Haemagglutination inhibition tests with the influenza virus strain A/Duck(Hungary)1/70 (Data supplied by Dr. SCHILD)

Immune serum	Titre of haemagglutination inhibition	
	Homologous strain	Duck(Hungary) 1/70
1. FPV (Dutch)	320	<10
Turkey(England)63	160	<10
2. "N"(Germany)49	640	<10
Quail(Italy)1117/65	320	<10
Pheasant(Italy)647/66	640	<10
3. Duck(England)56	120	<10
4. Duck(Czechoslovakia)56	240	<10
Duck(England)62	120	<10
5. Chicken(Scotland)59	320	<10
Tern(South Africa)61	160	<10
6. Turkey(Canada)63	80	40
Turkey(California)64	160	<10
Turkey(England)66	240	40
Turkey(Massachusetts)65	320	80
Turkey(Wisconsin)66	640	80
7. Duck(Ukraine)1/63	160	<10
8. Turkey(Ontario)6118/67	320	<10
Newcastle disease virus	320	<10

days, these too began to gain weight so rapidly that at 3 weeks they scarcely differed from the controls. Two ducklings died between 4 and 6 days after infection, showing changes of fibrinous airsacculitis and serositis. Pairs of ducklings killed every third day from the day of infection to 18 days all had a fibrinous inflammation of air sacs and serosa; the change was most pronounced between 3 and 6 days. The virus strain used for infection could be recovered from the respiratory tract lesions of both spontaneously and slaughtered ducklings. HI antibodies appeared in the serum of the infected birds after 8 days, the serum of in-contact control birds after 12 days; but titres were low in all four species.

Discussion

Five influenza virus strains, reacting with immune serum of three serotypes, were isolated from five outbreaks of respiratory disease among flocks in the period 1970—71. Along with three other influenza virus strains isolated

from guinea-fowls in 1969—70, the number of avian influenza virus isolations in the eastern part of Hungary during this two-year period new totals eight strains belonging to four serotypes (TANYI, 1972; Table III).

Table III

Antigenic relationships of avian influenza virus strains isolated at the Veterinary Institute of Debrecen in the period 1969—71

1. *FPV (Dutch)*

2. *"N" (Germany)49*
   Quail(Italy)1117/65 ——— GUINEA-FOWL(HUNGARY)1/69 (guinea-fowl layers: strain 148/69)
   GUINEA-FOWL(HUNGARY)1/70 (adult guinea-fowl layers: strain 130/70)

3. *Duck(England)56*
   DUCK(HUNGARY)2/70 (ducklings: strain 265/670) — GUINEA-FOWL (HUNGARY)2/69 (adult guinea-fowl: strain 237/69)
   DUCK(HUNGARY)71 (adult ducks: strain 144/71)

4. *Duck(Czechoslovakia)56*
   Duck(England)62
   DUCK(HUNGARY)3/70 (adult ducks: strain 349/70)
   DUCK (HUNGARY) 4/70 (adult ducks: strain 350/70)

5. *Chicken(Scotland)59*

6. *Turkey(Canada)63*
   Turkey(Wisconsin)66
   DUCK(HUNGARY)1/70 (ducklings: strain 235/70)

7. *Duck (Ukraine) 1/63*

8. *Turkey (Ontario) 6118/67*

Prototype strains are underlined; those of serotypes 2, 4 and 6 were not available, thus antigens and antisera prepared from strains serologically identical with the prototype were used instead. With the new isolates, the species, age and use of the bird together with our own reference number for the strain are indicated in brackets. Two isolates each from guinea-fowl and duck are antigenically related with two serotypes (transitory types).

The findings of experimental infections performed with two of the isolates were similar to those reported by ALLEN et al. (1970), HOMME et al. (1967), ROBERTS (1970) and SCHETTLER (1968). The observations suggest that infected ducks and with serotype 6 strains also guinea-fowl develop a fibrinous inflammation of air sacs and serosa, which subsides mere rapidly if the agent belongs to type 6 than if it belongs to types 3 or 4. Sporadic losses may occur even under relatively good conditions of management markedly increased if by feeding errors and management failures. The fact that virus could be reisolated from

birds with low antibody levels, if they had gross lesions (up to 18 days after infection) and was demonstrable in carcases of adult birds when flock mortality was relatively low, suggests a durable, often symptomless carrier state. Avian influenza, like many other diseases, depends greatly on predisposing factors, and since the elimination of the latter is likely to be a lengthy process the presence of influenza viruses in flocks and their extraordinary susceptibility to variation constitute a notable economic danger. The observation of concurrent infections (fowl cholera, aspergillosis) appears to be coincidental, but the possible activating role of influenza infection cannot be excluded. The fact that type 6 avian influenza virus strains possess a neuraminidase component in common with that of human A_2 influenza virus is of hygienic importance, as it suggests that certain avian species may play a direct or indirect role in the transformation of human influenza virus strains.

SUMMARY

A total of five avian influenza virus strains, two from growing ducks, three from adult birds outside the laying season, were isolated in eastern Hungary. The growing ducks had been respiratory symptoms and grossly they showed fibrinous airsacculitis, serositis and sinusitis. Among the adult ducks, isolations were made from minor outbreaks and sporadic individual cases.

The five isolates were found to belong to three serological types:

Type 3—4	Duck (Hungary) 2/70
	Duck (Hungary) 71
Type 4	Duck (Hungary) 3/70
	Duck (Hungary) 4/70
Type 6	Duck (Hungary) 1/70

The type 6 strain possesses a neuraminidase component identical with that of human type A_2 influenza virus.

Infection experiments with two of the isolates demonstrated that chicks, young guineafowl and turkey poults were also susceptible, but it was concluded the establishment and severity of the clinical disease depend not only on the bird species, but also on certain predisposing factors, above all on the conditions of feeding and management.

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ß-LACTOGLOBULIN AND IMMUNOGLOBULIN POLYMORPHISMS OF MOTHER'S MILK

By

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Papers dealing with the analysis of mother's milk proteins have mostly been content to describe the amount and amino acid composition of casein fractions in colostrum and in so-called "mature milk". Using paper electrophoresis, SCHÄFER (1951) demonstrated 4—5 different protein fractions and found that the fraction with the fastest electrophoretic mobility and heaviest staining intensity is β-lactoglobulin. He examined the proteins of cows' milk for the sake of comparison. Furthermore, SCHÄFER defined that the slowest, altogether flat, faintly staining zones are immunoglobulins.

With filter paper electrophoresis of various milk samples taken from various stages of the lactation period SAGER (1959) established that immunoglobulins are at peak concentration in the colostrum while β-lactoglobulins are present in comparatively smaller amounts, but that beginning from the eighth day of lactation the amount of immunoglobulins reduced whereas the β-lactoglobulin concentration increased.

For the present study both these protein fractions were examined in mother's milk on the basis of a quite numerous population, using different starch gel electrophoretic procedures.

Material and methods

Altogether 157 milk samples were examined, of which 137 were from the Obstetrics Department of the István Hospital, Budapest, while 20 originated from the department of Gynaecology at the City Hospital, Gödöllő. Each agglutination tube contained before sampling three drops of an EDTA-containing preservative solution and the samples were stored in +5 °C until use. Before electrophoresis the samples were centrifuged to remove fat. The $11 \times 14 \times 0.3$ cm gels consisted of 13% starch, hydrolysed in our laboratory.

The following buffer systems were employed: for determination of the fast-moving β-lactoglobulin fraction a Tris-citric acid gel buffer and H_3BO_3-LiOH vessel buffer (MEYER, 1966), and for the slow-moving immunoglobulin fraction POULIK's (1957) discontinuous Tris-citric acid gel and H_3BO_3—NaOH vessel buffer system. With the first system electrophoresis was carried out horizontally at about 10 V/cm output voltage for 4—4.5 hours, while in the second 12 V/cm output voltage was used until the borate boundary had migrated 10 cm from the insertion line. After electrophoresis the gel slices were stained with Amidoblack 10B dye solution.

Biochemical identification of these proteins examined was possible with-

out knowing their amino acid compositions, etc. Their characteristic could, however, only be concluded from their electrophoretic behaviours on the basis of the descriptions in the above mentioned papers.

Results

Polymorphism was found in the fast-moving protein fraction of mother's milk, which was identified with β-lactoglobulins. Its two homozygous variants were designated Lg^A and Lg^B; and heterozygous variants were present in the form of the AB phenotype and as the combination of the B band with a substantially slower Lg^C alleles (Fig. 1). Lg^A was comparatively rare (Table I),

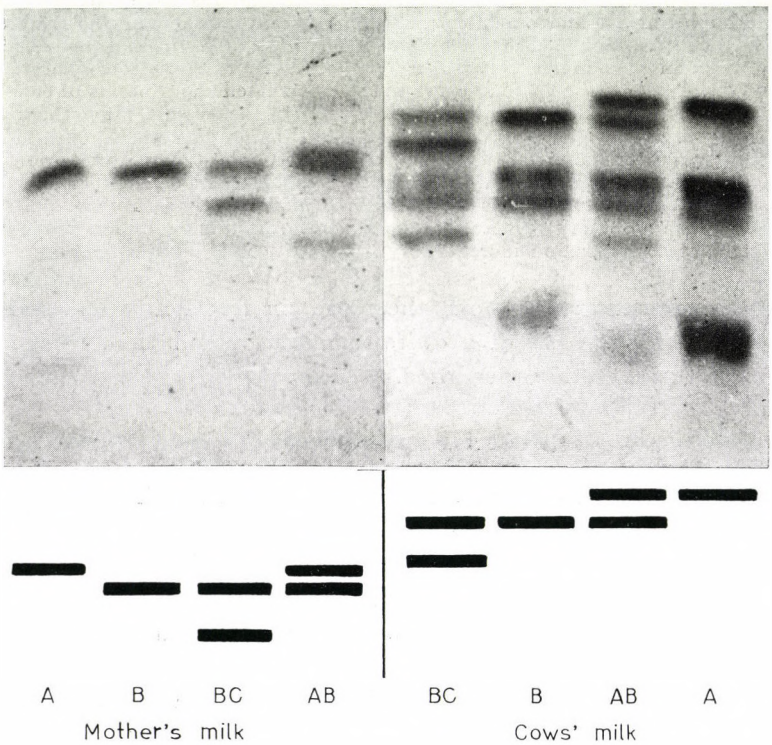

Fig. 1. β-lactoglobulin phenotypes of mother's milk and cows' milk

Table I

The phenotypical distribution of β-lactoglobulin phenotypes and the frequencies of the alleles

Number of samples	ß-lactoglobulin								
	A	%	B	%	C	AB	%	BC	%
157	10	6.3	134	85.3	—	10	6.3	3	2.1
	$Lg^A = 0.096$; $Lg^B = 0.895$; $Lg^C = 0.009$								

occurring in only 6.3% of the samples examined, in contrast to the 85.3% phenotypical frequency of Lg^B. The frequency of the AB phenotype is consequently also low. The homozygous C phenotype was not found in this material.

Polymorphism was also observed in the slowest-moving protein fraction, classified as immunoglobulin (Fig. 2). The three homozygous variants, Ig^A, Ig^B and Ig^C, migrated at different rates and each exhibited a diffuse protein band. Furthermore, two of the three possible heterozygous phenotypes were also found: AC and BC. With respect to the percentual distribution of the variants, the most frequent were the homo- and heterozygous forms of the Ig^C allele (Table II).

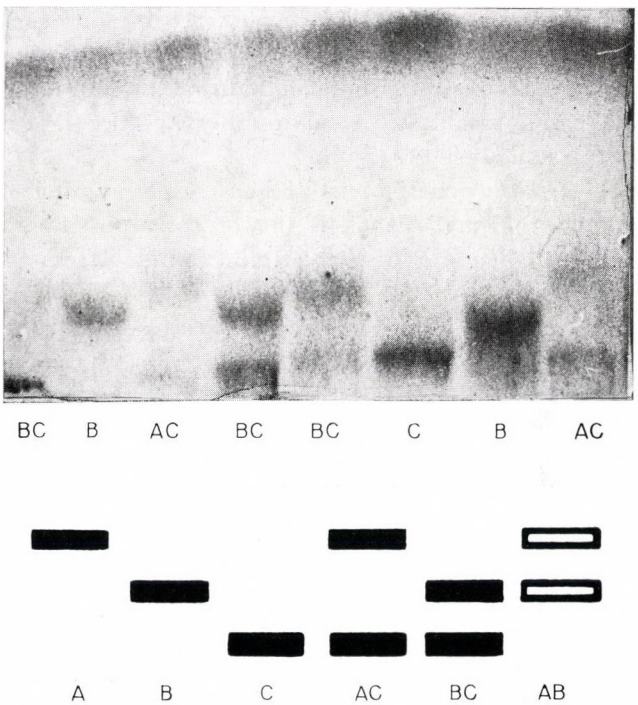

Fig. 2. Immunoglobulin phenotypes of mother's milk

Table II

The phenotypical distribution of immunoglobulin phenotypes and the frequencies of the alleles

Number of samples	Immunoglobulin									
	A	%	B	%	C	%	AC	%	BC	%
157	8	5.1	14	8.8	18	11.5	42	26.8	75	47.8
	$Ig^A = 0.185$; $Ig^B = 0.328$; $Ig^C = 0.487$									

Discussion

The fast moving, intensively staining fraction was classified as β-lactoglobulin on the basis of SCHÄFER's paper (1951), although the technique applied in our studies, including the electrophoretic technique and the buffer system, was quite different. The β-lactoglobulin bands of cows' milk obtained with the present method had a considerably faster electrophoretic mobility than the corresponding fractions of mother's milk (Fig. 1), but the bands stained to the same extent.

The immunoglobulin bands were likewise identified following SCHÄFER the slowest fraction after electrophoresis. It is very easy to follow from the variation of staining intensity of the electrophoretic bands the quantitative changes of this protein described by SAGER (1959). The staining intensity of the protein bands in this region also sheds light on the immunoglobulin concentration of the milk samples, although different stages of the lactation periods were not tested during sampling.

Determination of the genetical background is a very difficult problem in these protein polymorphisms. Although theoretically possible, the obstacles to the collection of an adequate number of milk samples from mother-daughter pairs, sisters, half-sisters or sib-pairs and perhaps, the samples of female twin pairs are practically unsurmentable. Genetical conclusions must therefore here be confined to a comparison of the corresponding polymorphism of the cows' milk in the case of β-lactoglobulins.

SUMMARY

Among 157 samples of mother's milk examined by starch gel electrophoresis, three variants were observed in β-lactoglobulin (Lg) region, the allelles being designated with the letters A, B and C. Polymorphism was also found in the immunoglobulin (Ig) region; three homozygous phenotypes, A, B and C, with different electrophoretic migration rates, and further two heterozygous phenotypes, AC and BC, were ascertained. The AB phenotype did not occur. The genetics of these polymorphisms were confined — on account of the practical difficulty of obtaining samples from mother-daughter pairs, sisters, half-sisters or twin pairs — to a comparison with the β-lactoglobulin polymorphism of cows' milk.

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CEREBROSPINAL VASCULAR WALL LESIONS CAUSING NERVOUS SYMPTOMS IN SWINE (HARDING'S CEREBROSPINAL ANGIOPATHY)

By

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Histological examination of the central nervous system is a common diagnostic means for the differentiation of infectious diseases, above all of viral conditions. Since 1962 periarteriitis-nodosa-like wall-lesions of cerebrospinal vessels have often been observed in specimens from swine that died or were emergency slaughtered showing nervous or even cerebral symptoms.

Literature

Though vascular wall lesions of swine have been widely studied, most authors have centered investigations only on the aorta, main arterial branches and coronary arteries. KRIEG (1968) established that proliferative processes of the arterial wall may appear in relatively young pigs and becoming more severe with advancing age they may eventually give rise to arterio- or atherosclerotic lesions in the aorta and coronary and cerebral arteries. The incidence of such changes does not notably differ in domestic swine and wild boar, except that severe lesions are more frequent in aged domestic swine. MAINGUET (1964) has explained the greater frequency and severity of lesions in the domestic pig as due to greater susceptibility ensuing from domestication. Quiet life, overfeeding and genetic factors have also been considered as predisposing to vascular wall disease (GRÜNBERG, 1965; VASTESAEGER, 1965).

Histological lesions of the superficial and deep cerebral arteries of swine were examined in more detail by BERG (1966), FRENCH et al. (1965), LUGINBÜHL (1966) LUGINBÜHL and JONES (1965). Next to the aorta, atherosclerotic lesions were most frequently found in the wall of intracranial arteries, about two-thirds of the examined swine showing stenotic thickening of the intima of brain vessels. The morphogenesis and nature of the cerebrovascular changes corresponded essentially with arterial lesions in other parts of the body, except that the intimal thickenings of the cerebral vessels usually contained less lipid and underwent a fibrosclerotic rather than an atheromatous transformation. Cases of cerebrovascular lesions giving rise to ulceration, thrombosis or haemorrhage were never reported. The atherosclerotic change of intracerebral vessels in swine is characterized by an increase of collagenous fibres in the adventitia or other layers of the vessel wall. In advanced fibrosis the vascular adventitia widens markedly, smooth muscle cells of the media are replaced by collagenous fibres and fibre synthesis causes the thickening of the intima. Vascular wall fibrosis seems to appear most frequently in arteries of the basal ganglia immediately after their entry into the cerebral parenchyma. No hyaline change, nor amyloid deposition has been observed in cerebral arteries of this species. Necrotizing arteritis, occasionally thrombosis in arteries and malacic areas were observed by CORNER and JERICHO (1964) in the brains of 11 pigs, while HARDING (1966) has described a cerebral angiopathy manifested by nervous symptoms in 66 swine from different sources. The greater part of the animals examined by HARDING exhibited the symptoms as early as 5 weeks after weaning. Microscopically the small arterioles of the central nervous system were found to be thickened, the smooth muscle cells of the media showed swelling and alteration, the subendothelium underwent a hyaline degeneration and infiltrating cells appeared in the perivascular area. Several animals had similar lesions in some vessels of the tongue, small intestine, pancreas, liver and eye.

A cerebrospinal angiopathy of unknown origin has recently been reported also from Belgium (HOORENS and THOONEN, 1967); Germany (FREESE and SANDERSLEBEN, 1968) and Austria (KÖHLER et al., 1971). In these cases a fibrinoid degeneration of the intima and media of arteries and arterioles and subsequent regressive changes of the central nervous system (oedema, malacia, cavitation), necrosis of brain cortex and vascular and perivascular cell infiltration were observed first in growing pigs showing signs of visual disturbance. KÖHLER et al. (1971) held the view that the aetiology of this condition as well as its relation to HARDING's cerebrospinal angiopathy, CORNER and JERICHO's necrotizing arteritis and periarteriitis nodosa remain to be clarified.

The incidence of periarteriitis nodosa in animals was first observed by LÜPKE (1906) among axis deer kept in the Favorite Park in Ludwigsburg where it consistently reappeared year after year. Occurrence of this lesion in other than cerebrospinal vessels of swine was reported by HARZER (1920), JOEST and HARZER (1921), KILSCHPERGER and STÜNZI (1946), OBERHAUSEN (1933), SWOBODA (1940) and PALLASKE (1943). In Hungary periarteriitis-nodosa-like lesions of renal vessels were detected in the course of abbatoir examinations (SZÁZADOS and NÁDOR, 1971).

At a congress held in 1954 in Stockholm periarteriitis nodosa was classified as a collagen disease.

Materials and methods

The examined specimens were mostly submitted to this institute for histological diagnosis of the cause of unusual and severe cerebral symptoms or on suspicion of Aujeszky's disease. The latter could always be excluded on the basis of animal experiments and virological and histological examinations.

Brains and spinal cords from a total of 98 swine were examined, most of which had been emergency slaughtered. Specimens were always secured from the same sites and cut in identical planes.

The Medulla oblongata, pons, corpora quadrigemini and the dorsolateral part of one hemisphere above the Ammon's horn were cut along the frontal plane, the Ammon's horn from one side was cut longitudinally, the cerebellum was excised in the median line along the plane of the longitudinal cerebral fissure and the cervical and lumbar intumescences of the spinal cord were cut transversely.

Brains from 12 swine were also examined in whole sections cut along the frontal plane. Whole brain sections were cut from the direction of the frontal lobe along the plane of the olfactory lobe, in the median line of the saeptum pellucidum anterior to the optic chiasma across the diencephalon in the plane of the pituary gland behind the optic chiasma across the mesencephalon in the plane of the Ammon's horns. Whole sections were also cut to examine the pons + cerebellum, the open (cranial) part of the medulla oblongata + cerebellum and the closed (caudal) part of the medulla oblongata. The lumbar and cervical intumescences of the spinal cord were examined in three different planes.

Specimens of brain and spinal cord were fixed in a 10% formalin solution to prepare frozen and paraffin-embedded sections. The sections were stained with Mayer's haemalaun and eosin staining technique for general examinations, collagenous tissue was demonstrated with Farkas—Mallory's or Cross-

mon's technique, elastic fibres were visualized with Weigert's resorcin-fuchsin staining while Sudan III and Fettrot staining were used for lipids. Fibrinoid necrosis was studied with Masson's trichrome staining method, medullary sheaths of nerve fibres were stained by the Heidenhain-Woelcke or Klüver-Barrera procedures and nerve cell structure was examined after Nissl staining as modified by Woight.

Incidence of the disease

All but one of the affected animals examined came from large herds. Incidence was sporadic; single cases of disease occurred at intervals of several days to months over periods of several months to one and a half years. We were able to examine a number of diseased swine on the spot in five herds and learned details about the clinical history of the herds. The number of cases in these herds was the following:

18	of	350	sows affected over a period of	4	months
6	of	80	sows affected over a period of	7	months
5	of	101	sows affected over a period of	6	months
12	of	200	sows affected over a period of	16	months
49	of	14-000	fattening pigs affected over a period of	2	months

In four herds exclusively one-year-old or older sows developed the disease. Some of the affected sows were pregnant, others were suckling. In one herd there had been an outbreak of Aujeszky's disease two months earlier which ceased only after repeated vaccination. In another herd swine fever vaccination had been carried out with a lapinized vaccine one month prior to the onset of this disaese. In the third herd vaccination against leptospirosis was performed two weeks after the first two cases of the vascular disease had been manifested. No vaccination was carried out in the fourth herd. In two herds widespread occurrence of corynebacillosis, involving phlegmon of the fetlocks, periarthritis and ulceration in various parts of the body was noted simultaneously with the vascular disease.

In the fifth herd consisting of fattening pigs the vascular wall disease affected 8—10 months old animals in 41 of the 173 fattener groups.

Symptoms

Affected swine usually showed no fever, their behaviour was dull or contrarily excited and the head was held in an oblique, drooping or rigid upward position. When moving, the animals either staggered or performed distinct compulsive movements. Other not infrequent symptoms were circular gait, disturbance of vision, tremor of muscles and eyes, retracing movements fits of convulsion and cutaneous hypersensitivity. The restless animal moved

aimlessly, at times stopping in the corner of the pen or leaning its head against the wall. Attempts were occasionally made to climb on the wall of the pen; some animals even succeeded in clearing it. Retracing and the upward position of the head sometimes made the pig to fall on its back, and prostrate animals were often bitten by their mates. Not infrequently it was because of such injuries that attention was paid to the disease. Sick animals lost their appetite partly or entirely. Severely ill swine were unable to stand but made vain attempts to rise or performed swimming movements and refused all food; sometimes they were dull to the degree of somnolence. These symptoms generally did not respond to drug therapy (antibiotic, vitamin or symptomatic) and had a fatal outcome in 4—6 days at the earliest or after a protracted course lasting up to 5—6 weeks; in the latter case many swine had to be emergency slaughtered. Occasionally, antibiotic or other treatment brought on a temporary improvement and even apparent recovery, but recidivated after a few weeks leading to death or emergency slaughter or normal slaughter during the period of temporary improvement.

Macroscopic pathology

At post-mortem examination the visceral organs either appeared nor mal, or chronic inflammation of serous membranes, liver cirrhosis and icteru were found. Occasionally the meninges were thickened and showed a smoky opacity. One swine each from two of the five herds under observation, one sow, the other a 3-month-old growing pig, had in addition scabies of the ear and a deeply extending inflammation of the auditory meatuses.

Pathohistological lesions

Vascular wall lesions reminiscent of periarteriitis nodosa lesions were present in the central nervous system of the examined animals. As a rule there was a pronounced mononuclear cell infiltration around the adventitia of the changed vessels. Wall lesions were shown above all by small arterioles and muscular-type arteries. They had no site of preference, usually appearing asymmetrically. Vascular injury was most frequent in the leptomeninx enveloping the brain and spinal cord (Figs 1, 2 and 3) though relatively rare in the spinal cord itself (Fig. 2). Lesions often occurred in the medulla oblongata (Figs 4, 5 and 6), pons and mesencephalon (Fig. 7) and in the pre- and postchiasmal diencephalic areas (Figs 8, 9, 10, 11), usually at the basal parts.

The vascular lesions were of sclerotic or fibrotic rather than atheromatous type. Necroses of various extent (Figs 5, 7 and 8), fibrinoid mural necrosis of the vascular wall (Fig. 1), fresh and organized thrombosis (Figs 5 and 8) and stenosis (Figs 10 and 11) or complete obliteration (Fig. 8) of the lumen were often seen.

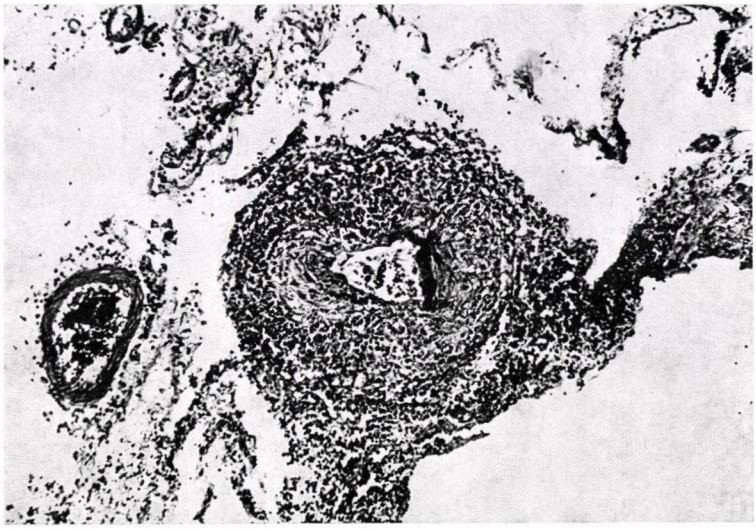

Fig. 1. Detail of cerebellar pia mater from an 18-month-old sow. Note the infiltrating cells and a severely degenerated artery in the leptomeninx. The arterial intima has degenerated and fibrinoid necrosis has become established in the degenerated media. Many mononuclear cells can be seen infiltrating the adventitia. (Haemalaun and eosin stain, 45×)

Fig. 2. Detail of lumbal intumescence of spinal cord from a 14-month-old sow. Several arteries passing in the septum close by the right dorsal horn show wall degeneration and a marked mononuclear cell reaction are visible between them. (Haemalaun and eosin stain, 45×)

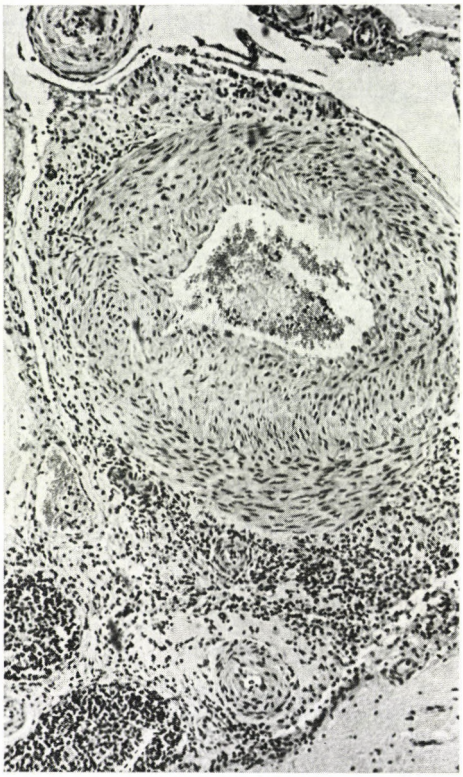

Fig. 3. Detail of leptomeninx from brain base in the chiasmal area, from a 17-month-old sow. The large artery shows signs of a regressed vasculitis. The thickened intima has become fibrotic in consequence of endarteriitis, while the media is narrowed. (Haemalaun and eosin stain, 95×)

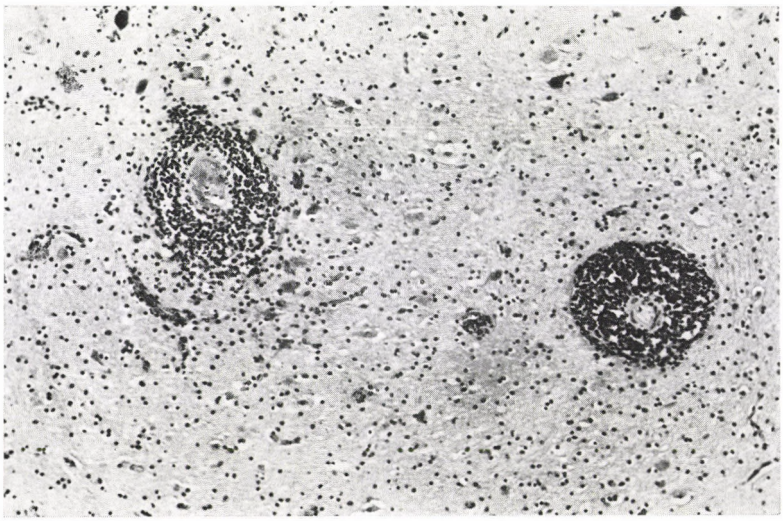

Fig. 4. Detail of medulla oblongata from a 13-month-old sow. Small arteries with degenerated walls are surrounded by a marked cellular reaction. (Haemalaun and eosin stain, 95×)

Different degrees of cerebrovascular injury were encountered. The mildest lesions occurred in the small arterioles; these showed degeneration, pyknosis and desquamation of endothelial cells, confluence of the border between intima and media, and different degrees of lymphocytic infiltration in the perivascular area (Fig. 6).

It was generally the lymphocytic infiltration which attracted the attention to the disease. The two inner layers of the vessel wall were characteristically oedematous, the elastic elements became obscure and the internal elas-

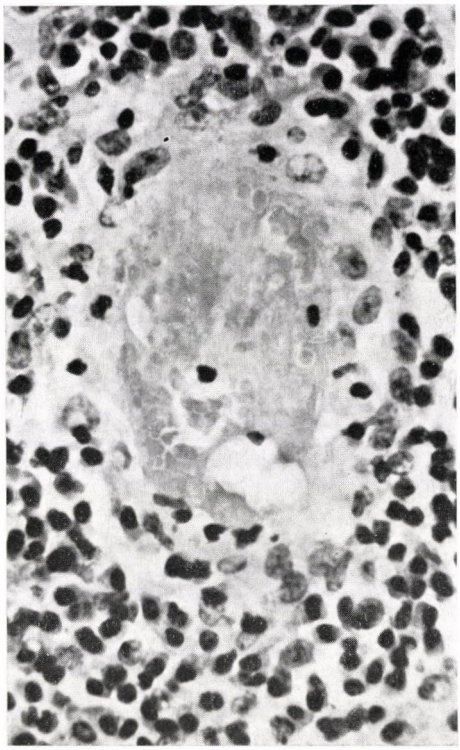

Fig. 5. Left arteriole from Fig. 4 at a 600× magnification. Thrombosis and mononuclear cell infiltration in the necrotic wall of the vessel

tic lamina appeared torn in places. In some small arteries, the necrotic intima and media had transformed to a homogeneous, structureless, slightly acidophilic tissue (Fig. 7); such arteries contained no elastic elements.

In case of more severe injuries the intima had thickened and transformed to a scar tissue rich in collagen elements and fibroblasts (Fig. 3). Intimal thickening was as a rule uneven, forming in places cushion-like protrusions which reduced the lumen and deformed the vessel wall (Figs 10 and 11). The smooth muscle cells of the media degenerated and became oedematous, cell

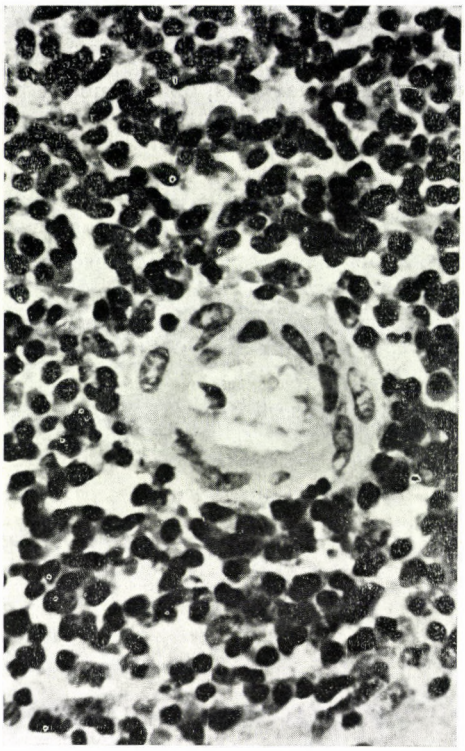

Fig. 6. Right small artery from Fig. 4 at a 600× magnification. The endothelium is necrotic, smooth muscle cells of the media are degenerated and 10—12 layers of infiltrating mononuclear cells can be seen in the adventitia

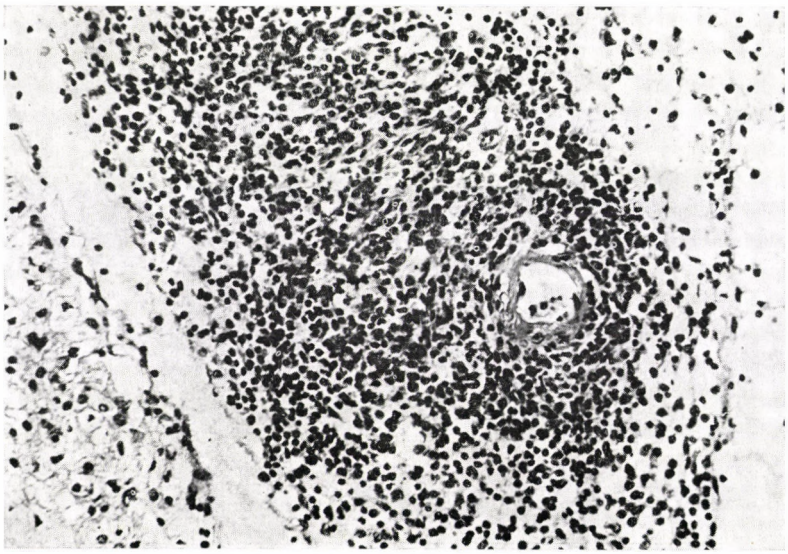

Fig. 7. Detail of mesencephalon from an 18-month-old sow. The necrotic arterial wall is surrounded by infiltrating mononuclear cells, the adjacent brain tissue is oedematous. (Haemalaun and eosin stain, 240×)

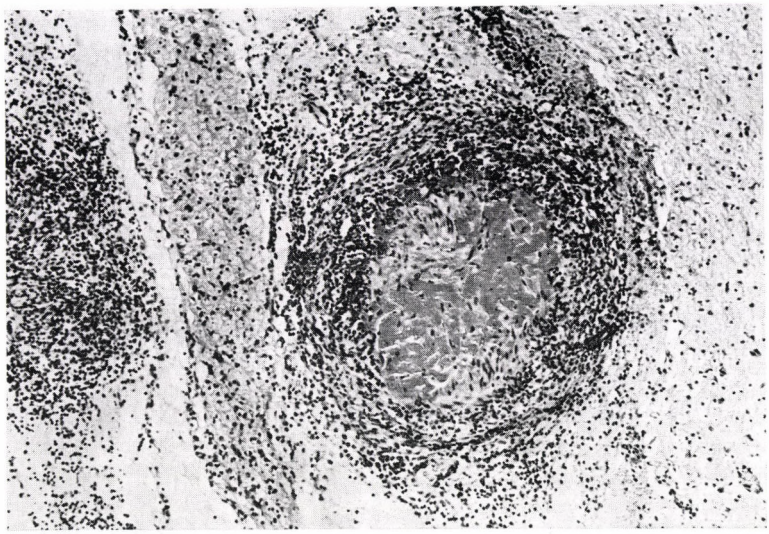

Fig. 8. Detail of diencephalon from a 14-month-old sow. An artery with necrotic wall is occluded by a thrombus and the surrounding brain tissue is oedematous. (Haemalaun and eosinstain, 95×)

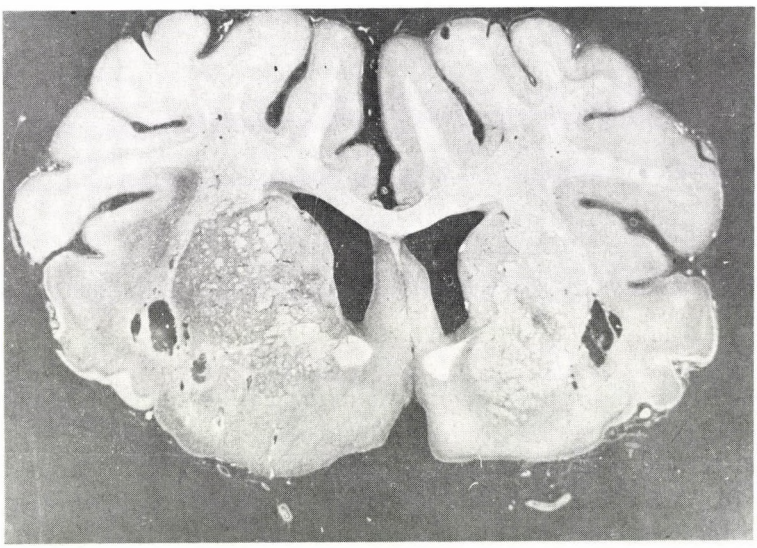

Fig. 9. Whole brain section from an 18-month-old sow cut in a frontal plane along the median line of the saeptum pellucidum. Cavities of various size are visible in the areas of the lentiform nucleus and external capsule on both sides of the brain base. (Masson's trichrome strain. Hand magnifying lens.)

margins and structure were indistinct, the nuclei were shrunk or puffed and showed signs of lysis (Fig. 6). In such vessels the smooth muscle cells of the media often underwent necrosis and transformed to a homogeneous, highly acidophilic substance which proved to be a fibrinoid necrosis by Masson's staining (Fig. 1). Amyloid necrosis could be excluded by examination in polarized light after staining with 1% Congo red. The vascular adventitia became wider and many infiltrating mononuclear cells appeared in it, above all round

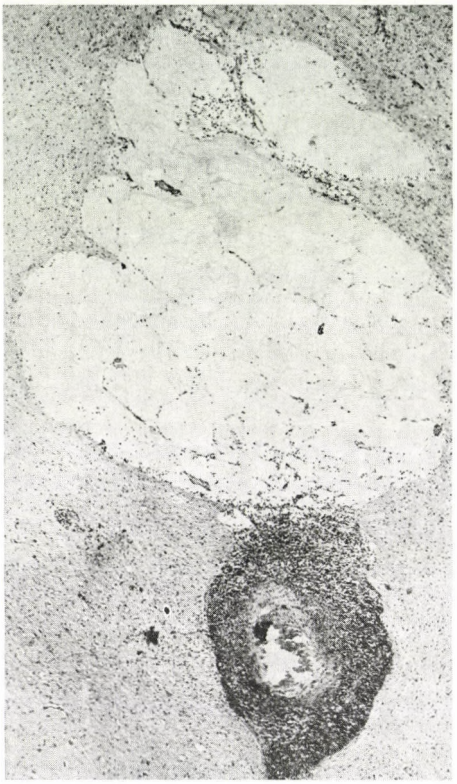

Fig. 10. Detail of Fig. 9 at a 45× magnification. Note the marked mononuclear cell infiltration around the adventitia of the severely injured artery and the large cavities, filled with exudation, in the surrounding brain tissue. (Haemalaun and eosin stain.)

cells, histiocytes, fibroblasts and occasionally a variable number of eosinophilic granulocytes (Figs 1, 4, 5, 6, 7, 8, 10 and 11). Chiefly fibroblasts migrated into the necrotic areas of the media from the adventitia. Fragmentation or disappearance of elastic elements was seen in the wall of such arteries on staining with Weigert's resorcine-fuchsin. The internal elastic lamina either became smooth or disappeared altogether, depending on the degree of injury, in contrast to the intact vascular areas, in which it was continuous, uniformly thick and undulating (Fig. 12).

All layers of the wall became oedematous and undifferentiable in some arteries, thus their contours and structure disappeared. The vascular wall thickened markedly causing a notable reduction of the lumen. In addition to the wall lesions described in the foregoing, necrosis of the intimal endothelium and thrombus formation took place.

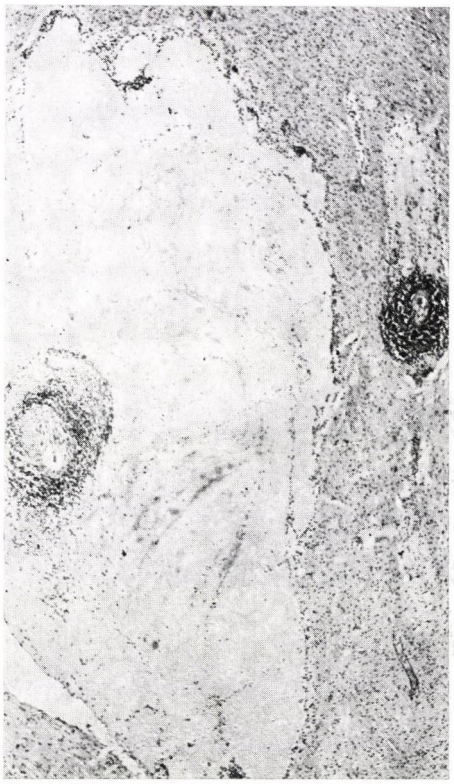

Fig. 11. Detail of Fig. 9 at a 45× magnification. Two arteries with degenerated walls are visible; a cavity filled by a gelatinous substance is present in the brain tissue around one artery. (Haemalaun and eosin stain.)

Occasionally severe haemorrhages were seen in the perivascular area of certain arteries. In other cases previous haemorrhage was indicated by the presence of many haemosiderocytes among other infiltrating mononuclear cells in the perivascular area and in all layers of the vessel wall. Infiltrating haemosiderocytes were also seen in perivascular brain tissue. Wall lesions of various extension were present along with acute or chronic perivascular haemorrhages.

Healed inflammatory processes were suggested by the presence of fibrotic or sclerotic lesions chiefly in certain larger arteries of the meninges (Fig.

3). Some layers of these vessels varied in width. The intima was broad and often also the media was traversed by fibrotic scar tissue (Fig. 3). The internal elastic lamina was in places thin and smooth, in places undulating, but thickened, while in other areas it exhibited elastosis. In some vessels the elastica was absent over large areas.

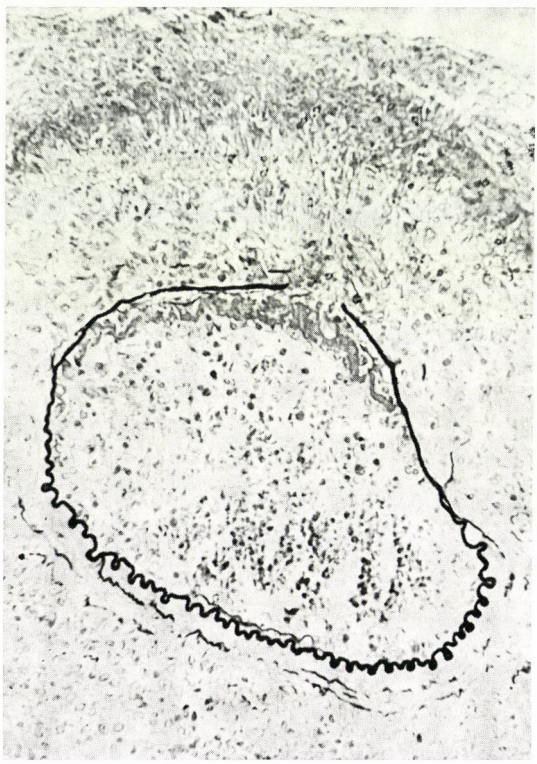

Fig. 12. Detail of grey matter from occipital lobe of a 12-month-old sow. Inflammatory cellular reaction is seen in the thickened meninges. The internal elastic lamina is smooth and shows a short discontinuity at one side of a large artery. (Resorcin-fuchsin stain, 240×)

In the surroundings of severe vascular injuries the brain tissue was usually also involved to judge from the variable degree of regressive nerve cell changes in these areas. Impaired nerve cells either showed chromatolysis or became pyknotic. In other cases, brain damage was indicated by rarefaction and porous structural alterations of the parenchyma or the appearance of smaller or larger cavities, filled by a serous or gelatinous substance, in the corpus striatum, internal and external capsule and lenticular nucleus (Figs 9, 10 and 11).

Occasionally the vascular changes were accompanied by a diffuse leptomeningitis of the serous type (Fig. 1), characterized by a marked cellular re-

action consisting of round cells, polyblasts, histiocytes, fibroblasts and eosinophilic granulocytes. The greater meningeal vessels in places showed severe wall lesions, occasionally even necrosis. Highly acidophilic serum was deposited in certain areas of the meninx while a delicate meshwork of fibrin was seen here and there in the leptomeninx.

Branches of the aorta and larger arteries and vessels of the viscera (liver, spleen, kidney, heart) were examined in only a few instances. Apart from slight fibrotic changes of the aortic intima, the visceral and other large arteries showed no lesions despite the severe concurrent damage to cerebrospinal vessels.

In none of the cerebral vascular wall lesions was neutral lipid demonstrable by lipid stain, nor crystalline Cholesterol by polarisation microscopy. In every case the lesions were of a fibrotic, sclerotic or necrotic nature rather than atheromatous. Since a marked mononuclear cell infiltration was always seen in the adventitia or in the perivascular area of the injured vessels, the wall lesions were regarded as periarteriitis-nodosa-like changes, which are vascular injuries characteristic of collagen diseases. The vascular changes probably arose by autosensitization, on an allergic basis.

Discussion

The above described wall changes of cerebrospinal vessels of swine are very reminiscent of periarteriitis nodosa lesions and cause nervous symptoms. The disease always involved the wall of small arterioles or muscular-type arteries. Although the wall lesions did not seem to have any site of preference, they occurred more often in certain brain areas than in others, such as before and behind the chiasma in the diencephalic area, chiefly at the basal part of the brain, in the arteries of the cerebral and spinal pia mater, as well as in the medulla oblongata, pons and mesencephalon. The injured vessels showed oedema, fibrinoid degeneration or necrosis of the wall and severe impairment of elastic elements. Above all the intima and media became involved, over areas of varying size. The injured vessels were surrounded either by a marked mononuclear cell infiltration reminiscent of periarteriitis nodosa or by acute and chronic perivascular haemmorrhages or signs indicative of an early phase of haemorrhages. Thrombosis regularly occurred in the injured vessels. These changes correspond not so much to a simple arteriosclerosis, progressing with age, as to a hyperergic periarteriitis-nodosa-like vasculitis. CORNER and JERICHO (1964) arrived at similar conclusions. Although wall lesions are often found in the sites characteristic of arteriosclerosis, these might well be attributed to the haemodynamic factors operative in the basic cerebrospinal disorder. Moreover, vascular injuries may also be found in the diencephalon, pons,

medulla oblongata and cerebellum, viz. in areas never involved by arteriosclerosis, to judge both from literary data and our own experience.

Cerebrospinal wall lesions differ from arteriosclerotic vascular injury not only in their site of localization, but also in the frequent occurrence of wall haemorrhages and thrombosis. We never saw necrosis of brain tissue in association with arteriosclerosis, but often observed injuries of perivascular nerve tissue, above all nerve cell changes probably due to circulation disorders. If the injury was extensive, the nerve tissue became porous, and the appearance of cavities filled by a liquid or gelatinous substance can probably be explained by lysis of nerve tissue. Post-vasculitis changes comprise stenosis reminiscent of chronic endarteriitis, and a marked fibrotic and sclerotic lesion of usually all vascular wall layers.

The varied herd history data permit no conclusion as to the nature of the factors responsible for eliciting this probably hyperergic vasculitis of the cerebrospinal vessels.

Human pathologists have advanced two theories to explain the aetiology of periarteriitis nodosa. One of these theories suggested by SELYE and PENTZ (1943) is based on the plentiful evidence that the organism responds to various stimuli by an alarm reaction, marked in part by increased secretion of adrenocortical hormones. Since repeated stimuli result in an adaptive thickening of the adrenal cortex, the consequent excessive hormone production, according to SELYE and PENTZ, elicits periarteriitis nodosa. Similar vascular lesions can in fact be elicited in rats by overdoses of adrenocortical hormone.

According to the other hypothesis, periarteriitis nodosa is a hyperergic vasculitis(RÖSSLE, 1937; GERLACH, 1923). RICH and GREGORY (1943) were able to reproduce the disease by repeated administration of horse serum to rabbits sensitized with horse serum. KIELSCHPERGER and STÜNZI (1946) established that repeated infections with *Erysipelothrix rhusiopathiae* might play a role in the development of cerebrospinal vascular disease.

Apart from onset, course and symptoms, the microscopic cerebrospinal lesions of the disease are also characteristic and differ from the encephalitic and meningeal changes elicited by infectious diseases (Aujeszky's disease, swine fever, infectious polioencephalomyelitis, etc.) or other causes (e.g. NaCl poisoning). Nevertheless, differentiation from the latter diseases always requires microscopic examination of the brain.

SUMMARY

Periarteriitis-nodosa-like vascular wall lesions, causing distinct nervous symptoms, were observed in the central nervous system of above 1-year-old sows and 8 to 12-month-old fattener swine. Incidence of the disease was sporadic; but losses were notable (8—49 adult swine per herd) over periods of 2—16 months. The course of the disease was either rapid, lasting a few days, or prolonged over several weeks, sometimes with temporary improvement, but the outcome was usually fatal or emergency slaughter had to be performed.

The lesions consisted of serous and cellular infiltration of the vessel wall, fibrinoid degeneration and necrosis of intima and media, injury or disintegration of their elastic elements, or chronic sclerotic processes, often accompanied by thrombosis and stenosis, or complete obliteration of the vascular lumen. Above all small arterioles and muscular-type arteries became involved. The lesions did not seem to have a site of preference, but they occurred most frequently in arteries of the cerebral and spinal leptomeninx, in the brain and much less often in the spinal cord. Other frequent sites of localisation were vessels of the medulla oblongata, pons, mesencephalon and diencephalic areas anterior and posterior to the chiasma, usually at the base of the brain.

In cases of severe vascular injury, the surrounding nerve tissue also became involved; the nerve cells showed regressive changes of different degrees, the brain tissue became rarefied and porous, while oedema and cavities of various sizes, filled by serous or gelatinous substance, appeared in the area of the corpus striatum and internal and external capsule. The cerebral processes were occasionally accompanied by a serous or cellular infiltration of the pia mater.

No similar changes were found in the aorta and visceral (splenic, hepatic, renal and cardial) vessels of the examined animals.

The cerebrospinal vascular wall injury has been regarded as a collagen disease and its causation has been attributed to an acute vasculitis of allergic origin.

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TOXICITY TESTING OF ENTEROCOCCUS STRAINS RESPONSIBLE FOR FOOD POISONING

By

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With the incidence of enterococcal food poisoning showing a rising trend of late, D-serogroup streptococci are beginning to acquire a growing importance in food hygiene. One sign of this is the recent classification of enterococcal components of the intestinal flora of man and animals with the indicator flora of certain food products, on account of their high resistance. The presence of large numbers of enterococci in food products directly after freezing, drying or mild heat treatment usually suggests a high degree of bacterial contamination prior to processing, yet the pathogenicity of these organisms is still a matter of dispute.

Clarification of the problem is hampered by the inconsistent results of feeding experiments performed on volonteers with isolates from reported cases of food poisoning (MOORE, 1955; SLANETZ et al., 1963; DEIBEL and SILLIKER, 1963; HARTMAN et al., 1965; SEDOVA, 1970). The probable explanation is that the establishment of food poisoning depends on several factors rather than one, like all other diseases caused by facultatively pathogenic microorganisms (PUSZTAI, 1967).

Certain authors have postulated a similarity between the mechanisms of staphylococcal and enterococcal food poisoning and toxin of similar action to staphylococcal enterotoxin has in fact been demonstrated from two strains of *Streptococcus faecalis var. zymogenes* (FUJIWARA et al., 1956).

The conflicting findings of feeding experiments on humans and the various difficulties of their performance and evaluation initiated our previous experiments aimed at an assessment of the frequency of occurrence in food products of enterococcus strains pathogenic for mice. We found that 18.8% of mice died after being inoculated intraperitoneally with 2 ml of 24-hour glucose broth cultures of D- streptococcus strains isolated from various foods (PUSZTAI and BIRÓ, 1965). The toxic action of several bacterial strains was demonstrated in a further series of experiments. Out of 50 enterococcus isolates, eight elicited well defineable microscopic lesions[1] indicative of hepato-toxic action after intraperitoneal administration to mice (PUSZTAI and VETÉSI, 1971).

A substantial contribution to the elucidation of certain aspects of enterococcal food poisoning has been made by phage typing (BEKERICH and HAUDROY, 1922, BAGGER, 1926; DUTTON, 1926; CIUCA et al., 1959; BELOIU et al., 1964; BROCK, 1964; BIRZU et al., 1966; HOCH and HERMÁN, 1971). This technique has already considerably extended the possibilities of detecting the source of an infection and increased the efficiency of prevention by enabling follow-up of the path of infection, and the good results obtained with certain Hungarian phage isolates promise a further broadening and refinement of its applications (HERMÁN and HOCH, 1971).

Table I

Characters of enterococci

Designation	Source of strains	Haemolysis in blood agar	Gelatin liquefaction	Tellurite resistance (1 : 2500)	Colorization on Barnes medium	Growth on agar containing 6,5% NaCl.	Mannitol	Inulin
E_1	S	α	—	—	white	+	+	—
E_6	SG	γ	+	+	red	+	+	—
E_{10}	SG	γ	—	+	red	+	+	—
E_{12}	V	γ	—	+	red	+	+	—
E_{13}	CN	γ	—	+	red	+	+	—
E_{18}	NSE	γ	—	+	red	+	+	—

Material and methods

The experiments reported in this paper were carried out with the aim of establishing whether certain enterococcus strains isolated from cases of food poisoning in Hungary are toxic for mice.

Nineteen strains, all originating from the collection of the National Institute of Nutrition, were examined. The majority (17 strains) had been cultured as presumed causative agents of food poisoning on the grounds that the food product responsible for the poisoning had contained them in a high concentration and also the course of the toxicosis seemed characteristic of these facultative pathogens. The remaining two strains had been isolated from cases in which the pathogen was present not only in the incriminated food product, but also in gastric contents obtained from the patient by stomach-pump. Two additional enterococcus strains originated from cases in which they represented only one component of a mixed flora in the toxic food product, in these cases the involvement of the enterococci was of course open to doubt.

With each strain 2 ml of a 1% glucose broth culture was inoculated intraperitoneally into two mice, weighing 35—40 g. Ten mice each inoculated by the same route with 2 ml sterile glucose broth and saline, respectively, served as controls.

Mice that died spontaneously or were killed in extremis during the experiment were autopsied, and their heart blood and various organs were examined bacteriologically. Infected animals showing no signs of disease and controls were killed after 72 hours for post mortem and bacteriological examination.

Livers from one of each infected pair and from several control mice were processed for histological examination. The liver specimens were fixed in a 8%

pathogenic and toxigenic for mice

Melicitose	Sorbitol	Melibiose	Raffinose	Esculin	Precipitin test with Group-D sera	Phage type	Identification of strains
—	—	+	+	+	+	670 963	*Str. faecium*
—	+	—	—	+	+	41	*Str. faecalis v. liquefac. iens*
—	+	—	—	+	+	41	*Str. faecalis*
—	+	—	—	+	+	NT	*Str. faecalis*
+	+	—	—	+	+	1, 5, 13, 41	*Str. faecalis*
+	+	—	—	+	+	1, 2, 10, 13, 41	*Str. faecalis*

Symbols used: S, sausage; SG, gastric rinsing fluid after ingestion of sausage; V, venison; CN, cooked noodles; NSE, noodles with crambled egg; NT, untypeable

neutral formaldehyde solution, embedded in paraffin and cut into about 6 μ thick sections. Sections were stained with haematoxylin and eosin, and some by the PAS, Gram and Gömöri's silver impregnation techniques. In every case, frozen sections were also prepared for lipid staining with Sudan III.

Results

The 19 strains studied could be divided into three groups on the basis of the experimental observations. Six strains caused a lethal disease in mice within 24—48 hours after intraperitoneal administration, 11 strains caused a non-lethal disease, and two strains produced no observable symptoms.

The six enterococcus strains of the first group had originally all been isolated as the unequivocal causative agents of food poisoning. The mice that died after infection with these strains displayed acute hyperaemic inflammation of the spleen, congestive hyperaemia of various organs, and acute heart dilatation on autopsy. The cultural and biochemical properties as well as the phage types of the bacteria reisolated from the carcases corresponded with those of the strains used for infection.

Four of the six strains (E_1, E_{10}, E_{12}, E_{13} in Table I) caused liver cell changes indicative of toxic damage. The nuclei of the impaired cells varied greatly in shape and size. Many were considerably enlarged, others elongated and deformed. Some cell bodies shrank and stained dark red with eosin (liver cell collapse). This latter change was especially pronounced in the liver of the mouse infected with strain E_{12}. Occasionally parenchymal cell necrosis was

Table II

Characters of enterococci pathogenic

Designation	Source of strains	Haemolysis in blood agar	Gelatin liquefaction	Tellurite resistance (1 : 2500)	Colorization on Barness medium	Growth on agar containing 6.5% NaCl	Mannitol	Inulin
E_3	S	γ	+	+	red	+	+	—
E_4	SS	α	—	—	white	±	+	—
E_5	S	γ	—	—	white	+	+	—
E_7	S	α	—	—	white	+	+	—
E_9	S	γ	+	+	red	+	+	—
E_{14}	SCh	β	—	—	white	±	+	—
E_{16}	FC	α	—	—	white	+	+	—
E_{20}	SCh	γ	—	+	red	+	+	—
E_{36}	NC	γ	—	+	red	+	+	—
E_{37}	VCF	γ	—	+	red	+	+	+
E_{39}	SCh	γ	+	+	red	+	+	—

noticed, and in one case thrombus formation took place in the hepatic vessels. With another strain, E_6, which had originally been isolated from the gastric washings of a person suffering from food poisoning, we observed signs of the activation of RES cells and the appearance of neutrophilic granulocytes in the liver of the inoculated mouse.

The 11 enterococcus strains of the second group caused an illness of varying severity but without fatal outcome. Animals killed 48—72 hours after infec-

Table III

Characters of enterococci

Designation	Source of strains	Haemolysis in blood agar	Gelatin liquefaction	Tellurite resistance (1 : 2500)	Colorization on Barness medium	Growth on agar containing 6.5% NaCl	Mannitol	Inulin
E_{11}	LS	α	—	—	white	+	+	+
E_{15}	SCh	α	—	—	white	+	+	—

but not toxigenic for mice

Melicitose	Sorbitol	Melibiose	Raffinose	Esculin	Precipitin test with Group-D sera	Phage type	Identification of strains
—	+	—	—	+	+	41	*Str. faecalis v. liquefaciens*
—	—	+	+	+	+	NT	*Str. faecium*
—	—	+	+	+	+	8, 670 963, 867	*Str. faecium*
—	—	+	—	+	+	NT	*Str. faecium*
+	+	—	—	+	+	41	*Str. faecalis v. liquefaciens*
—	—	—	—	+	+	8, 670 867, 963	*Str. durans*
—	—	+	—	+	+	8, 670 867, 963	*Str. faecium*
—	+	—	—	+	+	5, 7	*Str. faecalis*
+	+	—	—	+	+	NT	*Str. faecalis*
—	+	—	—	+	+	NT	*Str. faecalis*
+	+	—	—	+	+	NT	*Str. faecalis v. liquefaciens*

Symbols used: S, sausage; SS, smoked sausage; SCh, sheep cheese; FC, fermented curd; NC, noodles with curd; VCF, various cooked foods; NT, untypeable

tion did not show any characteristic gross lesions, but cocci could be reisolated from the carcases and they corresponded in every respect with those used for the infection (Table II). Microscopic examination of the livers of mice in this group revealed no toxic parenchymal cell damage, but, unlike in mice of the first group, there was often an acute fibrinous peritonitis.

not pathogenic for mice

Melicitose	Sorbitol	Melibiose	Raffinose	Esculin	Precipitin test with Group-D sera	Phage type	Identification of strains
—	—	+	—	+	+	8, 670 963	*Str. faecium*
—	—	—	—	+	+	8, 670 963, 1510	*Str. faecium*

Symbols used: LS, liver sausage; SCh, sheep cheese

The two enterococcus strains forming the third group had originally been isolated from food products having high total germ counts (10^7/g) covering a mixed bacterial flora. These strains produced no symptoms on intraperitoneal administration to mice. When killed 72 hours after inoculation, the animals showed only the acute dilatation of heart and lung resulting from extermination. Attempts to reculture the strains from organs, however, were successful (Table III). No toxic hepatic damage was seen on histological examination.

Control mice inoculated with sterile broth or saline showed no symptoms over a 72-hour period of observation, when the animals were exterminated. Post-mortem and bacteriological findings were negative, and no toxic parenchymal cell damage was seen during microscopic examination of the livers.

Discussion

The causative agents of food poisoning and food contamination are either toxin-elaborating microorganisms or else organisms producing pathogenic effects by an unknown mechanism (THATCHER and CLARK, 1968). To judge from both home and foreign statistics the facultatively pathogenic organisms belonging to the latter group are frequently responsible for what are called non-specific food poisonings. Orders regulating the reporting of such cases, however, differ from country to country. (In Hungary, all confirmed and even suspect cases must be compulsorily reported.) In the absence of an obligatory pathogen, any microorganism occurring in the incriminated food product at a sufficiently high concentration should be regarded as such, if there is evidence in literature of its causative role in food poisoning (ORMAY and NOVOTNY, 1970).

Investigations conducted during recent years have thrown much light on several aspects of non-specific food poisoning and infection. We now know that *Clostridium perfringens* and *Bacillus cereus* must be added to the list of proven agents of food toxicosis or infection (WHO, 1968). The role of other facultatively pathogenic microorganisms, including enterococci is, however, still a matter of dispute; feeding experiments on human volunteers have not helped to clarify this problem. Quite new research suggests that certain strains of *Str. faecalis var. liquefaciens* and *Str. faecalis v. zymogenes* have enteropathogenic properties (SEDOVA, 1970). It has also been demonstrated that the bacterium-free culture filtrates of pathogenic enterococcus strains do not cause disease. Ingestion of a large amount — several millions — of live germs is required to elicit the condition, as has also been affirmed by our previous findings.

Food poisoning elicited by facultatively pathogenic microorganisms as a rule takes a mild course, and recovery can be expected to be rapid. ORMAY

and NOVOTNY (1970) nevertheless report that a greater part of the patients affected by *Str. faecalis* food poisoning require hospital treatment.

Earlier studies in this laboratory had been performed to clarify whether enterococcal toxins having an action similar to staphylococcal enterotoxins can be demonstrated by inoculation of experimental animals. Certain enterococcus strains were found to cause hepatotoxic-like changes on intraperitoneal administration to mice. The present experiments were carried out to check the suitability of this model for toxicity assay of enterococcus strains. According to the histological examinations, four of the 19 strains studied caused hepatotoxic-like parenchymal cell damage in the liver of mice. No evidence of toxic property could be obtained for the remaining 15 strains, although these also had been isolated from food products responsible for poisoning. Lack of toxin production might have been due to the loss of this capacity during the several months that elapsed between isolation and experimental use. Our own experience, and that of other authors have been that enterococcal isolates from a given test material often contain several strains of which usually only one can be found after the subsequent transfers. It may be that this phenomenon was responsible for the lack of toxic property of several of the strains even through some of them caused disease or death of the experimental animals.

Determination of the biochemical properties and phage typing of streptococcal isolates from the patient's body and/or from the incriminated food can contribute greatly to an understanding of the aethiology of enterococcal food poisoning. Identification of the reisolated strains with the original ones on this basis can provide positive confirmation of their causative role in food poisoning. The importance of phage typing is further underlined by the fact that *Str. faecalis* and *Str. faecium* are often simultaneously present in the sampled materials.

Current studies with temperate phages of the transferability to non-pathogenic enterococcus strains of the genetic factor conferring pathogenicity (HERMÁN and HOCH, 1971) are expected to throw more light on the problem of enterococcal pathogenicity.

SUMMARY

The present studies were carried out to investigate the toxicity for mice of enterococcal strains isolated in connection with human cases of food poisoning.

Of the 19 type-D streptococcal isolates used only two originated from cases in which the causative organism could be demonstrated not only in the incriminated food, but also in the gastric contents of the patient. Four of the strains were found to cause toxic degenerative changes in the liver cells of infected mice, to judge from histological evidence. Thirteen strains caused an illness of varying degrees of severity and some of them even death but without any indication of toxic action, while the remaining two strains were non-pathogenic for mice.

In view of these findings it seems important that biochemical testing and phage-typing of isolates from food and patient be performed whenever there is a strong suspicion of enterococcal food poisoning, especially as *Str. faecalis* and *Str. faecium* are often simultaneously present in sampled material though in the majority of cases only *Str. faecalis* is the causative agent.

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EINIGES ÜBER DIE ALLOPLASTIK

Von

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Zusammenfassung

Nach Bestimmung des Begriffs der Alloplastik werden die gegenwärtig gebräuchlichen alloplastischen Substanzen kategorisiert sowie die die weitläufige Anwendung der metallenen alloplastischen Materien hemmenden Faktoren und die vorteilhaften Eigenschaften der Kunststoffe bzw. die den „gewebefreundlichen" Substanzen gegenüber gestellten Anforderungen dargelegt. Unter den aufgearbeiteten 400 Literaturangaben werden einige grundlegende bzw. zusammenfassende Mitteilungen eigens hervorgehoben.

Im Rahmen der Erläuterung der eigenen Untersuchungen werden die über die bei 430 Versuchstieren und etwa 200 klinischen Patienten mit den Mitarbeitern gemeinsam durchgeführten experimentellen und klinischen Untersuchungen berichtenden Publikationen zusammengefaßt. Die im Zusammenhang damit aufgearbeiteten Themen beziehen sich auf die vorteilhaften Eigenschaften der Polyester-Nähfäden, die günstigen histologischen Ergebnisse der unter Anwendung von Tantalklammern verfertigten maschinellen Nähte, die neuen Operationsverfahren, bei denen anläßlich von Magen-Darmtrakt-, Blutgefäß-, Urogenitalapparat- und Gallengang-Eingriffen der Gewebeklebstoff Histoacryl verwendet worden war, die Anwendungsmöglichkeiten der filmbildenden Substanz Plastubol in der veterinärmedizinischen Chirurgie und die Vorteile des fibrinhaltigen Wundstreupulvers. In der Folge werden die Ergebnisse der sich auf das Gewebeverhalten und die Absorption der Bioplaste beziehenden Untersuchungen sowie die Antigeneigenschaften der Bioplaste ausführlich beschrieben.

Unter Alloplastik versteht man die zum Ersatz der zerstörten oder fehlenden Gewebe des Organismus mit fremden leblosen Substanzen dienende Methode. Hierbei kommen Materien zur Anwendung, die der Organismus toleriert und die sich zur Versorgung verschiedener schutz- bzw. statisch-mechanischer Aufgaben eignen.

Diese Bestrebungen blicken auf eine ziemlich lange Vergangenheit zurück. Schon die alten Ägypter haben zum Ersatz von Schädeldachdefekten Edelmetall angewandt. PETRONIUS implantierte in den Organismus 1565 Metall. Zur Vereinigung der geschädigten Gefäßenden haben NITZE 1897 ein Elfenbeinrohr, WARDZ 1908 ein Gummirohr, TUFFIER ein paraffiniertes Silberrohr und CARREL Glas-, Aluminium- bzw. Goldrohre angewandt. Der Erfolg dieser Verfahren erwies sich aber nur als provisorisch. FRANKEL war der erste, der 1894 zum Ersatz von Schädeldachdefekten einen Kunststoff — und zwar Zelluloid — angewendet hat, aber auch diese Methode bot keine endgültige Lösung. Die Ursache der Erfolglosigkeit dieser ersten Versuche lag darin, daß die verwendeten Materien nicht gewebefreundlich waren.

Nach der Jahrhundertwende hat sich die Zahl der ohne Schädigung des Organismus anwendbaren, sog. »gewebefreundlichen« Substanzen bereits in bedeutendem Maße erhöht. Dies ist in erster Linie nicht einmal der stets zunehmenden Produktion der gewebefreundlichen Metalle und Metall-Legierungen zu verdanken, sondern vielmehr der sich in verhältnismäßig kurzer Zeit vollzogenen Verbreitung der gewebefreundlichen Kunsstoffe, welche der riesigen Entwicklung der chemischen und biochemischen Forschungen zufolge auf dem Markt erschienen.

Die gegenwärtig gebräuchlichen alloplastischen Materien können in zwei große Gruppen

eingeteilt werden: Metalle bzw. Metallegierungen und Kunststoffe. Das wichtigste Anwendungsbereich der Metalle ist die Knochenchirurgie, Metallnetze finden beim Verschluß von Bauchbrüchen eine Verwendung. Metallklammern sind Bestandteile von Magen-, Darm- und Gefäßnähmaschinen. Die Metalle werden entweder in unlegierter (z. B. Tantal) oder in legierter Form (z. B. Vitallium) verwendet. Die meist gebräuchlichen Metallegierungen sind die aus Eisen verfertigten rostfreien Stähle, die Nickel- und Chromhaltige Edelstahllegierung, das Chrom, Molibden und Kobalt enthaltende Vitallium und schließlich die Titannickel-Legierungen.

Auf dem Gebiet der Alloplastik nehmen die Kunststoffe allmählich die Stelle der früher allgemein angewandten Metalle ein. Dies ist mit den bekannten nachteiligen Eigenschaften der Metalle zu erklären: Einerseits sind einige von ihnen toxisch (Kupfer, Magnesium, Eisen, Aluminium, Bronze), anderseits melden sich bei ihrer Anwendung Korrosionsprobleme, außerdem erleiden einige Metalle eine Elektrolyse (Gold, Silber, Aluminium); hierzu kommt noch der hohe Preis gewisser Metalle und die Aufarbeitungsschwierigkeiten. In der Knochenchirurgie und zur Verfertigung verschiedener Exoprothesen gibt es aber trotzdem auch heute noch kein besseres Material als Metall.

Der bei der Herstellung angewandten Grundsubstanz entsprechend lassen sich die Kunststoffe in zwei große Gruppen — natürliche und künstliche Kunststoffe — einteilen. Unter die letzterwähnten gehören die bioplastischen Substanzen, deren Grundsubstanz aus natürlichem Eiweiß besteht, die aber in ihrer äußeren Erscheinung kunststoffartige Produkte sind.

Die Vorteile der Kunststoffe — gegenüber den Metallen — können im folgenden zusammengefaßt werden: niedrigeres spez. Gewicht, schlechtere Wärmeleitfähigkeit, dielektrische Eigenschaft, sie verursachen keine Metallose im Organismus; die meisten von ihnen verfügen über eine bedeutende Reiß-, Stoß- und Biegefestigkeit bzw. eine gute Färbbarkeit und tolerieren sogar die wiederholte Wärmesterilisierung; Transport und Speicherung der Kunststoffe sind einfach, ihr Preis ist niedrig.

Den hinsichtlich der Gewebe indifferenten, sich zu chirurgischen Zwecken eignenden Kunststoffen gegenüber werden laut Bornemisza (1960, 1963, 1964, 1968) folgende Anforderungen gestellt: Der Kunststoff darf keine Fremdkörperreaktion im Organismus auslösen, keine allergische Reaktion bzw. Tumorbildung verursachen, Sterilisierung und Bearbeitung sollen einfach sein. Zumeist wird gewünscht, daß der Kunststoff seine ursprüngliche Form bzw. seine mechanischen usw. Eigenschaften andauernd beibehalte, in anderen Fälle ist es jedoch vorteilhaft, wenn die alloplastische Substanz mit der Zeit resorbiert wird und sich die Resorptionsdauer bedarfmäßig regulieren läßt.

Die wichtigsten, bei der Auswahl der in den Organismus eingepflanzten, der ständigen Einwirkung der Gewebesäfte ausgesetzten, körperfremden, leblosen Implantate organischen oder unorganischen Ursprungs berücksichtigenden biologischen Anforderungen sind nach Auber und Antal (1962), daß das eingepflanzte Material keine Gewebeschädigung verursache, von chemischem Standpunkt aus widerstandsfähig sei und von physischem Standpunkt aus den Anforderungen der Inanspruchnahme entspreche.

Durch die körperfremden Substanzen wird — selbst wenn sie von gewebefreundlicher Natur sind — von ihrer Quantität und Qualität abhängig, im Organismus eine gewisse Umgebungsreaktion ausgelöst, wichtig ist nur, daß sie den Heilprozeß nicht hemmen und über eine geringe gewebereizende Wirkung verfügen.

Die weitläufige Verbreitung der alloplastischen Substanzen warf eine ganze Reihe theoretischer und praktischer Fragen auf. Obwohl die Ergebnisse der alloplastischen Methoden auf dem Gebiet der Gewebevereinigung bzw. des Gewebsersatzes als vorzüglich zu bezeichnen sind, gibt es immer noch zahlreiche ungelöste Probleme. Nach der Ansicht von Petrowskij und Wenediktow (1967) vermag die Alloplastik den Ersatz der verletzten bzw. zerstörten Gewebe gegenwärtig noch nicht ideal zu lösen, d. h., daß Mängel und Kontraindikationen auf diesem Gebiet keine Seltenheiten sind. Heinze hat in seiner 1955 erschienenen Mitteilung darauf hingewiesen, daß bisher noch keine, sämtlichen Gesichtspunkten, vor allem der Toleranz und der Belastungsfähigkeit der Gewebe entsprechende körperfremde Substanz entdeckt worden ist.

Im Laufe der vergangenen 15 Jahre erschienen sowohl in der einheimischen (Auber und Antal, 1962, Bagdy und Mitarb., 1963, Bornemisza, 1960, 1963, 1964, 1968, Csillag und Jellinek, 1958, Furka, 1967, Gyurkó, 1967, 1969; Kovács, 1959; Nemes, 1970; Sipos, 1956) wie auch in der ausländischen (Gottlob und Blümel, 1965, 1966, 1967, 1967; Gritsman, 1964, 1966; Gritsman und Mitarb., 1968; Häring und Mitarb., 1966; Heinze, 1955; Hudeman, 1959; Inou und Mitarb., 1962, 1965; Matsumoto und Mitarb., 1967, 1967, 1967, 1968; Ota und Mitarb., 1965; Petrowskij und Wenediktow, 1967) Literatur zahlreiche, die Problematik der Alloplastik behandelnde grundlegende oder zusammenfassende Mitteilungen. Im Laufe der Untersuchung alloplastischer Materien haben auch wir mehr als 400 Literaturangaben aufgearbeitet (Somogyvári, 1971).

Eigene Untersuchungen

Unlängst erschien eine unserer Mitteilungen über die Untersuchung der allo- und bioplastischen Substanzen. Das Ziel dieser Arbeit war die experimentelle und klinische Erprobung der anläßlich einiger chirurgischer Eingriffe in der letzten Zeit angewandten bzw. experimentell verwendeten alloplastischen Materien (Nähmaterial, Klebstoffe, zur Wundbedeckung dienende filmbildende sowie bioplastische Präparate) ferner die Analyse des Gewebeverhaltens dieser Substanzen. Aufgrund der Gegenüberstellung der klassischen und neuen chirurgischen Ersatz- bzw. Hilfsmaterien trachteten wir der humanmedizinischen und veterinärmedizinischen Chirurgie neue experimentelle Angaben und pathohistologische Beobachtungen zu liefern.

Im Laufe dieser Untersuchungen wurden bei 430 Versuchstieren (Pferd, Rind, Schwein, Hund, Kaninchen, Meerschweinchen und Albinoratte) Operationen durchgeführt, Beobachtungen ermittelt, außerdem wurden mit einigen alloplastischen Materien etwa 200 zum Krankengut der Klinik gehörende Tiere behandelt. Die Operationsergebnisse wurden mit Hilfe von zahlreichen Röntgenaufnahmen kontrolliert und das Sektionsmaterial der experimentellen Eingriffe anhand von etwa 5000 pathohistologischen Präparaten bewertet.

Unsere Untersuchungen sowie die die ausführliche Erläuterung derselben enthaltenden Mitteilungen umfassen die Vergleichsuntersuchungen der Polyester-Kunststoff- und der herkömmlichen Nähmaterialien (B. Kovács und Somogyvári, 1969, 1969, 1969), die Bewertung der bei den Magen-Darm-Nähmaschinen verwendeten Tantal-Metallnähte (Egry und Mitarb., 1969), die operative Erprobung des Histoacryl-Gewebeklebstoffes bei verschiedenen Eingriffen (Verdauungskanal: Egry und Somogyvári, 1970, 1971; Somogyvári und Egri, 1971; Blutgefäße: Dubecz und Mitarb., 1971, Szentgáli und Mitarb., 1969, 1971; Urogenitalorgane: Breier und Mitarb., 1971, 1971; Gallengänge: Somogyvári und Mitarb., 1972), die veterinärmedizinische Anwendung von Plastubol (zur Wundbedeckung dienendes synthetisches Präparat) (B. Kovács und Mitarb., 1969, 1970), die experimentelle Untersuchung eines fibrinhaltigen Wundstreupulvers (B. Kovács und Mitarb.. 1969, 1970), die Resorption der Bioplaste aus dem subkutanen Bindegewebe (B. Kovács und Mitarb., 1965, 1965) sowie aus der Markhöhle der Röhrenknochen Horváth und Somogyvári, 1971, Somogyvári und Horváth, 1971), die sich auf den Bioplast-Schwamm (Gerendás und Mitarb., 1966) und auf die nahtstärkende Bioplast-Knöpfe (B. Kovács und Mitarb., 1967) beziehenden Angaben und schließlich die Ergebnisse der zwecks Klärung der Antigeneigenschaften der Bioplaste vorgenommenen Untersuchungen (Horváth und Mitarb., 1969, 1969).

Folgerungen

Die im Laufe der Untersuchungen ermittelten Folgerungen lassen sich in folgendem zusammenfassen:

Das einheimische Polyester-Nahtmaterial (Rico-Verbandwerke) verfügt im Vergleich zu den bishergebräuchlichen Nahtmaterialien über manche vorteilhafte Eigenschaften: Es toleriert sogar mehrere Sterilisierungen im Autoklav ohne Schädigung, wegen seiner geringen Kapillarität ist die Drainagewirkung unbedeutend, die Dehnung ist geringer als die von Polyamid, die Reißfestigkeit dagegen ausgezeichnet; das Produkt kann mit den meisten allgemein gebräuchlichen Chemikalien behandelt werden, industrielle Verunreinigungen lassen sich leicht und restlos entfernen.

Der entsprechenden Reißfestigkeit und Knotenfestigkeit zufolge sichert das Polyester-Nahtmaterial selbst im Falle einer bedeutenden Spannung einen zuverläßlichen Wundverschluß, welcher Umstand sich in der veterinärmedizinischen Chirurgie als besonders vorteilhaft erwies.

Laut der Ergebnisse der histologischen Vergleichsuntersuchungen, wobei es sich um die gleichzeitige Prüfung der herkömmlichen Nähmaterialien (Catgut, Seidenfaden) handelte, waren die besten Ergebnisse in bezug auf das Polyester-Nahtmaterial zu erhalten. In der Umgebung dieser Nähte hörten die entzündlichen Prozesse rasch auf und auch die Reparationserscheinungen schritten unbehindert voran. Das im Laufe der Reparation gebildete Angio-Fibroblastgewebe drang in die Substanz der aus mehreren Fäden zusammengedrehten Nähte ein, teilte diese in Bündel, sodann in einzelne, alleinstehende Fäden; in den späteren Präparaten ließ sich bereits beobachten, daß das Gewebe die Fadensubstanz vollkommen umsponnen (organisiert) und in das anstelle des Eingriffs gebildete Narbengewebe eingebaut hat. In der Umgebung der kontrollhalber untersuchten klassischen Nähmaterien entwickelte sich in sämtlichen Fällen eine umfangreichere Gewebereaktion, außerdem waren sogar mehrere Wochen nach der Operation — obwohl sich die Fäden teilweise bereits abgekapselt haben — entzündliche Zellelemente vorzufinden, welche für die häufig beobachtbaren operativen Spätkomplikationen (Fadeneiterung, Außtoßen der Nähte, Fistelbildung) verantwortlich sind.

Die bei den menschlichen Operationen gebräuchlichen Magen-Darm-Nähmaschinen haben wir im Hundeexperiment, anläßlich von Magenresektionen und Darmanastomosen geprüft: Die angewandten Tantalklammern sicherten — im Vergleich zu den klassischen Nähmaterien — eine genauere Wundvereinigung und lösten eine geringere Gewebereaktion aus; diesen Umständen zufolge sind nach Anwendung von maschinellen Nähten die Wundheilung beschleunigt und die Reparationsvernarbung geringer.

Wie darauf die histologischen Untersuchungen hingewiesen haben, besteht zwischen den Reparationsprozessen der operativen Wunden, davon abhängig, ob maschinelle Nähte oder klassische Methoden angewandt worden

waren, ein wesentlicher Unterschied. In den mittels Nähmaschine vereinigten Operationswunden bildet sich in der Umgebung der Tantalklammer in Kürze ein definitiver Zustand aus (bindegewebige Abkapselung), während sich rund um die mit dem klassischen Nähmaterial vereinigten Wundränder noch in der postoperativen Spätphase eine entzündliche Reaktion abspielt und die Abkapselung mit der Bildung einer bedeutenden Bindegewebemenge einhergeht.

Das Problem der Gewebevereinigung kann, obwohl im Laufe der Zeit sowohl was die Qualität der Nähmaterien, wie auch was die Nahttechnik anbelangt, große Fortschritte zu verzeichnen sind, auch heute noch nicht als gelöst betrachtet werden. Die Entdeckung der Zyanoakrilate trug anscheinend dazu bei, daß die Gewebevereinigung ohne Naht, d. h. das Gewebekleben eine zugängliche Methode geworden ist. Heute wird bereits eine sich stets erweiternde Skala der Zyanoakrilat-Derivate hergestellt. In unseren Versuchen kam Butyl-2-zyanoakrilat — ein unter dem Namen Histoacryl in den Handelsverkehr gebrachtes Produkt der Firma BRAUN Melsungen — zur Anwendung. Die histologische Untersuchung der Gewebeklebungen verschiedener Organe hat es bestätigt, daß sich Histoacryl als gewebefreundliche Art verhält und langsam (im Verlauf von 5—7 Monaten) resorbiert wird. Die Umgebungsreaktion des in dünner Schicht aufgetragenen Klebstoffes ist gering, genauer gesagt geringer als die der bisher angewandten Nähmaterialien, so daß auch die Reparationsvernarbung verschwindend klein ist.

Die in den oben angeführten Mitteilungen beschriebene Darmanastomose-Operation ist eine neue, einfache, etwa eine 50%ige Zeitersparnis ermöglichende Methode. Ein, besonders vom Standpunkt der Asepsis aus, wesentlicher Vorteil des Verfahrens liegt darin, daß die Anlegung der Seit-zu-Seit-Anastomose durch eine kleine Inzisionsöffnung gelingt, d. h. daß sich die Eröffnung des Darmlumens auf einem größeren Abschnitt erübrigt.

Mit der in den diesbezüglichen Arbeiten beschriebenen Technik kann die zur Vereinigung der Blutgefäße dienende End-zu-End-Anastomose mit der Kombination von Richtungsnähten und Kleben angelegt werden. Die Anzahl der Richtungsnähte hängt von verschiedenen Faktoren — Maß der Spannung, Typ der Gefäße, Dicke der Gefäßwand, Weite der Gefäße und intravaskuläre Druckverhältnisse — ab. Der Verschluß der longitudinalen Inzisionen kann entweder mit Stütznähten und Kleben, oder nur mit Kleben oder durch Anlegung eines Venenflecks bzw. Fasziastreifens leicht, rasch und strikturfrei gelöst werden.

Bei Ureteroperationen sichert das biologische Kleben sowohl beim Verschluß der Ureterotomien, wie auch bei der End-zu-End-Vereinigung der Ureterstumpfe ein befriedigendes Resultat. Die Entwicklung der nach Anwendung der herkömmlichen Methoden in der postoperativen Phase auftretenden Komplikationen (Nahtinsuffizienz, Fistelbildung, Harninfiltration) kann durch das biologische Kleben vermieden werden. Die Vereinigung der Wundränder bei,

die Harnblasenwand betreffenden Eingriffen (Zystostomie, Resektion) läßt sich sogar unter ungünstigen Operationsverhältnissen (z. B. Cystitis haemorrhagica) durch kombinierte Anwendung von Stütznähten und Klebstoff, einfach lösen.

Zur chirurgischen Versorgung der extrahepatischen Gallengänge hat sich das biologische Kleben gut bewährt; der wesentliche Vorteil der Methode liegt darin, daß die Entwicklung von Gallensickerung-bedingter postoperativer Peritonitis, Gallenfisteln bzw. der Verschluß des Lumens der Gänge mit ihrer Hilfe zu vermeiden ist.

Laut unserer Erfahrungen hat sich Plastubol zur Bedeckung der mit Nähten vereinigten Operationswunden vorzüglich bewährt. Wie darauf die histologischen Untersuchungen hingewiesen haben, verlaufen Wundheilung und Kruspenbildung unter der Filmschicht ungestört.

Zur Bedeckung von größeren Hautdefekten, mit Nähten nicht vereinigbaren offenen Gewebeverletzungen bzw. sekundär heilenden Wunden kann Plastubol nicht oder nur mit gewissem Vorbehalt empfohlen werden. Bei der Behandlung eitriier Wunden, Verbrennungen II. und III. Grades sowie Hautplastiken ist Plastubol kontraindiziert. Die gemeinsame Anwendung von Wundstreupulvern und wundbedeckenden Spray-Präparaten ist nicht zweckmäßig.

Im Laufe der Erprobung des fibrinhaltigen Wundstreupulvers zeigten die klinischen und histologischen Untersuchungen, daß das unter dem Namen Fibrofurin in den Handel gebrachte Wundstreupulver (welches in einem Fibrin-Thrombin-Vehikel die antibakterielle Verbindung Furidin enthält) über mehrere vorteilhafte Eigenschaften verfügt: Es fördert die rasche Entwicklung des die Wundhöhle ausfüllenden Fibrinnetzes, beschleunigt seiner leukotaktischen Reizwirkung zufolge die Krustenbildung und die Entwicklung des Granulationsgewebes, außerdem schützt es die Wunde von der Infektion und fördert die Heilung der infizierten Wunden.

Unsere histologischen Untersuchungen ergaben, daß die Bioplaste von gewebefreundlicher Natur sind und verhältnismäßig rasch bzw. restlos resorbiert werden. Die Resorption der verschiedenen Präparate — aus dem Blutplasma von Mensch und Rind stammendes Fibrin und Albumin sowie Muskelmyosin — vollzieht sich unter identischen Gewebeerscheinungen, am frühesten wird Myosin resorbiert, sodann das Fibrin und zuletzt das Albumin. Resorptionsprozeß und -dauer werden durch die angewandte Preßtechnologie (plastifizierende Mittel, Druck- bzw. Temperaturwirkung) nicht beeinflußt, von der Dauer der Formaldehydvorbehandlung abhängig, läßt sich aber die Resorptionszeit beliebig regulieren.

Im Resorptionsprozeß spielen die Leukozyten, vor allem die neutrophilen Granulozyten, sowie die sich vermehrenden Histiozyten und Fibroblasten eine Rolle. Der Abbau und der Transport der abgebauten Substanzen ist das Ergeb-

nis der fermentativen (Lyse) und zellulären (Phagozytose) Prozesse. Die Begleiterscheinung der Resorption ist die Entwicklung der inneren zellreichen und äußeren faserreichen Reaktionszonen. Anstelle des resorbierten Bioplasts bleibt als Ergebnis der Reparation etwas Bindegewebe zurück.

Die Bioplast-Knöpfe eignen sich zur Verstärkung der Nähte. Wir haben diese Formstücke zum Verschluß der Bauchwand von Schweinen, Rindern und Pferden angewandt; mit Hilfe der in »U«-Form eingelegten lockernden Nähte und Bioplast-Knöpfe konnten auch erweiterte Bruchpforten verschlossen werden, die mit der klassischen Methode, ohne Rezidivgefahr nicht zu vereinigen gewesen wären.

Zur Ausfüllung von Wundhöhlen oder irgendeiner anderen Ursache zufolge enstandenen Höhlen hat sich der Bioplast-Schwamm gut bewährt. Laut der histologischen Ergebnisse dringen die Mesenchymzellen in die Schwammstruktur ein, und der Schwamm wird in kurzer Zeit resorbiert.

Im Laufe der Untersuchung der in die Markhöhle der Röhrenknochen angebrachten »Nägel« konnte festgestellt werden, daß die den Resorptionsprozeß begleitenden Erscheinungen dieselben sind wie die im Zusammenhang mit dem Abbau der in andere Gewebe des Organismus eingepflanzten Bioplaste beobachteten Vorgänge. Die Untersuchungen ergaben ferner, daß in der Gewebereaktion der sog. rasch resorbierbaren Bioplaste die Zellelemente bereits vom Anfang an eine intensive Tätigkeit ausüben, während im das sog. langsam resorbierende Bioplast umgebenden Angio-Fibroblastgewebe die Faserbildung in den Vordergrund tritt, so daß zu Beginn eine bindegewebige Demarkation entsteht. Dies bedeutet, daß es eine längere Zeit in Anspruch nimmt, bis die Substanz der langsam resorbierenden Bioplaste durch die Gewebesäfte und die Leukozyten in einen, zur Resorption geeigneten Zustand gebracht wird.

Die Bioplaste lösen im Organismus, als natürliche Eiweiße eine immunologische Reaktion aus. Laut unserer experimentellen Resultate enthalten die keiner vorangehenden Wärmebehandlung unterworfenen Bioplastpräparate die einzelnen Antigen-Determinanten des Fibrinmolekels in einem Zustand, der es ermöglicht, daß sie im artfremden Organismus eine Antikörperproduktion auslösen. Wie darauf die Untersuchungen hingewiesen haben, meldet sich diese Wirkung anläßlich der Anwendung wärmebehandelter Bioplaste nicht.

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CHANGES IN PLASMA CORTICOSTERONE LEVEL AND THEIR RELATIONSHIP TO THE SERUM VITELLIN FORMATION IN LAYING HEN

by

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It was demonstrated in a previous paper (KEMÉNY et al., 1966), with the aid of ^{3}H, that the rate of biosynthesis of corticosteroids in the adrenal cortex of the domestic fowl showed significant differences according to age. The rates of incorporation of ^{3}H-pregnenolon as precursor into corticosterone were as follow: 4.1 (day-old chicken), 3.0 (adult cock), 1.5 (laying hen) per cent, respectively. The result of this *in vitro* experiment indicated that the adrenals of the laying hen utilized considerably less labelled pregnenolon than those of chicken or cock. What appears to be the most likely explanation of the observed differences is that the biosynthesis of corticosteroids is inhibited during the laying cycle. The inhibition may be caused either by the decreased activity of enzymes responsible for synthesis or by the inadequate production of precursor. The latter assumption is supported by the findings of several authors (LASKOWSKI, 1935; HOSODA et al., 1955; MCINDOE, 1959; MUKHERJEE and BHOSE, 1968). These authors demonstrated that ostradiol inhibited the hepatic biosynthesis of cholesterol. On the other hand, the same hormone stimulated the synthesis of a special phospholipoprotein, named serum vitellin, in the liver.

As a step toward the understanding of the mechanism, further experiments were carried out to answer the question, whether is there any change in cortical cholesterol level as well as plasma corticosterone concentration when vitellin appears in the sera of laying hens. The cholesterol and corticosterone values were compared to those of non-laying hens and day-old chickens.

Materials and methods

Animals

The investigations were carried out on forty-five 5 to 7-day-old chickens, six pullets aged 4 months, two adult cocks and nineteen hens of 2 years old. Nine of the 19 were laying. At the time of investigation, an egg with calcified shell was present in the uterus and the ovaries had mature follicles in large quantity.

Collection and preparation of samples

The birds were sacrificed by the severance of the cervical blood vessels and heparinized blood samples were collected. After centrifugation, the blood plasma was used for hormone assay. Since at the accurate determination of

corticosterone at least 10—20 ml of plasma is needed, the blood samples of 15—155 to 7-day-old chickens were mixed and the hormone level was measured from this pool. Consequently, each value in this age group represented the mean of 15 chickens. The corticosterone concentration in the plasma of older birds was determined individually. After extermination, the adrenals were removed, weighed and homogenized with known volume of 0.9 per cent saline, then centrifuged at 2000 r. The supernatant was used for the determination of cholesterol concentration.

Analytical procedure

The corticosterone was extracted from plasma by the method of BUSH (1961) and the tetrazolium blue reaction was performed according to the description of CHEN et al. (1963). The optical density of formazan developed was read in Spectromom 360 typ. photometer at 560 mμ.

The aliquots of supernatant were assayed for cholesterol as described by BOUTWELL (1964). The colorimetric method measures the total (free + esterified) cholesterol concentration.

Serum vitellin was determined by the serological test of HOSODA et al. (1955). Adult rabbit was immunized with the plasma of laying hen: 1 ml of plasma was added to equal volume of Freud adjuvant and this mixture was injected subcutaneously in rabbit every seventh day for 4 weeks. On the 28th day, the rabbit was exterminated and the immune serum obtained was mixed with 1 : 10 diluted sera of cocks, laying and non-laying hens.

For the determination of serum proteins, electrophoretic analyses were carried out in acryl-amide gels by the method of ORNSTEIN (1964) and DAVIS (1964).

The data were evaluated for statistical significance by conventional methods ("*t* test").

Results

Table I presents the data of plasma corticosterone concentrations. The highest levels were found in young chickens. Lower values were observed in the plasma of pullets aged 4 months. The concentration of plasma corticosterone in non-laying hens was about one-third, in laying hens about one-fifth than that of young chickens. The differences according to age are statistically significant ($P < 0.01$).

Since it is known that the cholesterol is the precursor of corticosteroids, the changes of cholesterol concentration were also measured in the adrenals. The results shown in Table II indicate that no differences were observed between the cholesterol levels in adrenals of young chickens and non-laying hens.

Table I

Changes in plasma corticosterone concentration of domestic fowl according to age

5 to 7-day-old chicken	Pullet	Non-laying hen	Laying hen
Corticosterone (μg per 100 ml of plasma)			
18.0*	9.2	5.1	4.4
15.0*	8.0	4.4	4.6
13.0*	10.7	5.0	4.6
	8.2	5.8	1.2
	14.0	5.9	2.8
	8.5	6.2	1.3
		6.0	2.6
		6.3	3.0
		6.8	
		5.8	
Mean $\pm$ S.E. 15.0	9.7 $\pm$ 0.93	5.7 $\pm$ 0.24	3.1 $\pm$ 0.48

* The value was determined from the mixed blood samples of 15 – 15 chickens.

Table II

Cholesterol concentration of adrenal glands in 5 to 7-day-old chickens, non-laying and laying hens

Chicken	Non-laying hen	Laying hen
Cholesterol (mg per 100 mg of wet tissue)		
1.8	0.9	0.6
2.4	1.6	0.5
1.5	1.8	0.7
1.3	1.2	0.7
2.0	2.1	1.2
1.5	2.4	1.3
1.4	1.0	1.1
1.5	2.2	0.8
		0.8
Mean $\pm$ S.E. 1.7 $\pm$ 0.12	1.6 $\pm$ 0.20	0.8 $\pm$ 0.03

In contrast, the cholesterol concentration was significantly ($P < 0.01$) lower in laying hens than that either in young chickens or non-laying hens.

A serological test was used for detecting the presence of serum vitellin. There was no agglutination observed when the sera of cocks or non-laying hens

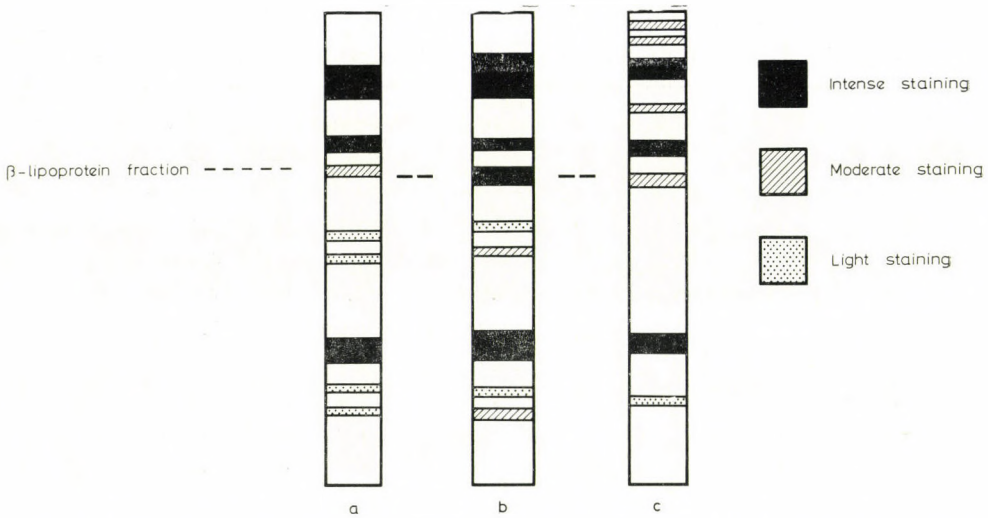

Fig. 1. Acryl-amid gel electrophoretic pattern of serum proteins *a*, Non-laying hen; *b*, Laying hen; *c*, Cock

were mixed with the immune sera. In contrast, in case of laying hens a highly positive (+++) vitellin response was found, indicating the presence of serum vitellin during oviposition.

Figure 1 shows the result of the acryl-amide gel electrophoresis of sera obtained. It can be seen that there was an increase in the β-globulin region of the laying hens compared to that of non-laying hens and cocks. This observation also confirms the presence of serum vitellin in the actively laying fowl, since vitellin is related to the β-lipoprotein fraction.

Discussion

Our earlier finding that the rate of incorporation of precursor is highest in the adrenal cortex of day-old chickens (KEMÉNY et al., 1966) has been confirmed in this study by measuring the data of circulating corticosterone. The elevated corticosterone level in the plasma of young chickens indicated a very active hormone synthesis. By advancing age, a gradual decrease in the plasma corticosterone concentration could be observed. On the other hand, with the exception of the laying hen, no changes were detected in cholesterol content of adrenals according to age. The mean levels of cholesterol in young chickens and non-laying hens (1.6—1.7 mg, 100 mg of wet tissue) seem to be sufficient to satisfy an adequate rate of hormone synthesis. It is most likely that the relatively low cholesterol content in adrenal cortex of the laying hen may have contributed to the decreased rate of hormone synthesis.

Pioneer studies by LASKOWSKI (1935) have shown that the serum of the laying hen is characterized by the presence of phosphoprotein, named serum

vitellin, because of the similarity of its chemical properties to the ovo-vitellin. Later it has been demonstrated by HOSODA et al. (1955) that the sera of chicks treated with diethylstilboestrol also contain serum vitellin. MCINDOE (1959) analyzed by electrophoretic method the sera of laying hens and chicks injected with oestrogens. In both sera a significant rise in the β-lipoprotein fraction was observed. This fraction which contains serum vitellin is characterized by a relatively low cholesterol and very high triglyceride content. MUKHERJEE and BHOSE (1968) reported that the rate of cholesterol biosynthesis in the liver decreased after oestradiol treatment. It was demonstrated that the site of this action was the decreased conversion of acetate to mevalonic acid, an important intermediate of cholesterol biosynthesis. It seems probable that the increased oestrogen release from the ovaries in the laying hen (MATHUR and COMMON, 1966) supresses the conversion of acetate to cholesterol in the adrenal gland too. Since cholesterol acts as precursor in the biosynthesis of corticoids, its reduced level in the laying hen (0.8 mg/100 mg) is insufficient to produce a plasma corticosterone concentration similar to that of the non-laying hen.

The decreased corticosterone level in the plasma of laying hens is an indicator of the hypofunction of the adrenal cortex. In this condition, a decreased resistance to a variety of environmental stressors, such as changes of temperature and humidity, etc. can be observed. JUDGE et al. (1968) demonstrated in mammals that the animals with inadequate production of corticoids were incapable of maintaining physiological homeostasis when subjected to mild stressors. WELTER et al. (1967) found that White Leghorn hens when exposed to temperature stress produced eggs with reduced shell thickness.

SUMMARY

A total of 72 domestic fowls, selected according to age and physiological status, were studied to follow the biosynthesis of corticoids. The groups of birds were as follow: 5 to 7-day-old chickens, pullets, aged 4 months, adult cocks, non-laying and laying hens. Plasma corticosterone, adrenal cholesterol and serum vitellin were determined.

It was observed that plasma corticosterone concentration gradually decreased by advancing age. The lowest levels were found in the plasma of laying hens. The concentration of cholesterol, precursor of corticosterone, was of the similar order and magnitude in the adrenals of different age groups studied. The only exception was the adrenal gland of the laying hen, where the level of cholesterol was about half than that of the other groups ($P < 0.01$). The presence of a phosphoprotein, named serum vitellin, was also characteristic of the laying hens.

The decreased plasma corticosterone concentration in laying hens is the indicator of stress condition. The decreased rate of corticosterone biosynthesis can be explained with the reduced level of precursor cholesterol in the adrenal cortex. It seems probable that the inhibitory effect of oestrogens is responsible for the inadequate cholesterol production in the laying hen.

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BIBLIOGRAPHIA

ELLIS, P. LEONARD: *Orthopedic Surgery of the Dog and Cat.* 2nd Edition. B. Saunders Company, Philadelphia, London, Toronto, 1971. Bp.

The four parts and eighteen chapters of this book cover all aspects relating to the surgery of orthopedic disorders. The first part (four chapters) goes over the fundamental ground, dealing with the anatomy of the skeleton, the normal course of bone-fracture repair, and the materials and equipment employed in orthopedic operations. The techniques of dressing and fixing limbs, including the various types of splints and pins and the manner in which they are applied, are discussed next, while the part ends with a consideration of prostheses of the femoral head and detailed comments on internal and external modes of fixation.

The subject of the second part (three chapters) is the surgical treatment of fractures. Especially ample space is devoted to an account of the different forms of femoral fracture, though this is not to the detriment of the coverage on handling of fractures to the pelvis, spine, skull and mandibula.

The third part (three chapters) is an account of luxations. Detailed descriptions are given of various techniques of treating luxation of the hip, but equal space is devoted to patellar luxations and there are plentiful data on luxations of the mandibula and vertebral spondyles.

The remaining miscellany of orthiopedic disorders receives due attention in the final part. First to be discussed are traumas to soft tissues that are concomitant with bone-fracture (e. g. lesions of nerves, blood vessels, tendons) and the consequent need to pay appropriate surgical attention to them. A separate chapter reviews techniques of limb and digit amputation, while the chapter on disorders of the vertebral disks is outstanding and the detailed synopsis of hip dysplasia particularly topical. The last chapters deal with osteochondritis, ulnar dysplasia, osteochondritis dissecans and osteoarthropathia.

Special mention should be made of the 286 excellently reproduced illustrations which supplement the text and of the exceedingly useful summaries of literary data closing each chapter. The book should prove a valuable addition to the bookcase of the general veterinary surgeon and is an indispensable reference work for all concerned with small animal orthopedics.

L. TAMÁS

PINNIGER, R. S. (ed.): *Jones's Animal Nursing.* Pergamon Press, Oxford—New York—Toronto—Sydney—Braunschweig 1972. 471 pages, 127 illustrations.

The amazing affection of British people towards their pets is a matter of common knowledge although a pet population explosion has recently been noted also in some other countries. The love of animals is well reflected in high standard requirements regarding the treatment of animals in the approved veterinary hospitals throughout the United Kingdom. Some ten years ago, the Council of the Royal College of Veterinary Surgeons (RCVS) introduced a scheme for the recruitment, training and registration of Animal Nursing Auxiliaries (ANA), who would be employed by veterinary surgeons mainly in the small animal practice. The ANA is not allowed to carry out practice of veterinary medicine and surgery but is expected to do nursing of animals under the supervision of a veterinary surgeon or practitioner, to make the preparations for operations, to assist with anaesthesia, side-room tests, radiography, etc.

This amplified second edition of a first highly successful work covers the syllabus of subjects in ANA examination courses, thus it is regarded as most comprehensive text and reference book for trainees who wish to pass examinations to qualify for Registered Animal

Nursing Auxiliary (RANA). The book combines the work of twenty distinguished authors. It is divided into ten chapters covering a fairly wide range of topics on small animals, mainly on dogs, cats, cage birds and other small animal species, in a depth necessary for RANA.

The first chapter of this book on nursing deals with the anatomy and physiology stressing the relationship and interdependence of these two disciplines. The chapter is a fine example of conveying many informations in a nutshell and so enabling the students to understand the normal structure and function of the animal body.

Chapter two devoted to principles of animal management, hygiene and feeding gives useful advice on the restraint and handling of small animals, and offers a basic knowledge on the housing, nutrition, administration of medicaments to patients in the small animal practice. A subchapter on genetics and animal breeding is also included; the latter has a rather academic scope.

The third chapter discusses the first aid. The treatment of haemorrhage, wounds fractures, dislocations, sprains, strains, loss of consciousness, poisoning, etc. are covered in this section.

Chapter four presents a guide line how to run a diagnostic laboratory and describes diagnostic aids, methods of simple routine laboratory tests on the blood, urine and faecal samples. Unfortunately, the quality of the few microphotographs included in this chapter are not up to the general high standard of other illustrations.

In the next three most extensive chapters, the theory and practice of medical (Chapter 5), surgical (Chapter 6), and obstetrical as well as paediatric nursing (Chapter 7) is dealt with. In addition to some more general subchapters, such as dispensing, administration of various medicines, anaesthesia, general care of in-patients, post-operative and post-partum activity, etc., an extensive cover is given to the specific duties of RANA in cases of most common diseases or other conditions. All necessary instructions are incorporated in the text well representing the advance in small animal practice, still the compendium does not go beyond the needs of the auxiliaries.

The eighth chapter is concerned with radiography. Although the adjustment of the X-ray apparatus and the exposure of the film is usually carried out by the veterinary surgeons, the nursing staff may be called on to assist in this work, to process the exposed film and later to care and file the radiographs. How to do this work efficiently is described in this chapter.

In the ninth chapter, the legal position of RANA under the Veterinary Surgeons Act of 1966 is summarized, and chapter ten gives a short cover to the veterinary profession, including its history, the veterinary training, the veterinary surgery as a career, veterinary hospitals, etc.

For students who may wish to get information in a greater depth book references are provided at the end of some chapters. Though this book on nursing is comprehensive indeed, the reviewer wonders if it were worth of consideration to amplify the next edition by adding one more chapter on the recognition of the various breeds of species which the auxiliary is bound to meet in the small animal practice.

The book is a storehouse of valuable practical informations for ANA trainees, and may also be useful for veterinary students attending the practice. As a reference book, it may be of interest for postgraduate veterinary surgeons, although it is unlikely the practising veterinary surgeon who will gain much from it. Some selected chapters may deserve attention of the breeders of small animals, pet-shop personnel, and owners of kennels and catteries.

I. VARGA

HOFMANN, W.: *Entzündliche Erkrankungen des Myokard der Tiere.* Ferdinand Enke Verlag. Stuttgart, 1971. 176 pages, 69 illustrations. DM 49.80

Myocardial disease is a subject to which veterinary science devoted rather scant attention until quite recently, yet its claims for special interest are considerable, as it is manifested in no small measure as an economic problem in modern animal husbandry. HOFMANN has therefore done us a very valuable service by producing this exceptionally absorbing monograph. His work basically falls into two parts, on the one hand thoroughly covering the literature relating to the incidence of myocarditis, and on the other presenting findings from a series of histological investigations on hearts from 250 animals of 14 species that had died from various causes, as well as from experiments conducted on white mice with foot-and-mouth disease and rabies viruses to study myocarditis of viral origin.

The basis for the detailed descriptions of the different myocardial lesions is a well-arranged table summarizing the various forms of inflammatory processes in this tissue. As

classification of the changes is made for nosological convenience, this means that comprehensive pictures can be built up of myocarditides of bacterial, viral, fungal or parasitic causation, and also those arising in consequence of toxicosis or allergy or appearing as concomitant phenomena. Where HOFMANN can give an account of his own work in the the course of his masterly summary of the literature, he assesses it in detail against the available evidences and does not hesitate to commit himself to standpoints on disputed questions. He sometimes finds it useful and even necessary to include insights that have been gained from human medicine, so the reader also acquires a view of approaches from the angle of comparative pathology. Of interest in this connection is the particular emphasis laid on the significance of oedema of the hypolemmal cavity, the interstitial spaces, capillary endothelium and the cell nucleus in the pathogenesis of virally caused myocarditis.

One topic to which HOFMANN pays special attention is the elucidation of the nature of the muscular nuclei with linear arrangement and Anitschkow's cells. He convincingly resolves the long-debated question of whether Anitschkow's cells are of myogenic or mesenchymogenic origin by arguing that in fact two cell forms are concerned. His conclusion is that the cells in muscle fibre correspond to myocytes with altered nuclei, whereas Anitschkow's cells of the interstitium are essentially histiocytes.

This work, with its 69 excellent, well-chosen light and electron micrographs, can be recomended to anyone involved in pathological research, because no more succinct yet still comprehensive survey of myocarditis in animals is known to the reviewer.

A. KARDEVAN

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VARIA

THE FIRST ISSUE OF A NEW QUARTERLY PERIODICAL

WORLD ANIMAL REVIEW,

has recently been published by the Food and Agriculture Organization of the United Nations.

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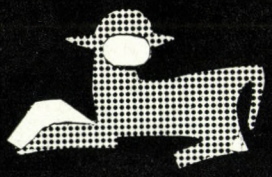

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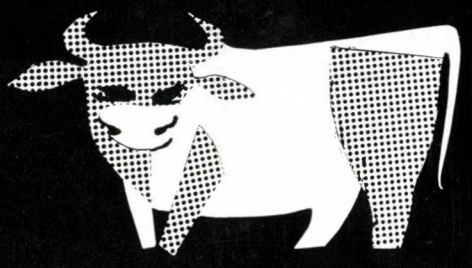

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ACTA VETERINARIA

ТОМ 22—ВЫП. 3

РЕЗЮМЕ

EIMERIA MRIGAI SP. N. (EIMERIIDAE: SPOROZOA) ИЗ ANTELOPA CERVICAPRA

Б. П. ПАНДЭ, П. П. С. ЧАУЭН, Б. Б. БАТИЯ и Г. С. АРОРА

При изучении содержимого прямой кишки **Antelope cervicapra** обнаружены характерные ооцисты с шапочкой, которые на основании морфологических признаков и характера споруляции, дальше, морфологии ранних гаметоцитов в гистологических срезах авторы выделили в новый вид **Eimeria mrigai sp. n.** Этот вид хорошо отличим от близких ему видов из диких **Cervidae** и **Bovidae** и домашних жвачных. Это третий вид рода **Eimeria**, описанный из **Antelope cervicapra.**

НОВЫЙ РОД И ВИД КРИПТОСПОРИДИДНЫХ КОКЦИДИЙ ИЗ ИНДИИ

Б. П. ПАНДЭ, Б. Б. БАТИЯ и П. П. С. ЧАУЭН

При вскрытии 4-х щенят 13—15-дневного возраста в содержимом прямой кишки, соскобах из тощей кишки и срезах той же кишки с участков с гиперимией обнаружены ооцисты, напоминающие представителей **Cryptosporidium** (аспоровые, тетразоичные), локализация которых однако была вне клеток. Поскольку типичные ооцисты обнаруживались и в мазках и срезах тех же животных, авторы учли обоснованным выделить для этих ооцист новый род **Hoareosporidium**, который на основании вышесказанного отличается от рода **Cryptosporidium.** Описываются разные эндогенные стадии развития этого нового вида — **H. pellerdyi sp.n.**

ИЗУЧЕНИЕ СНАБЖЕННОСТИ ПОГОЛОВЬЕВ ВЕНГЕРСКОГО КРУПНОГО РОГАТОГО СКОТА МАГНИЕМ

ДЬ. ТЁЛДЕШИ, ДЬ. БАРОЧАИ и И. ХОРВАТ

Изучался общий образец мочи от 8—10 животных из 775 стад крупного рогатого скота на содержание магния. Средний показатель магния на государство равнялось 491 **ppm**, он же отдельных комитатов — 452 **ppm.** На основании пробы Диксона среди показателей нет с заметной разницей. Нет корреляции в территориальном распределении кислых почв и концентрации **Mg** в моче крупного рогатого скота. Очень строгий нижний предел концентрации — 200 **ppm** — при повторных исследованиях не обнаружен только в 0,9%-ов хозяйств. Из этого можно заключить, что в минеральные кормодобавки для крупного рогатого скота не нужно добавлять в условиях Венгрии магния. Очень редко наблюдаемый весенний недостаток в магнии можно прекратить мероприятиями на месте.

РОЛЬ PSEUDOMONAS AERUGINOSA В ХРОНИЧЕСКИХ МАСТИТАХ АКТИНОМИКОТИЧЕСКОГО ХАРАКТЕРА У КОРОВ

И. САЗАДОШ и И. КАДАШ

За время 4-х лет (1968—1971) среди убойных и вынужденно убитых коров обнаружено 19 случаев хронического мастита, которые в гистологических срезах показывали актиномикотический характер. При одновременном бактериологическом изучении вымян (гной абсцессов, стенки абсцессов, соединительная ткань между абсцессами, кажущаяся неповрежденной железистая ткань) изолированы чистые культуры **Pseudomonas aeruginosa.**

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ВЕНГЕРСКИХ И ЗАРУБЕЖНЫХ ОСОБОГО СЕРОТИПА РОЖИСТЫХ ШТАММОВ

ДЬ. КУЧЕРА

Автором проведено сравнительное серологическое изучение 20 зарубежных и 25 венгерских типовых рожистых штаммов при помощи пробы двойной агар-жель-диффузии и преципитации при использовании автоклавированного антигена.

Данные изучения показали, что в типизации штаммов **Erysipelothrix rhusiopathiae** из разных уголков мира, имеется не малая путаница: оказывается, что штаммы той же метки отличаются серотипом, или штаммы, по-разному обозначенные, одного серотипа. Так, штаммы Муразе, отмеченные как C, D не соответствуют штаммам C, D Хойнера, но тождественны с штаммами II. (J) и I. (1) Кастро и с штаммами L, M Кучера. Штамм Муразе E не соответствует штамму E Кучера, но дает перекрестную реакцию со штаммом H Ювалд. Штаммы Муразе F и Кучера O представляют один серотип, подобно Кастро III (K) и Кучера I.

Обоснованным является отличать следующие изученные серотипы: A, B (Дедье); A_2, B_2, C, D (Хойнер); P (Трущински); E, G, J, K и без метки (Кучера).

Кроме этого автор изолировал из миндалин здоровой свиньи рожистый штамм нового серотипа, отличающийся от известных серотипов.

Ради прекращения этой путаницы назрело время унификации серологического типового обозначения рожистых штаммов. Учитывая последовательность описания отдельных серотипов (если это возможно), автор рекомендует отличать их не буквами, а арабскими цифрами пока от 1 до 15. Отличие штаммов того же серотипа в антигенной структуре рекомендуется отмечать малыми буквами. Серологическую принадлежность рожистых штаммов, не обладающих типово-специфическим антигеном и, таким образом, не вызывающих образования антител в кролике даже против себя, согласно предложению можно бы и в дальнейшем обозначать буквой N. Эта система обозначения рожистых серотипов обеспечивает удобную регистрацию для новых, в будущем обнаруженных серотипов.

Согласно сказанному серологическая типизация бациллов рожи свиней показывает следующую картину: тип 1a = A360 (A, Дедье); тип 1b = EW2 (A_2, Хойнер); тип 2a = B4 (B, Дедье); тип 2b = NF_4 (B_2, Хойнер); тип 3 = Витлинг (C, Хойнер); тип 4 = Доггершабе (D, Хойнер); тип 5 = P—190 (C, Муразе); тип 6 = P-32 (D, Муразе); тип 7 = P-43 (E, Муразе); тип 8 = P-92 (F, Муразе); тип 9 = kaparék (E, Кучера); тип 10 = P (P, Трущиньски); тип 11 = = IV 12/8 (G, Кучера); тип 12 = 4 PR (S4). (III или K, Кастро); тип 13 = Pécs-56 (J, Кучера); тип I4 = Iszap-4 (K, Кучера); тип I5 Pécs-3596 (без метки, Кучера).

ПРОСТОЙ, ТАК НАЗЫВАЕМЫЙ МИКРОТЕСТ ДЛЯ ВЫЯВЛЕНИЯ ВИРУСНЕЙТРАЛИЗИРУЮЩИХ АНТИТЕЛ И ОПРЕДЕЛЕНИЯ ЗНАЧЕНИЯ ТИТРА ВИРУСОВ

КИШАРИ Я. и БАРТА А.

Авторами разработан очень быстрый и дешевый микрометод выявления вируснейтрализирующих антител на пластмассовой пластинке Такачи. Однослойные тканевые культуры в U-образных выемках пластинки оказались удобными для культивации вирусов и оценки вирусом вызванных цитопатогенных изменений при помощи инвертного микроскопа. При помощи микрометода при малой затрате труда и средств можно определить содержание антител в очень большом количестве образцов крови.

ОБНАРУЖИВАЕМОСТЬ ВИРУСА ИНФЛЮЭНЦЫ А СРЕДИ УТОК ВЕНГРИИ
(Типы 3, 4 и 6 вируса птичьей инфлюэнцы)

Я. ТАНИ

Из уток восточной Венгрии изолировано 5 штаммов вируса птичьей инфлюэнцы: два из утят, три из взрослых птиц вне сезона кладки. У утят наблюдались респираторные симптомы, а при вскрытии наблюдались фибринозное воспаление воздухоносных мешков, серозит и синузит. Среди взрослых уток симптомы наблюдались только спорадически.

Изолированных пять штамма принадлежат к трем серологическим типам:

Тип 3—4	Duck (Hungary) 2/70
	Duck (Hungary) 71
Тип 4	Duck (Hungary) 3/70
	Duck (Hungary) 4/70
Тип 6	Duck (Hungary) 7/70

Штамм типа 6 содержит нейраминидазный компонент, идентичный таковому вируса человечьей инфлюэнцы типа A_2.

Экспериментами по заражению доказано, что к вдум среди пяти штаммов восприимчивы цыплята, молодые цесарки и индюшата, но заражение и клиническое проявление болезни зависит не только от вида птицы, а от наличия специальных предрасполагающих факторов, в первую очередь от условий кормления и содержания.

ПОЛИМОРФИЗМ БЕТА-ЛАКТОГЛОБУЛИНА И ИММУНОГЛОБУЛИНА МОЛОКА ЖЕНЩИНЫ

И. ХОРВАТ, П. ШОШ и МАРИЯ КАРДОШ

Электрофорезом на крахмальном желе авторами изучался полиморфизм бета-глобулина и иммуноглобулинов молока женщины. Белки отождествлялись согласно Шеферу (1951) и Загер (1959). В бета-лактоглобулине кроме двух гомозиготных полос (А и В) выявлена и комбинация АВ. Предполагают, что существует вариант С, но поскольку это редкий вариант, в относительно небольшом количестве образцов молока его не обнаружили. Выявлен и полиморфизм иммуноглобулинов. Три, разной скоростью поступающие гомозиготы обозначены буквами А, В и С; кроме этих обнаружены еще две гетерозиготные — АС и ВС — комбинации. Комбинация АВ не обнаружена.

Расшифровка генетического фона полиморфизма изученных белков — из-за невозможности одновременного изучения образцов молока материнских—дочерних пар — ограничивалась на сравнение с подобным полиморфизмом белков молока коровы.

ИЗМЕНЕНИЯ СТЕНОК КРОВЕНОСНЫХ СОСУДОВ МОЗГА СВИНЬИ, СОПРОВОЖДАЮЩИЕСЯ КЛИНИЧЕСКИМИ СИМПТОМАМИ (ЦЕРЕБРОСПИНАЛЬНАЯ АНГИОПАТИЯ ХАРДИНГА)

А. СЕКИ и И. САБО

В центральной нервной системе племенных свиноматок и 8—12-месячных откормочных свиней авторами обнаружены изменения стенки сосудов, напоминающие **periarteriitis nodosa**, которые сопровождались клиническими признаками. Заболевание регистрировалось спорадически, но в некоторых пологовьях за время 2—16 месяцев все-таки обусловливали значительный отход (8—49) взрослых свиней. Болезнь после нескольконедельного или недельного течения при постепенном обострении признаков или переходном улучшении состояния заканчивалась обычно гибелью или вынужденным убоем животного.

Это изменение стенки сосудов центральной нервной системы авторы включают в группу коллагенных заболеваний и их истосчником считают острое воспаление на аллерги ческом фоне.

Изменения характеризуются фибринозной, клеточной инфильтрацией стенки сосудов, фибриноидным перерождением и некрозом интимы и медии, повреждением или распадом эластических волокон или хроническими склеротическими процессами. Альтеративные изменения стенки сосудов часто сопровождаются тромбозом, сужением или закупоркой просвета. Обычно повреждаются малые артериолы и мышечного типа артерии. Нет определенной локализации изменений; они обнаруживаются в первую очередь в артериях лептоменинкса головного и спинного мозгов, в головном мозгу и реже в спинном мозгу. Кроме этого изменения обнаруживаются в продолговатом мозгу, в мосту, в среднем мозгу, перед хиазмой и после нее, обычно у основания мозга.

При тяжелых случаях в окресности изменившихся сосудов наблюдаются разной степени регрессивные изменения нервных клеток, прорежение ткани мозга, на территории **corpus striatum** и **capsula interna** и **externa** имеется отек, появляются полости, наполненные серозной или студенистой массой. Процессы в мозгу иногда сопровождаются серозной или клеточной инфильтрацией мягкой мозговой оболочки.

Аорта и кровеносные сосуды других органов (селезенки, печени, почек и сердца) изученных свиней подобных изменений не показывали.

ИЗУЧЕНИЕ ТОКСИЧЕСКОГО ЭФФЕКТА ЭНТЕРОКОККОВЫХ ШТАММОВ, ВЫЗЫВАЮЩИХ АЛИМЕНТАРНОЕ ОТРАВЛЕНИЕ

Ш. ПУСТАИ, Ф. ВЕТЕШИ и Р.-нэ ХОХ

Спорной является роль стрептококков серологической группы D в этиологии алиментарных отравлений. В раньших экспериментах авторы наблюдали токсическое действие одной части энтерококковых штаммов, изолированных без отбора из разных видов пищевых продуктов, при внутрибрюшном введении экспериментальным мышам. В настоящей работе изучалось токсическое действие энтерококковых штаммов на мышах, изолированных в случаях алиментарных отравлений.

Среди 19 стрептококковых штаммов D, вводившихся внутрибрюшно мышам, только два происходили из таких случаев, когда патогенные микробы удалось изолировать как из мышцы, так и из желудочной полоскательной жидкости больного. Среди 19 штаммов, употреблявшихся для прививки, гистологически патогенность доказана только у 4-х стрептококковых штаммов D; печеночные клетки экспериментальных мышей показывали регрессивные изменения.

13 стрептококковых штаммов D вызывали то слабое, то тяжелое заболевание, иногда даже гибель среди экспериментальных мышей, но не вызывали изменений, указывающих на токсический эффект; 2 штамма были непатогенными для мышей.

На основании своих результатов авторы подчеркивают, что при подозрении на отравление энтерококками — если это возможно — необходимо определить биохимические свойства и тип фага как из пищи, так и из конзумента изолированных стрептококковых штаммов. Это обосновано уже и потому, ибо микробы **Str. faecalis** и **faecium** часто обнаруживаются вместе в пробах, но алиментарное отравление вызывает обычно **Str. faecalis**.

ПРОБЛЕМЫ АЛЛОПЛАСТИКИ

К. ШОМОДЬВАРИ

Определяется понятие аллопластики, группируются современные аллопластические материалы, обсуждаются факторы, тормозящие широкое распространение металлических аллопластических веществ и выгодные свойства пластмасс. Приводятся требования, предъявляемые к «тканедружным» материалам.

Собственные и в соавторстве выполненные исследования охватывают данные экспериментов на 430 экспериментальных и 200 животных, лечившихся на клинике. В суммарном резюмировании данных исследований указывается на свойства швейных нитей из полиэстера и благоприятные гистологические результаты при употреблении машинных швов из танталовых скобок. Приводится опыт употребления гистоакрилового тканевого склеивающего материала при операциях на желудке, кишках, кровеносных сосудах, мочеполовых сосудах, желчном проходе. Оценивается возможность применения

в ветеринарной хирургии образующего фильм материала — Пластубола. Приводятся полезные свойства фибрин содержащих дустов на раны. Обсуждаются данные исследований по поведению и рассасыванию в тканях. Наконец дискутируется об антигенных свойствах биопластов.

ИЗМЕНЕНИЯ КОНЦЕНТРАЦИИ КОРТИКОСТЕРОНА В ПЛАЗМЕ И ЕГО ВЗАИМОСВЯЗЬ С ОБРАЗОВАНИЕМ ВИТЕЛЛИНА В СЫВОРОТКЕ НЕСУШЕК

МАРИЯ КОЗМА и А. КЕМЕНЬ

На 72 головах куры домашней, подобранных согласно весу и физиологическому состоянию, изучалась последовательность биосинтеза кортикоидов. Животных разбили на следующие группы: цыплята 5—7-дневного возраста, молодняк 4-месячного возраста, взрослые петухи и кладущие и некладующие несушки. Определялись: сывороточный кортикостерон, холестерол надпочников и сывороточный вителлин.

Обнаружено, что концентрация кортикостерона в сыворотке с возрастом постепенно уменьшается. Наименьше кортикостерона выявлено в сыворотке кладущих несушек. Концентрация холестерола в надпочечниках, предшественника кортикостерона, в разных возрастных группах показывала подобный характер изменения. Исключением явились только надпочечники кладущих несушек, где уровень холестерола не превышал даже половины его уровня других групп ($P < 0{,}01$). Наличие одного фосфорпротеина, именуемого сывороточным вителлином, было тоже характерным у кладущих несушек.

Понижение концентрации кортизона в сыворотке кладущих несушек является показателем стрессного состояния. Падение темпа в биосинтезе кортикостерона можно объяснить понижением уровня его предшественника — холестерола — в коре надпочечников. Не исключено, что ответственным за несоответственную продукцию холестерола у несушек является тормозящий эффект эстрогенов.

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THE GUT BACTERIAL FLORA OF HEALTHY EARLY WEANED PIGLETS, WITH SPECIAL REGARD TO FACTORS INFLUENCING ITS COMPOSITION

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Many studies have been made of the modifications in the intestinal flora of young pigs brought about by certain factors, and above all by early weaning, a practice that is now becoming widely adopted (*e.g.* Soyka, Lloyd and Sweeney, 1960; Smith, 1961, 1965; Tadd and Hurst, 1961; Pesti, 1962a,b; Kenworthy and Crabb, 1963).

Similar examinations have been conducted in this institute, though we have chosen to pursue the alterations of the gut flora in early-weaned, clinically healthy, rather than in diseased animals, in order to fill the gaps in our knowledge about their relation to weaning at different ages.

Material and methods

The litters farrowed by five Large White sows from a breeder herd were observed until the piglets reached 54 days of age. A total of 36 of the piglets were killed for complete bacteriological assay of the intestinal tract. Three of the five sows (A—C) were removed for farrowing to the climatic chambers of the institute, in which an optimal room temperature of 16—18 °C and a relative humidity of 70—80% were provided. In the creep, the temperature was raised to 26—32 °C and air flow maintained at 0.1—0.2 m/sec until weaning. After weaning, the infrared lamps were no longer used, and the animals were kept at a room temperature of 24—26 °C, with a relative humidity of 60—75%. Air quality was satisfactory throughout the experimental period.

The offspring of the remaining two sows (D, E), a total of 18 piglets, were farrowed and reared on the farm, at a farrowing pen temperature of 10—15 °C, a relative humidity of 85—90% and exposed to a higher air flow than was permitted in the climatic chambers, viz. under essentially poorer environmental conditions than the first three groups.

Piglets of litters A, B and C were weaned at the ages of 14, 21 and 28 days, respectively, the farm-reared piglets at 21 and 28 days. Animals of

all groups were thereafter fed the same bacteriologically checked pig starter formula (PHYLAXIA). No prophylactic or therapeutic medication was administered during the experimental period.

The sequence of exterminations for bacteriological study is shown in Table I. (Animals marked with "*" contracted *E. coli* infections during the experiment and the observations made on them are not considered here; the changes in their intestinal flora corresponded broadly with those described elsewhere in the literature). The animals were stunned and killed by bleeding, then following abdominal incision their stomach and intestines were removed under conditions of surgical sterility. Before the bowels were excised, both ends as well as the centre of the stomach were ligated to separate the cardiac and pyloric regions.

Table I

Experimental arrangement

	Groups	Age of weaning (days)	Age of exterminated pigs (days)									
Climatic chambers	A	14	10	13	15	18	24	44				
	B	21	17	20	22	25	31	44				
	C	28	24	27	29	32	34	44				
Farm	D	21	17	20	22*	25*	28*	31	34	36	39	44
	E	28	24	27	29	32	34	36*	40*	44*		

Samples of gastro-intestinal contents, collected so as to contain about one-quarter mucosal scrapings, were taken from the cardiac and pyloric regions of the stomach, duodenum, jejunum, anterior and posterior ileum, caecum, colon and rectum, and immediately transferred to appropriate bacteriological media; pH was determined with a Calmann—Grahnert (Dresden) MV-11 electric pH-meter. One gram of each sample was ground in 9 ml Mitsouka buffer (MITSOUKA, 1965), the suspension diluted in serial tenfold steps and 0.1 ml from each dilution step was inoculated into the different media. The media used, the times and conditions of incubation, and the primary isolates obtained are summarized in Table II. Anaerobic culturing conditions were furnished in glass jars, utilizing NaOH and pyrogallol, following the technique of PELZAR (1957). Readings of cultures were complemented by microscopic examination and, occasionally by further culturing.

Numerical data for bacterial counts are presented as arithmetic means calculated from the base 10 logarithms of the counts of each component. Statistical analyses of the correlation between bacterial counts and the different factors were checked by Student's t-test.

Table II

The culture media, primarily isolated bacteria and incubation methods

Culture media	Isolated bacteria	Incubation's		
		condition	temp.	tim
Nutrient agar	Aerobic sporeforming	aerobic	37 °C	48 h
Nutrient blood agar	haemolytic coli	aerobic	37 °C	48 h
Crystalviolet-lactose brom-thymolblue agar	coliforms	aerobic	37 °C	24 h
Edward's agar (modified by NYIREDY, SZABÓ 1968)	D streptococci	aerobic	37 °C	48 h
AGAT medium (RAIBAUD et al. 1961)	D streptococci	aerobic	37 °C	48 h
Tomato juice agar	lactobacilli	aerobic	37 °C	48 h
SA medium (by TAKÁCS 1968) inoculated with heat treatment samples (at 80 °C; 10′)	spores of clostridia	anaerobic	39 °C	72 h
SA medium with 1000 γ/ml D-cycloserine tartrate (FÜZI et al. 1968)	clostridia (vegetative forms)	anaerobic	39 °C	72 h
RCM agar (FULLER—LEV, 1964)	bacteroides, clostridia	anaerobic	37 °C	4 days

Results

Though the stomach ligation technique permitted separate sampling of the pyloric and cardiac regions, the results presented in Table III reveal no significant differences between the bacterial flora and pH in the two areas. It should be noted, however, that the variance coefficients were fairly high

Table III

Bacterial flora in the oesophageal and pyloric parts of the stomach

log 10/g	Stomach I	Stomach II
Lactobacilli	7.74	7.18
Streptococci	5.93	5.44
Coliforms	2.96	2.39
Clostridia	3.03	3.06
Clostridium spores	2.75	2.50
Aerobic sporeformers	4.40	4.46
pH	4.18	3.85

($k = 3$--30%). Since the flora components had originally been expected to differ considerably, especially with regard to counts at different pHs, an attempt was made to identify whether, among the 60 samples available, there was any indication of an association of particular bacterial flora compositions with pHs (Fig. 1). The histograms indeed suggest a clear inhibitory effect on coliforms in the pH range 0.5—2.5, but such conditions were only encountered in six instances. In most cases (36 samples) the pH lay between 2.5 and 5.4. There was a less marked effect of low gastric pH on streptococci, but the count

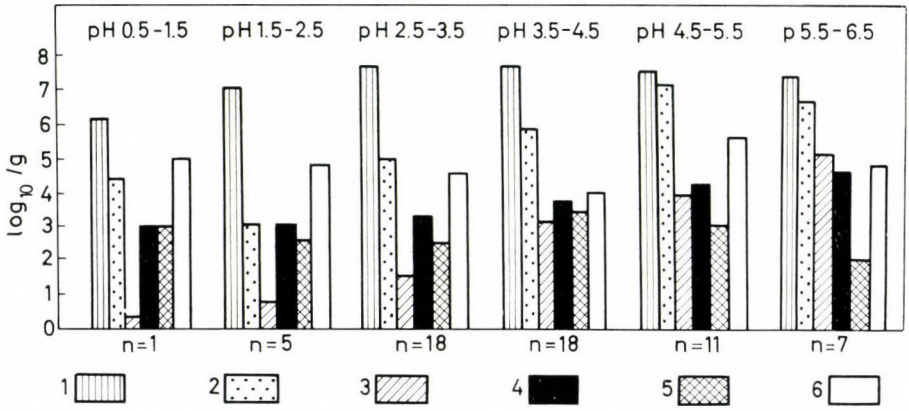

Fig. 1. Bacterial flora of the stomach at different pH ranges. 1, lactobacilli; 2, streptococci; 3, coliforms; 4, vegetative cells of clostridia; 5, spores of clostridia; 6, aerobic sporeformers bacteria; n, number of samples

of acid-tolerant lactobacilli hardly varied and the counts of aerobic and anaerobic spore-formers behaved irregularly at different pHs.

In four cases, the volume of gastric contents was very low; average bacterial counts calculated from eight samples were:

lactobacilli	= 6.32;	streptococci	= 5.15;
coliforms	= 3.20;	clostridia	= 2.20;

sporogenic aerobes = 4.00 (pH 5.02)

Thus lactobacillus and clostridium counts were lowered with respect to the mean values presented in Table III, while the pH was significantly above average.

The experiments were so designed that the effect of time of weaning on the gut flora could be established. Nevertheless, it was found that where all piglets of a group remained healthy in the weaning period, as was the case with all but litter D, no significant displacements could be detected in the

relative proportions of the different flora components; individual variations of the flora composition were minimal and not generally characteristic of the piglets studied. It appears, therefore, that the bacterial flora components investigated do not reflect at all the radical dietary change undergone at weaning.

Although the gastric contents of farm- and chamber-reared piglets contrasted in macroscopic appearance, the former being greenish-grey with an admixture of litter straw, the latter consisting purely of grain meal, there were no statistically verifiable differences at the microscopic level.

Small intestine

The bacterial counts determined in luminal contents taken from the duodenum, jejunum and the two segments of the ileum are shown in Table IV. The tendency they display to rise nearer the large intestine is especially con-

Table IV

Bacterial flora of the small intestine

log 10/g	Duodenum	Jejunum	Ileum I	Ileum II
Lactobacilli	6.87	6.98	7.65	8.18
Streptococci	5.13	5.48	6.68	7.42
Coliforms	3.41	3.81	5.50	6.32
Clostridia	1.53	1.37	3.00	4.22
Clostridium spores	1.00	0.69	2.09	2.44
Aerobic sporeformers	4.00	3.18	4.63	5.22
Bacteroides	—	—	1.45	2.12
pH	6.13	5.86	6.80	7.00

spicuous in the ileum, the proximal and distal portions of which differed considerably in respect of the flora composition. The fullness, pH and bacterial population of the ileum proved to be more stable compared to those in more proximal segments of the small intestine.

Comparison of gastric and duodenal data reveals an inhibitory effect of the duodenal juice on those bacterial types which resist the selective effect of the lower stomach pH. Thus, the reduction of lactobacillus count among others, is clearly due to the shift of the pH in the alkaline direction. The most typical alteration, however, was the marked fall in clostridial counts; certain authors have attributed this to the inhibitory influence of the bile acid salts, others to the combined effect of lipids and the rapid passage of intestinal contents (FULLER and MOORE, 1967). Clostridial growth inhibition affected chiefly

the vegetative forms, the absence of which is characteristic for this particular intestinal segment. In the distal segments of the small intestine, the count of the vegetative forms rose distinctly, while that of spores moderately, which suggests that clostridia multiply actively lower down the digestive tract (SINKOVICS, 1971).

Asporogenic anaerobes *(Bacteroides)* were also isolated from caudal small-intestinal segments, but only sporadically and in relatively low numbers.

The collective term *Bacteroides* designates those Gram-negative, chiefly coccoid organisms which form dewdrop-like colonies with little, if any, haemolytic activity on the RCM-medium described by FULLER and LEV (1964) and incubated under anaerobic conditions for

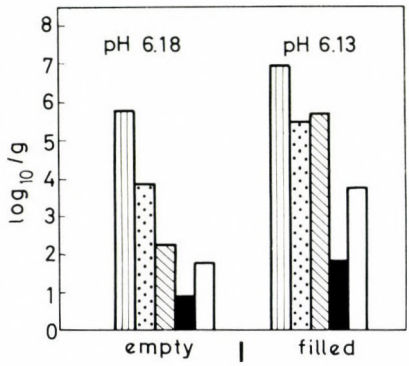

Fig. 2. Bacterial flora of empty and filled jejunal portions

4 days at 37 °C. Using this method, FULLER and LEV and SMITH and CRABB (1961) classified most such organisms as Bacteroidaceae, a few as *Veilonella*. Bacteroides occur chiefly in the large intestine and several authors regard them as the dominant components of its flora. According to the literary data, apart from polymorphous forms, rod shapes predominate in adult swine; in piglets we found principally coccoid forms, in accordance with the observations of KENWORTHY and CRABB (1963), who regard the coccoid shape as dominant up to 40 days of age.

Changes in the small-intestinal bacterial flora were most marked in the jejunal portion, where they depended principally on the fullness of the lumen. In those segments which were empty or contained very little chyme — so that sample material consisted for the most part of a small amount of mucus along with dead epithelial cells scraped off the mucosa — the bacterial population was usually low. In properly filled segments, however, there was a numerical increase of all examined bacterial components, and above all coliforms, but without any significant alteration of pH (Fig. 2). This accords well with the old medical experience, that indigestion of bacterial origin can be favourably influenced simply by fasting. Unburdening of the gastro-intestinal tract during the weaning period proved to be a useful measure also in our earlier piglet studies (BIRÓ et al., 1968).

Comparison of the pre- and post-weaning bacterial flora in the small intestine revealed only minor numerical changes without any effect on the ratios of the components to one another. Occasionally the streptococcal count in the jejunum and anterior ileum fell after weaning, reaching the degree of significance ($p = 1\%$) in the case of litters B and C. The small intestinal pH was likewise not notably affected by weaning.

More obvious were the disparities between the small intestinal flora of piglets reared under unlike environmental conditions. Piglets reared on the farm (viz. under less favourable hygienic conditions) carried a significantly higher ($p = 5\%$) coliform population in all segments except the jejunum, in

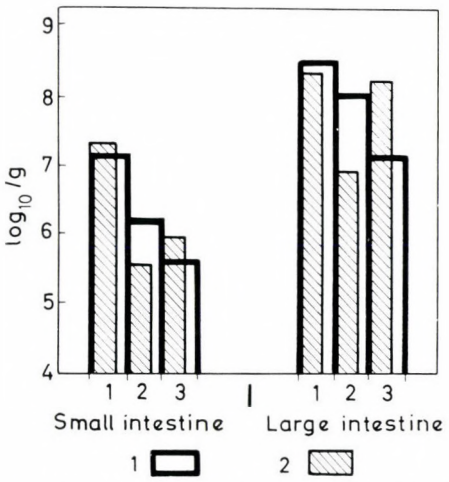

Fig. 3. Intestinal flora of suckling pigs in climatic chambers and in farrowing houses. 1, lactobacilli; 2, streptococci; 3, coliforms; 1, climatic chambers; 2, farrowing houses

which, as already noted, the bacterial flora was influenced chiefly by the fullness of the lumen. Also clostridial counts were generally, although not significantly, higher.

In the attempt to identify an environmental influence, the main components of small- and large-intestinal flora were examined during the lactating period, viz. as long as the principal nourishment of the piglets was the sow's milk. The results are summarized in Fig. 3. As can be seen, there were striking disparities not fully explicable by a change of numerical relations, already prior to the stress imposed by weaning. The characteristic lactobacillus–streptococcus–coliform ratio of the normal flora was modified markedly in the two environments: in farm-reared piglets the coliform count exceeded that of streptococci in both small and large intestine. It should be emphasized again that none of the animals included in this study showed symptoms of illness prior to weaning, and even afterwards only few of the farm-reared piglets

developed oedema disease. Piglets in the climatic chambers, which were of the same breed and raised according to the same management technology, exhibited no clinical disease throughout.

Large intestine

The counts of floral components found in samples from the caecum, mesocolon and rectum (Table V) demonstrate that differences between segments here were less pronounced than in the small intestine. The small fluctua-

Table V

Bacterial flora of the large intestine

log 10/g	Caecum	Colon	Rectum
Lactobacilli	8.26	8.41	8.52
Streptococci	7.81	7.98	7.91
Coliforms	7.05	7.19	7.03
Clostridia	5.27	5.33	5.83
Clostridium spores	3.18	3.46	3.58
Aerobic sporeformers	4.44	5.42	5.54
Bacteroides	5.36	6.44	5.41
pH	6.22	6.56	6.81

tions in bacterial population along each segment also suggest a greater stability of the flora than in the small intestine. As the large intestine was always uniformly filled, no comparison could be made between the bacterial contents of empty and full segments. Here again the flora did not notably alter on weaning, but marked differences were found in relation to environmental conditions. Piglets kept on the farm under conventional conditions of winter rearing (litters D and F) possessed significantly ($p = 5\%$) higher coliform counts in the large intestine than those kept in the climatic chambers. Statistical analysis of the streptococcal, lactobacillar and clostridial counts revealed an occasional tendency of the latter to rise. The practically identical composition of the flora in the anterior and posterior segments of the large intestine indicates that the faecal bacterial population is already established, by and large, in the caecum, as suggested earlier by PESTI (1962). The mean count of asporogenic anaerobes is low compared with figures given in the literature, but this may have been a consequence of the culturing technique adopted.

Discussion

The bacteriological status of the stomach and small and large intestines are strongly contrasted, as would be expected from the anatomical and physiological distinctness of these functional units. The differences between segments demonstrated in this study are, on the whole, in good agreement with those reported in the literature, but still findings for certain segments deserve more detailed discussion.

Originally, it had been expected that the gastric pylorus and cardia would deviate notably with regard to both bacterial flora and pH, in view

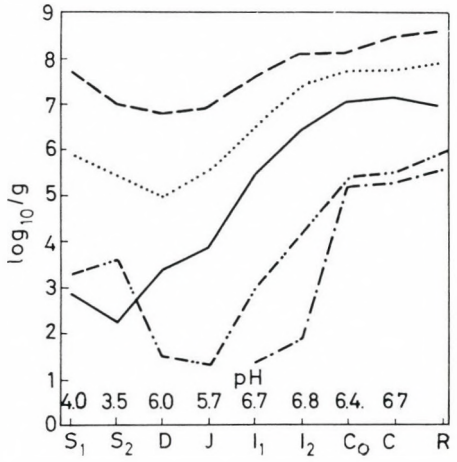

Fig. 4. Bacterial flora of alimentary tract in healthy young pigs. S_1, stomach (oesophagic part); S_2, stomach (pyloric part); D, duodenum; J, jejunum; I_1, ileum (upper part); I_2, ileum (lower part); C, caecum; C_0, colon; R, rectum; --------- lactobacilli; ..... streptococci; ——— coliforms; — — · · — · · — — clostridia; — · — · — · — bacteroides

of their functional separateness. However, it turned out that any differences were not significant statistically — as indeed evidence from recent physiological studies on the gastric function of swine would suggest (HÖLLER, 1970).

Among the four small-intestinal segments studied, the duodenum contained reduced numbers of all floral components, apart from coliforms, compared to the stomach: apparently, the inhibitory factors operative in the duodenum affect coliform organisms the least. Coliform counts in fact tended to rise posteriorly from the gastric pylorus, whereas lactobacillar, streptococcal and — especially — clostridial counts tended to fall (Fig. 4). There is reason to suppose that the interaction of the selective forces operative in the digestive tract of the healthy pig is an important factor in protecting against *E. coli* infection and gastro-enteritis due to *Clostridium perfringens* C.

Bacterial counts augmented somewhat in the post-jejunal segments of the intestine, and already in the terminal portion of the ileum the lactobacillus–streptococcus–coliform ratio reached the value generally characteristic of the large intestine. The fairly steady numerical increase of the bacterial components studied continued up to the caecum, and the balance established there remained practically unchanged along the remaining length of the large intestine.

It seems, nevertheless, that specific internal or external factors can affect the characteristic composition of the intestinal flora in certain segments.

The selective inhibitory effect of gastric pH was not generally manifested, because in the prevailing pH range (2.5—4.5), coliform counts still reached 10^2—10^3/g. The low pH (0.5—2.5) measured in a few instances, though, undoubtedly suppressed coliform growth. No pH-related change of bacterial flora could be detected in the small and large intestines.

A statistically significant relationship between the fullness of the gut and the bacterial flora could only be demonstrated in the small intestine (Fig. 2). In jejunal segments filled with chyme, coliform counts were three exponents higher than in empty portions at a practically identical pH; for bacteria other than coliforms this difference did not exceed 0.5—1.0 exponents.

Weaning, being a critical physiological period for the piglet, is often accompanied by an abrupt multiplication of *E. coli* in the digestive tract, resulting in oedema disease. Of the piglets we investigated, six developed oedema disease after weaning and would probably have died had they not been killed for experimental purposes. The bacteriological examination of the intestinal tract of these animals revealed the well-known alterations characteristic of oedema disease.

Where no *E. coli*-infection occurred after weaning, either among the exterminated piglets or among their litter-mates, weaning had no notable influence on the bacterial flora. The counts of the various components and their relative proportions alike remained practically unmodified. Minor changes only, consisting of a significant decline in streptococcal counts, were recorded in two segments of the small intestine of piglets reared in the climatic chamber and weaned at 21 or 28 days of age (litters B and C). Considering the drastic dietary switch ensuing upon weaning and the associated stress, however, it cannot be excluded that other alterations did take place, affecting either only certain types of the bacterial groups examined, or possibly types and groups not studied at all.

The disparity between environmental conditions was clearly the sole factor whose effect could be followed up consistently in all examined segments of the gut. The bacterial populations in the small and large intestines of piglets reared in conventional and controlled environment contrasted already during

lactation (Fig. 3). The lactobacillus–streptococcus–coliform ratio was generally normal for chamber-reared piglets, whereas in farm-reared age-mates it showed a shift in favour of the coliforms. In litter D and later in litter E the outcome of this was oedema disease shortly after weaning.

The unfavourable effect of poor environmental conditions on the digestive tract can be seen most clearly from Fig. 5. Among farm-reared piglets all small-intestinal segments except the jejunum contained significantly larger populations of coliforms than the small intestine of their age mates reared in climatic chambers. Consideration of the known factors suggests that the higher coliform counts are due not only to an increased intake of bacteria from a less

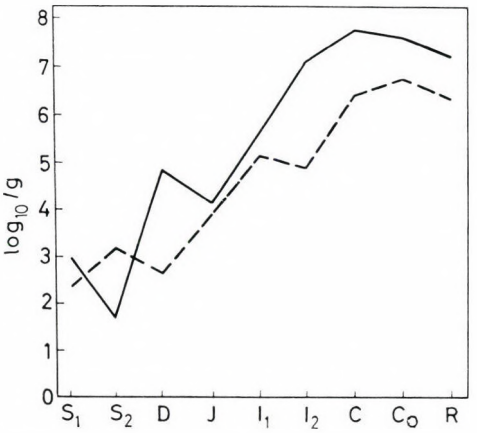

Fig. 5. Coliform bacteria in the alimentary tract of pigs in climatic chambers and in farrowing houses. ——— farrowing houses; - - - - - - - climatic chambers

hygienic environment, but also to a weakening of the various intestinal bacteriostatic effects. The fact that exclusively farm-reared pigs became diseased, underlines the importance of environmental influences on the gut and, it is hoped, will stimulate investigations into the joint effect of internal and external milieu.

SUMMARY

The gastric and intestinal bacterial floras of healthy piglets of five Large White sows were examined before and after early weaning at 14, 21 or 28 days of age. Piglets of three litters were farrowed and reared in climatic chambers, while those of the remaining two litters on the farm, under conventional conditions of management.

The pH and bacterial flora did not differ significantly in the cardiac and pyloric parts of the stomach. Conditions in the duodenum had a marked inhibitory effect on all examined bacterial components except coliforms, suppressing especially clostridial growth. Bacterial counts rose gradually in the more posterior segments of the small intestine, and in the terminal portion of the ileum they approximated the values characteristic of the large intestine. Bacteriological status and pH were essentially similar along the caecum, mesocolon and rectum.

Inhibition of coliform growth by a low gastric pH (1.5—2.5) was clearly demonstrable in a few cases, but the usual pH range (2.5—4.5) had no noticeable effect. No pH-related alterations of bacterial flora could be detected in the more posterior segments. The fullness of the jejunum was found to be positively correlated with the bacterial population: the greater the amount of chyme, the higher the number of bacteria, and above all of coliforms.

Weaning in itself did not notably affect the bacterial flora of either stomach or intestines. Environmental conditions, however, had a demonstrable influence on the bacteriological status of all examined segments. Farm-reared piglets carried a significantly larger coliform population in both small and large intestine than their age-mates reared in climatic chambers and exhibited an irregular lactobacillus–streptococcus–coliform ratio already during lactation.

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STUDY OF FELINE VIRUS DISEASES

I. EXAMINATION OF VIRUS STRAINS ISOLATED FROM UPPER RESPIRATORY TRACT INFECTIONS OF CATS

By

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VERGE and CRISTOFORONI (1928) were the first to suggest the causative agent of feline panleucopenia is a filtrable virus, but its precise nature remained uncertain until JOHNSON (1964) isolated it in feline kidney epithelial cell culture. Formerly several viruses had been held responsible for the causation of panleucopenia, until JOHNSON's isolate (which he himself regarded as an adeno- or papovavirus) was classified as a parvovirus by the Temporary Committee for Nomenclature in 1970. This classification was based on the physical and chemical properties of the agent and on the electron microscopic determination of the virion diameter (25 mμ).

It should be mentioned that JOHNSON and MARGOLIS (1967) observed a relationship between feline ataxia, a disease originally regarded as congenital, and feline panleucopenia. The agent isolated from ataxic cats was found to have the same physical and chemical properties as the panleucopenia virus, while the inclusion bodies present in their cerebellum corresponded with the Cowdry type A intranuclear inclusions occurring in panleucopenia.

Another long-known viral cat disease is feline pneumonitis, described by BAKER in 1944. The agent of this disease, which belongs to the *Bedsonia (Miyagawanella)* group, is pathogenic for several species, including albino mice, hamsters and guinea pigs, and grows well in the chorioallantoic membrane of the chick embryo; the elementary bodies produced by the virus can be demonstrated in impression smears of the membrane.

The recent great progress made in veterinary virology has resulted in independent isolations of viruses from feline upper respiratory tract diseases in the United States from 1958 (CRANDELL and MAURER, 1958; CRANDELL et al., 1960; CRANDELL and MADIN, 1960; CRANDELL and MILLER, 1962; PATRICIA et al., 1968; SCOTT et al., 1970a, b) and in Europe from 1963 (PIERCY and PRYDIE, 1963; BÜRKI, 1965; PRYDIE, 1966). Following these authors, it is customary to differentiate the herpesvirus-induced infectious feline rhinotracheitis from picornavirus infections; though clinically the two diseases are so similar that their diagnostic differentiation often rests solely upon virus isolation.

The herpesvirus strain C-27 (FVR-1) isolated by CRANDELL and MAURER deserves special mention in this respect, because the symptoms caused by it led to the recognition of the new disease enbity generally known as feline viral rhinotracheitis (FVR).

The first isolations of herpesvirus to be made in Hungary were the strains BK 1/63 and BK 2/63, recovered from the throat of two dead cats by HORVÁTH et al. (1965), who regarded the disease in question as infectious rhinotracheitis.

The feline herpes- and picornavirus strains isolated by the various authors have generally originated from animals with clinical or subclinical upper respiratory tract infections. The isolates mostly possessed a medium degree of virulence, causing a catarrhal change of the upper airways in only a portion of the susceptible cats. It is of historical interest that certain early isolates from fatal panleucopenia cases were mistaken for the aetiological agent until this was properly identified. As feline viral diseases often result from mixed infections, usually the fast-growing and intensively cytopathic picornavirus strains are isolated first. Among others BOLIN's isolate (1957), originally described by him as a feline panleucopenia virus, was later identified as a picornavirus. The agent described by FASTIER (1957) as kidney-cell degenerating virus, was in due course identified as a non-pathogenic picornavirus strain not forming intranuclear inclusion bodies.

SCOTT et al. (1970) isolated, under similar circumstances a reovirus from the intestine of a cat that died of panleucopenia. The isolate was a chloroform-resistant, thermostable strain with double-stranded RNA and had a medium degree of virulence for cats.

The growing interest in cats as domestic pets in Hungary presents the opportunity for frequent observation of feline upper respiratory disease. As up to now only herpesvirus infections have been described in this country, further investigations into the problem seemed justified, all the more that acute upper respiratory infections of cats by picornaviruses have been reported from abroad. The present investigations were therefore aimed at clarifying the nature and role of the pathogenic feline viruses occurring in Hungary.

Materials and methods

Test material. Sterile nasal, pharyngeal, conjunctival and rectal swabs from diseased cats were each placed in 2 ml Hanks' solution containing antibiotic and antimycotic. The swab fluids were centrifuged at 5000 r.p.m. for 30 minutes and 0.2 ml aliquots of supernatant were inoculated into 3 tube cultures of renal epithelial cells.

Tissue culture. Renal epithelial cells from 6—12 weeks old cats were used to set up the tissue cultures. The cells were collected after digestion in PBS and Hanks' solution containing 0.1—0.15% trypsin. The growth medium was a mixture of Hanks' solution containing 15% calf serum and 0.5% lactalbumin hydrolysate and an equal volume of bovine amniotic fluid, to which 100 IU penicillin, 100 mcg (μg) streptomycin and 100 IU mycostatin were added.

The virus isolates were diluted in serial tenfold steps and 0.2 ml aliquots of each dilution inoculated into 3 replicate tube cultures. They were incubated for 1 hour at 37 °C, then 0.2 ml maintenance medium was added to each and incubation continued. The results were read after 1—7 days. The 50% tissue culture infective dose ($TCID_{50}$) was calculated according to REED and MUENCH (1938). If isolation failed in the first passage, up to five blind passages were made before a negative result was accepted.

Immune serum was prepared in cats with the strain M_3. Basic immunization was carried out by subcutaneous administration of 5 ml undiluted M_3 suspension reinforced by a further 15 ml dose one month later. The cats were bled out two weeks after the second immunizing dose and the sera were stored at —20 °C until use.

For virus neutralization tests all sera, including control serum from healthy cats, were inactivated at 56 °C for 30 minutes. The tests were usually

carried out with 30—300 $TCID_{50}$/0.2 ml virus (Table III). Horse and sheep sera served as serum controls and Aujeszky's disease virus as the virus control. Serial twofold dilutions of serum were added to various $TCID_{50}$ doses of virus (Table III) in equal final volume and the mixtures were incubated (in roller tubes for one hour at 37 °C). After incubation, 0.2 ml portions of the mixture were transferred to 3 roller tubes or stationary cultures.

Examination of physical and chemical properties

Thermosensitivity was determined from the titre reduction of 1 : 10 dilutions of the virus isolates after one-hour incubation in a water bath at 50 °C Cation stability tests were performed by the method of WALLIS and MELNICK (1962).

Sensitivity to pH 3 was tested according to PLUMMER (1963) in McIlvaine buffer, after one-hour incubation at 37 °C. Bile sensitivity was tested by THEILER's method (1957).

Resistance to trypsin was determined by titrating mixtures of equal volumes of 1 : 10 diluted virus and 0.25% trypsin solution, after incubation for one hour at 37 °C. Chloroform-sensitivity tests were performed after BÖGEL and MAYR (1962) with 50% analytical grade chloroform. Virus-chloroform mixtures were stored overnight at +4 °C before titration. Nucleic acid type was determined by adding 100 mcg (μg) 5-iodo-deoxyuridine (IUDR) per ml virus suspension. The trypsin-treated, chloroform-treated and IUDR-treated materials were diluted in serial tenfold steps, 0.2 ml portions of each dilution were transferred to 3 tube cultures and after appropriate incubation the titres were calculated by the method of REED and MUENCH.

Cytopathic effects caused by the isolates were studied after staining the cultures with haemalaun and eosin, Giemsa's stain or floxintartrazine. Occasionally also the Feulgen reaction was utilized. The cell monolayers were fixed (in situ) in Bouin solution, embedded in celloidine, and removed from the tube for examination on slides.

Results

Positive isolations of virus were made from nasal, pharyngeal or conjunctival swabs of 12 cats from a total of 53 (22.6%) brought to the Outpatient Clinic of the University of Veterinary Science during the past year with symptoms of upper respiratory disease. The isolates were designated by "M" (for macska = cat) plus a serial number in sequence of isolation, irrespective of later typing results. Strains M_1, M_2 and M_3 were examined in more detail, because these differed from one another in cytopathic effect and other properties.

Of the 12 isolates, M_1, M_{11} and M_{12} were found to belong to the herpesvirus group, the rest to the picornavirus group (Table I).

Table I

Clinical data on cats from which isolations were made

Designation of strain	Symptoms	Origin of isolate				Group of virus
		NC	PH	C	R	
M_1	Listlessness, infirmity, subnormal temperature, no catarrhal sign	—	+	—	—	herpes
M_2	Sneezing, hoarseness, purulent nasal discharge	—	+	—	—	picorna
M_3	Sneezing, reddening of throat	+	+	+	—	picorna
M_4	Violent sneezing, reddening and purulent exudation of conjunctiva, reddening of throat	+	+	—	—	picorna
M_5	Sneezing, reddening and purulent exudation of conjunctiva, reddening of throat	—	+	—	—	picorna
M_6	Vomiting, inappetence, infirmity, subnormal temperature	—	+	—	—	picorna
M_7	Listlessness, inappetence, sore and reddened throat	—	—	+	—	picorna
M_8	Fever, sneezing, dyspnoea	+	—	—	—	picorna
M_9	Sneezing, serous nasal discharge, reddened throat	+	+	—	—	picorna
M_{10}	Reddening of conjunctiva, profuse lacrimation, sneezing, diarrhoea	—	+	—	—	picorna
M_{11}	Conjunctival catarrh, serous nasal discharge, stertorous respiration	—	—	+	—	herpes
M_{12}	Salivation, reddened throat, severe dyspnoea, high fever	+	—	+	—	herpes

NC, nasal cavity; PH, pharynx; C, conjunctiva; R, rectum

Strain M_1 was isolated from the pharynx of a cat presenting no catarrhal symptoms. The primary isolate caused cytopathic changes in the renal cell culture after 4 days. In unstained cultures, rounding of cells was seen first focally, then over increasingly larger areas, until the complete cell layer became detached from the tube wall. The rounded cells were highly refractive and sharply contrasted even on direct examination. In stained preparations, polykaryocytes (syncytia) and Cowdry type A acidophilic intranuclear inclusion bodies were observed (Fig. 1). Inclusions could already be detected by direct examination prior to the appearance of the cellular changes (Fig. 2). In subsequent passages the cytopathic effect developed earlier, within 24—48 hours, and was well visible on direct examination.

Stability tests showed that strain M_1 was sensitive to heat, pH 3, trypsin, chloroform and could not be stabilized with magnesium chloride. Treatment with IUDR for 24 hours resulted in a fall of the virus titre to zero, indicating a DNA-type nucleic acid. On the basis of these properties, strain M_1 was classified as a herpesvirus (Table II).

Table II

Titre changes of virus strains M_1, M_3 and M_9 in stability tests

Treatment	Herpesvirus	Picornavirus	
	M_1	M_3	M_9
Titre of untreated virus	6.5*	7.5	3.75
Chloroform	0	5.5	4.5
Heat	1.75	1.5	< 1
Heat + 1 M $MgCl_2$	< 0.2	< 0.5	< 1
pH 3	< 2	< 2	< 2
Trypsin	< 0.2	3.25	3.25
Sodium deoxycholate	< 0.2	2.75	n.t.
IUDR	0/24 hours	8.0/24 hours	4.75/24 hours

* log 10 $TCID_{50}$/0.2 ml; n.t., not tested

Eight strains of variable properties (M_2, M_3 . . . M_9) were isolated from nasal, pharyngeal and conjunctival swabs of cats affected with sneezing mucopurulent nasal discharge and throat pain. The diseased cats were from 2 months to 5 years old. The cytopathic effect of these isolates was similar to that of strain M_1 in unstained cultures. First, single cells became rounded and refractive until by 24—48 hours the change involved the entire cell population, resulting in detachment of the monolayer from the tube wall. If undiluted virus suspensions were used for inoculation, the cytopathic change developed as early as 5—6 hours. In stained preparations, rapid rounding of single cells, cytoplasmic eosinophilia and simultaneous nuclear degeneration were seen.

Examination of the stained cultures, however, revealed certain differences between the cytopathic effects of the isolates. Strains M_2, M_3 . . . M_8 caused a pyknotic degeneration and shrinking of the nucleus much earlier than strain M_9; a short-lived prepyknotic phase could be clearly observed with the latter (Fig. 3). Cytoplasmic degeneration also differed with strain M_9, as this produced a granulation consisting of eosinophilic clumps of various sizes in affected cells (Fig. 3).

Strains M_9 and M_3 were for these reasons examined in more detail. Both strains were sensitive to heat treatment at 50 °C and to pH 3, and neither

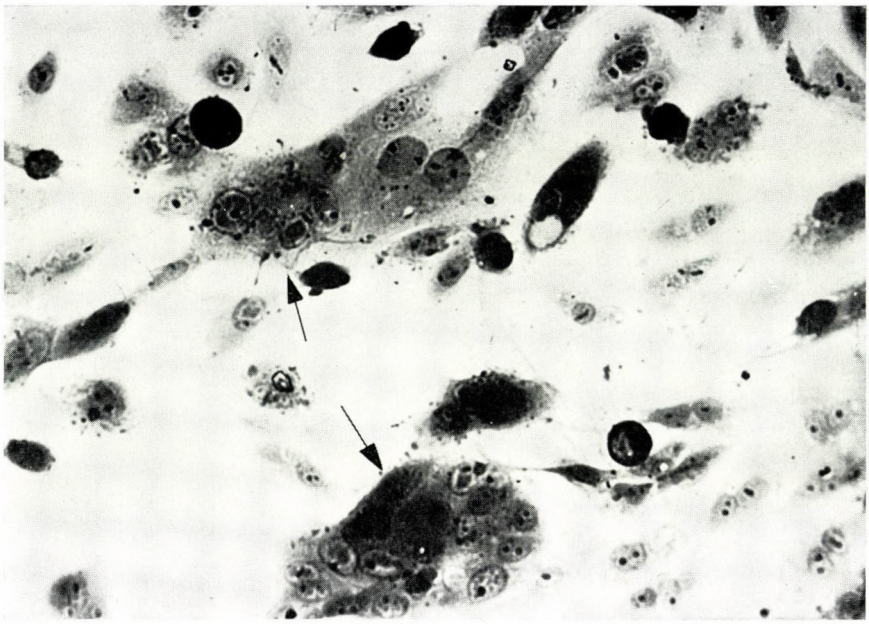

Fig. 1. Cytopathic change caused by strain M_1. Arrows point to polykaryocytes (syncytia) containing Cowdry type A intranuclear inclusion bodies. (Haematoxylin and eosin, ×640)

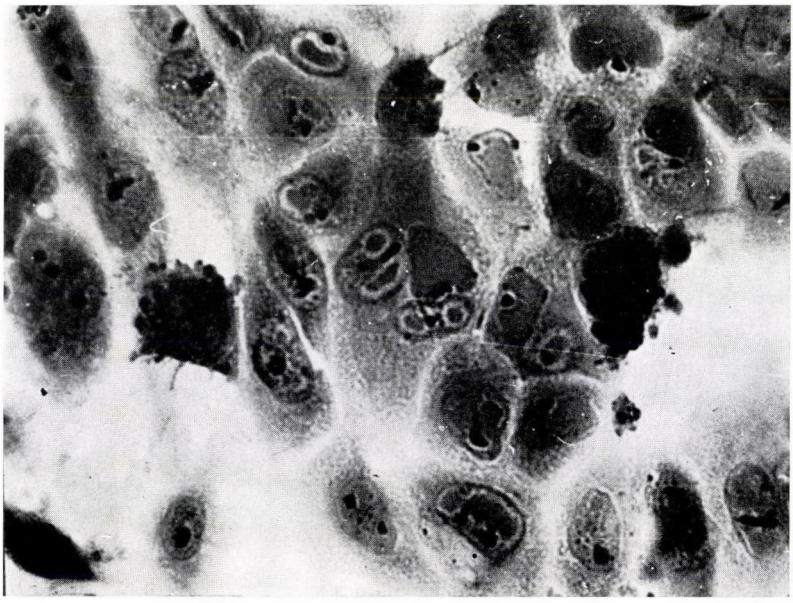

Fig. 2. Early appearance of intranuclear inclusion bodies not visible by direct examination: strain M_1 in feline kidney epithelial cell culture. (Haematoxylin and eosin, ×800)

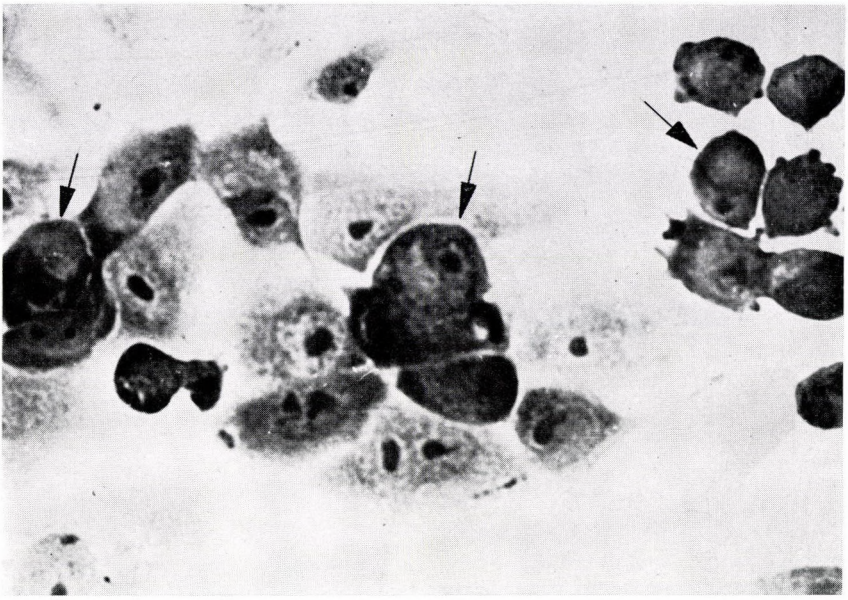

Fig. 3. Nuclear changes caused by strain M_9. Arrows point to semilunar, eccentric nuclei with sharp-contoured nucleolus. (Haematoxylin and eosin, ×800)

could be stabilized with magnesium chloride. But while strain M_9 proved to be resistant to chloroform, strain M_3 consistently showed a titre decrease by two logarithmic exponents after such treatment. Again, strain M_9 resisted treatment with trypsin and sodium deoxycholate, whereas the titre of M_3 fell markedly on both treatments. The two strains also differed in pathogenicity:

Table III

Results of neutralization tests with anti-M_3 cat immune serum and normal cat serum

Designation of strain	$TCID_{50}$/0.2 ml	Titre of M_3 hyperimmune serum	$TCID_{50}$/0.2 ml	Titre of normal serum
M_2	50	1 : 80		
M_3	300	>1 : 128	30	1 : 16
M_4	30	1 : 89		
M_5	30	1 : 89		
M_6	30	>1 : 128		
M_7	200	>1 : 128		
M_8	100	1 : 64		
M_9	50	1 : 2	30	0
Control M_1	30	0	30	0

M_9 was pathogenic, M_3 only mildly pathogenic for the cat. Treatment with IUDR did not affect the proliferation growth of either strain, indicating that both of them are RNA-viruses (Table II).

In view of the dissimilar behaviour of these two strains in resistance tests, the non-herpes isolates were further tested for serological relationship. For this purpose a hyperimmune serum to strain M_3 was prepared in cats and used for neutralization tests with strains M_2, $M_3 \ldots M_9$ in renal cell culture. The neutralization titres obtained (Table III) show that strain M_9 is definitely antigenically unrelated, while strains M_2, $M_4 \ldots M_8$ are clearly related to M_3. The serological type of strain M_{10} has yet to be established.

On the basis of physical and chemical properties and cytopathic effect, strains M_3 and M_9 are regarded as picornaviruses, probably of the rhinovirus group.

Discussion

A relatively large number of pet cats are treated in the Outpatient Clinic of the University of Veterinary Science, often for respiratory diseases. Positive isolations of virus from the upper respiratory tract were made from 22.6% of such patients of ages between 2 months to 5 years.

Of the 12 isolates, three were identified as herpesviruses, the remaining nine as picornaviruses. All three herpes isolates proved to be identical with the feline rhinotracheitis virus originally isolated by CRANDELL and MAURER (1958) in the United States and earlier in Hungary by HORVÁTH et al. (1965). The picornavirus strains could be divided into two serological subgroups, represented by strains M_2, $M_3, \ldots M_8$ and the single strain M_9, respectively.

The two picornavirus strains (M_3 and M_9) examined up to now in more detailed tests could be classified as rhinoviruses on the basis of their pH-sensitivity. The titre reduction observed with strain M_3 on treatment with chloroform remains to be explained, because picornaviruses are generally resistant to this reagent. Strain M_9 differed from the other picornavirus isolates not only antigenically, but also in its resistance to trypsin and sodium deoxycholate. Similar variable behaviour of certain picornavirus strains in the latter respect has already been reported by PATRICIA et al. (1968). Strains M_3 and M_9 also contrasted to some extent in their cytopathic effects, and M_9 was distinctly pathogenic for the cat, whereas M_3 only mildly so.

The pictures caused by feline herpes- and picornaviruses can not be distinguished from one another solely on a clinical and pathological basis, and the only reliable diagnostic differentiation entails virus isolation and neutralization tests with serum from the diseased animal. According to RÖHRER et al. (1968), Cowdry type A intranuclear inclusions are only temporarily present in the respiratory epithelium of cats suffering from infectious feline rhinotracheitis and do not appear at all in experimental infections.

In view of the findings presented here, closer investigations into the upper respiratory diseases of cats would seem of value also in this country.

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SUMMARY

Twelve virus strains were isolated from respiratory tract discharges of 12 among 53 cats (22.6%) treated for catarrhal disease of the upper airways during the last two years. Three isolates (strains M_1, M_{11} and M_{12}) were identified as herpesviruses, while the remaining nine (M_2, M_3, . . . , M_{10}) were tentatively classified as rhinoviruses of the picornavirus groups. Strains M_3 and M_9 were examined in more detail.

Strain M_9 differed serologically both from M_3 and the seven other picornavirus strains tested. It also differed from M_3 in respect of cytopathic effect and did not show the latter's sensitivity to treatment with trypsin, sodium deoxycholate and chloroform.

Of the three strains tested for pathogenicity M_1 and M_9 proved distinctly pathogenic for the cat, while M_3 was only mildly pathogenic.

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STUDIES ON THE HISTOPATHOLOGIC CHANGES OF ACUTE MAREK'S DISEASE

By

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The acute form of Marek's disease, first differentiated from the classical one by BIGGS et al. in 1965, is now recognized as a serious problem in several countries. Many reports (BIGGS, 1966, 1967; BIGGS and PAYNE, 1967; PAYNE and BIGGS, 1967; PURCHASE and BIGGS, 1967; MALIK, 1969; WITTER et al., 1970; FLETCHER et al., 1971) published in the last years, however, have dealt chiefly with its virological aspects. Relatively little attention seems to have been devoted to the microscopic lesions and pathogenesis.

Now that diagnosis of acute Marek's disease is a routine task in our institute for several years, it was felt that closer investigations should be carried out into the characteristics of the disease, first of all to aid the diagnostic work.

Materials and methods

Field investigations were performed at a poultry farm where acute Marek's disease had been occurring for several years. Poultry house V of this infected farm was stocked with 11 800 one-day-old broiler (Plymouth and Cornish) chicks hatched from eggs disinfected with formalin gas. After prior clinical observation altogether 87 of the birds were killed for pathological, bacteriological and histological examinations according to the following schedule: three chicks were killed daily up to 10 days of age, and three each on two occasions every week from 11 days up to marketing at 76 days.

In every case the following specimens were histologically examined: brain, the cervical and lumbar intumescences of the spinal cord, the left and right brachial and ischiatic nerves between the axillary and knee joints the spleen, appropriate parts of liver, the kidneys, heart and bone marrow from the proximal end of the femur. The proventriculus, testes, thymus, bursa of Fabricius and skin were additionally examined if they appeared grossly changed. The organ-blocks were fixed in 10% neutral formalin solution and embedded in paraffin.

Brains were studied in complete longitudinal sections, the cervical and lumbar intumescences were cut in the frontal plane, and sections from peripheral nerves were cut longitudinally.

Table I

Incidence and severity of microscopic lesions in different organs of chickens 1—76 days old

Bone marrow	1	+	+++	+	+	++++	+++	−	+	+
	2	++	+	+	++	++	+	++	++	+
	3	++	+++	+	+	++	++	+	++	+
Kidney (left)	1	+	++	+	++	+	++++	+	++	+
	2	++	+	+	+	+++	+	+	+	+
	3	+++	+++	++	+	++	+	−	+	+
Kidney (right)	1	+	+++	++	+	+	+++	+	+	+
	2	++	++	++	+	+++	+	+	+	+
	3	++	+++	++	+	++	+	+	+	+
Liver	1	++	++	+	++	+	++++	++	+	++
	2	++	++	+	+	+++	++	+	++	+
	3	+++	++	+	+	+	+	+	+	+
Heart	1	−	++	+	+	+	++	++	+	+
	2	+++	+	+	+	++++	++	+	++	+
	3	+++	+++	++++	++	++	++	+	+	+
Brachial nerve (left)	1	++	−	−	−	−	+++	⊖	−	⊖
	2	+	+	−	−	+++	+++	−	⊖	−
	3	+	⊖	−	−	+	+	−	−	+
Brachial nerve (right)	1	+	−	−	+	−	+	+	⊖	⊖
	2	+	−	−	⊖	+++	+++	−	+	−
	3	++	−	−	−	−	+	−	⊖	⊖
Ischiatic nerve (left)	1	+	+++	−	⊖	−	++	++	++++	−
	2	++	−	+	−	+++	+++	−	++	−
	3	+	−	−	+	+	+	−	+	++
Ischiatic nerve (right)	1	+	++	−	−	−	+++	⊖	++	+
	2	++	+	−	+	+++	+++	+	−	+
	3	++	−	−	−	−	++	+	−	++
Lumbar intumescence	1	−	+++	+	+	−	−	+	+++	−
	2	+	−	−	−	+++	++	−	+	−
	3	++	−	−	+	+	+++	−	−	−
Cervical intumescence	1	−	+	−	−	−	+	+	++	−
	2	++	+	++	+	−	+	−	−	−
	3	+	+	+	−	−	+++	−	+	++
Brain	1	+	+	+	++	+	+	+	+++	+
	2	+++	+	++	+	++++	++	+	++	−
	3	+	+	+	+	+	+++	+	+	+
		76	69	67	62	60	55	53	48	46

The severity of changes is graded by crosses, as follows: + mild, focal lesion; ++ more cognizable in islets; +++++ affected tissues no longer recognizable; ⊕ oedema of certain

For general information the sections were stained with Mayor's acid haemalaun and eosin. The cytoplasmic ribonucleic acid component of the infiltrating cells was stained with methyl green-pyronine and Mayer's acridine orange technique. The reticular fibres of the parenchymatous organs were demonstrated by Gömöri's silver impregnation technique and the nuclear inclusions according to the Farkas—Mallory procedure.

originating from a flock with a history of acute Marek's disease

pronounced focal lesion; +++ diffuse cell infiltration; ++++ affected tissue only re-
peripheral nerves with mild lesion; ⊖ oedema without lesion

Results

Rearing losses in the flock during the 76-day-period of exposure amounted to 4.66%. About half of the deaths occurred as a consequence of weakness after hatching at the age of up to 10 days and the rest from 38 days mainly from the acute form of Marek's disease.

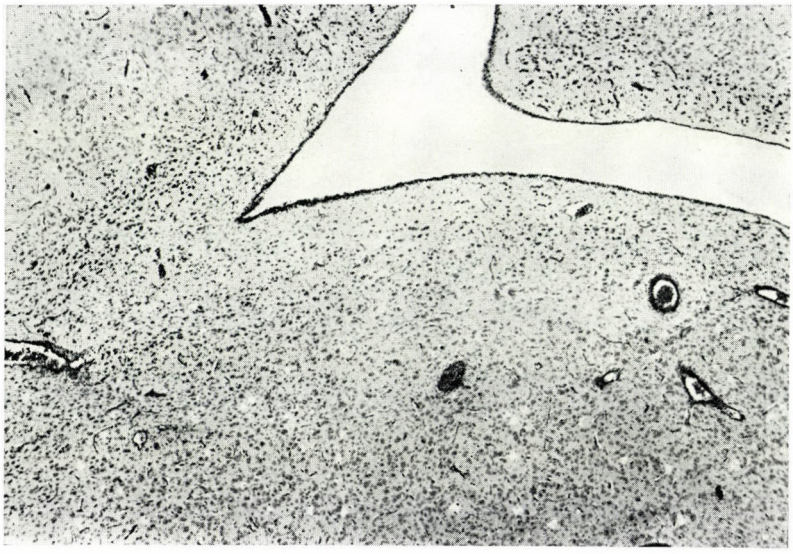

Fig. 1. Detail of optic lobe from the brain of a 41-day-old chicken exposed by contact to Marek's disease agent. Note the lymphoid cell infiltration around several vessels of both the white and grey matter (Haemalaun and eosin, ×45)

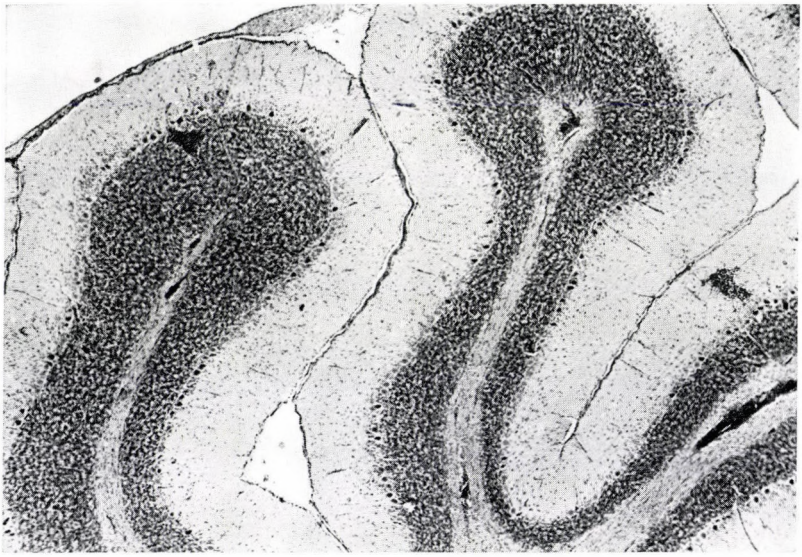

Fig. 2. Detail of cerebellum from 41-day-old chicken exposed by contact to Marek's disease agent. Perivascular accumulation of lymphoid cells are visible in both white and grey matter (Haemalaun and eosin, ×45)

Symptoms were first observed at 41 days when 2—3% of the birds showed mostly minor motoric disturbances (instable gait, stumbling), though a few developed complete paralysis.

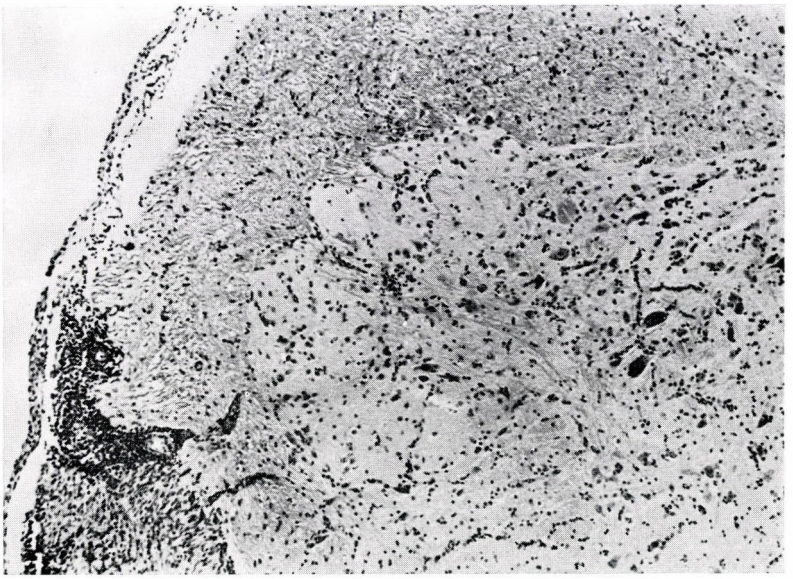

Fig. 3. Detail of cervical intumescence from a 41-day-old chicken exposed by contact to Marek's disease agent. Note lymphoid cell infiltration along the efferent nerve root in the white matter (Haemalaun and eosin, ×95)

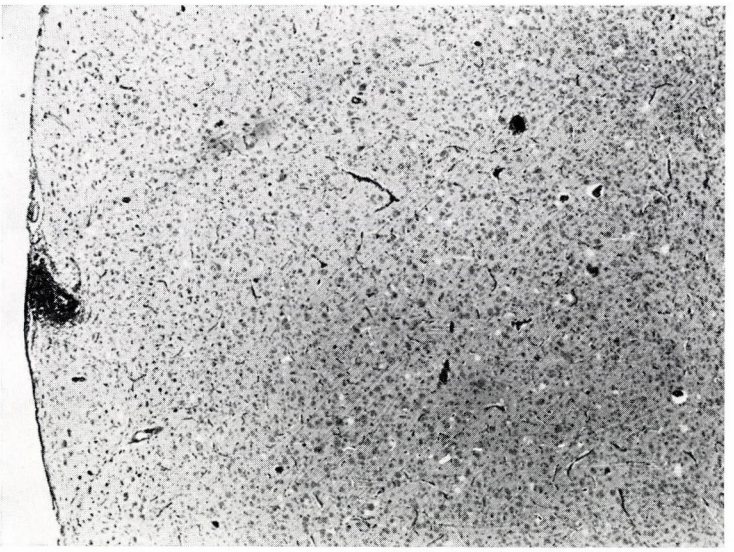

Fig. 4. Detail of brain from a 41-day-old chicken exposed by contact to Marek's disease agent. A focal lymphoid cell infiltration is visible within and below the meninx (Haemalaun and eosin, ×45)

At necropsy pathological lesions were detected in about 40% of the birds examined; these lesions, which were slight and involved one or occasionally more organs, appeared from 41 days of age.

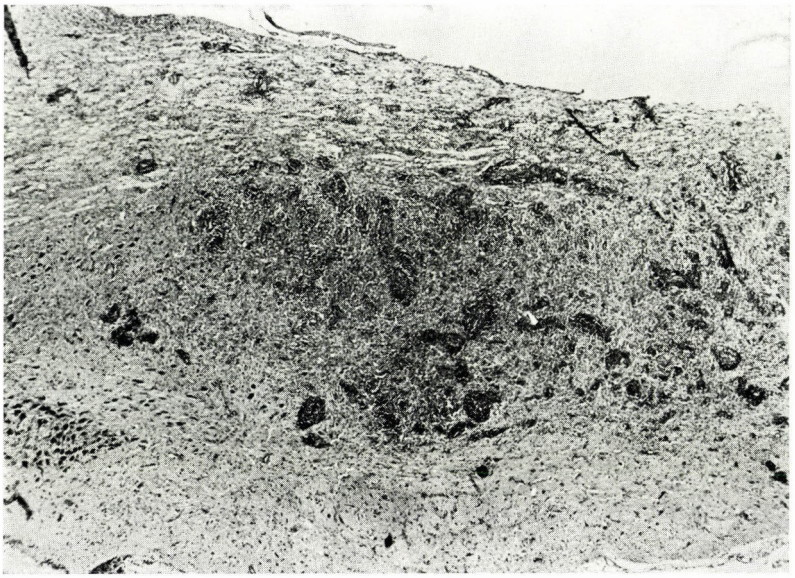

Fig. 5. Detail of medulla oblongata from a 69-day-old chicken exposed by contact to Marek's disease agent. Neoplastic-like changes are visible in the white and grey matter (Haemalaun and eosin, ×45)

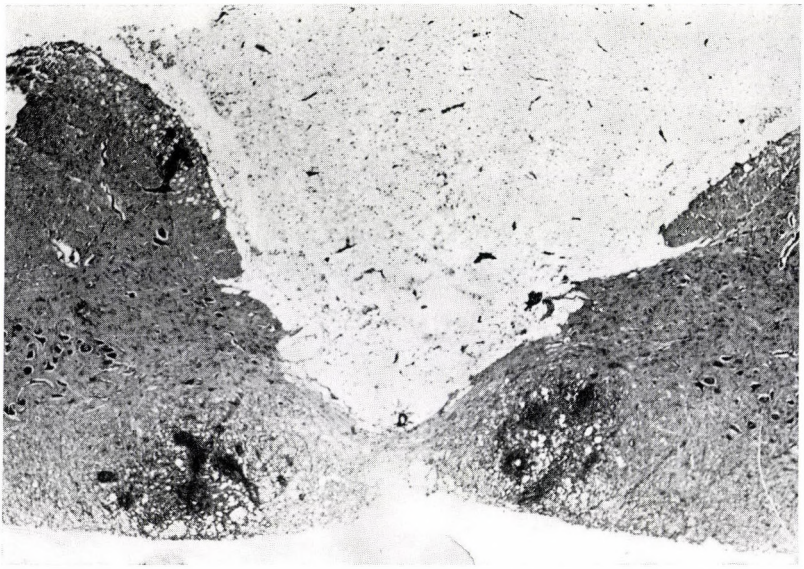

Fig. 6. Detail of lumbar intumescence from a 69-day-old chicken exposed by contact to Marek's disease agent. Exhibiting a neoplastic-type infiltration in the white matter (Haemalaun and eosin, ×45)

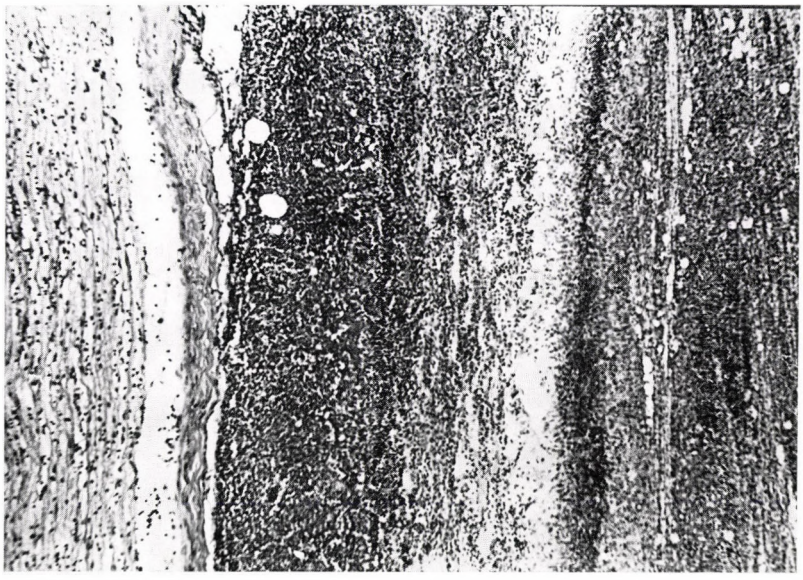

Fig. 7. Detail of ischiatic nerve from a 60-day-old chicken exposed by contact to Marek's disease agent showing the concomitance of markedly infiltrated and non-infiltrated nerve fibre bundles (Haemalaun and eosin, ×95)

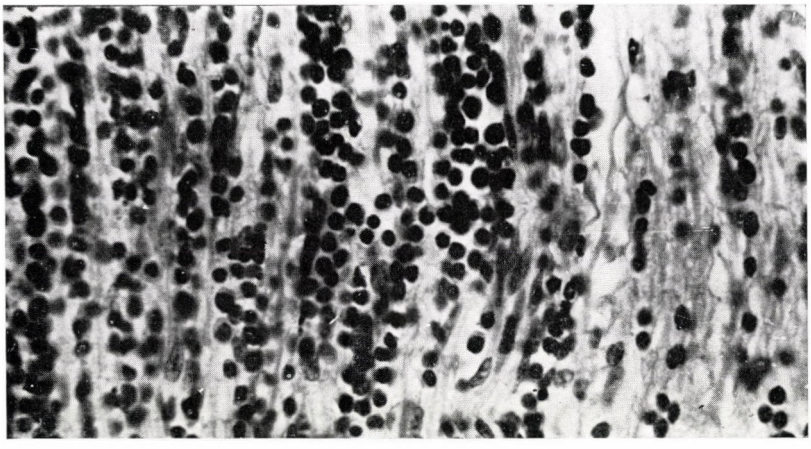

Fig. 8. Detail of ischiatic nerve from a 46-day-old chicken exposed by contact to Marek's disease agent presenting endoneural infiltration by rows of lymphoid cells (Haemalaun and eosin, ×600)

No bacteria were isolated from the affected tissues.

Histological examinations were begun only when the total material had been collected and were conducted first on the specimens from the oldest birds. The results are summarized in Table I. The severity of changes is graded by crosses as follows:

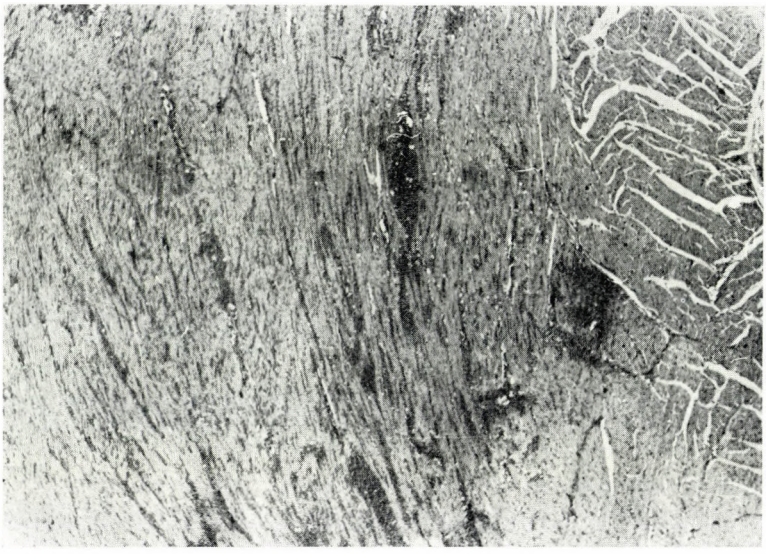

Fig. 9. Detail of myocardium from a 76-day-old chicken exposed by contact to Marek's disease agent. A focal lymphoid cell infiltration is visible among the muscle fibres (Haemalaun and eosin stain, ×45)

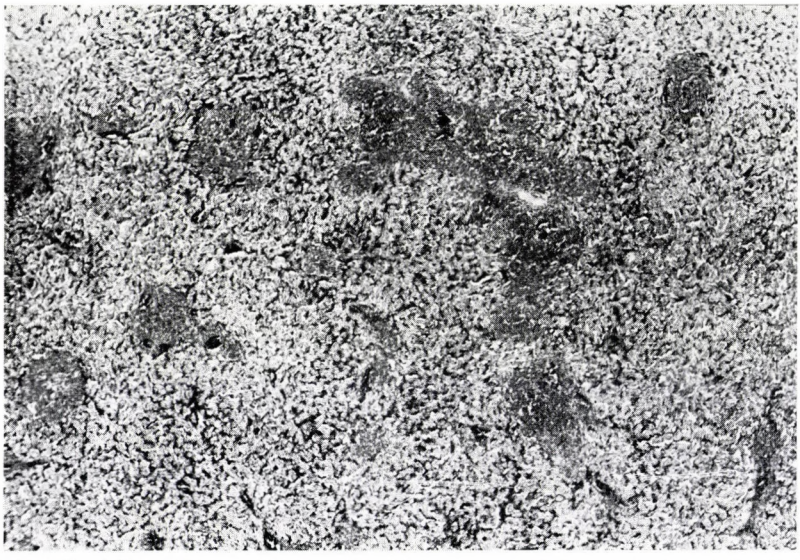

Fig. 10. Detail of liver from a 60-day-old chicken exposed by contact to Marek's disease agent. Infiltrating lymphoid cells are present in the parenchyma and around the walls of the larger vessels (Haemalaun and eosin, ×45)

\+ mild, focal lesion
\+\+ more pronounced focal lesion
\+\+\+ diffuse cell infiltration

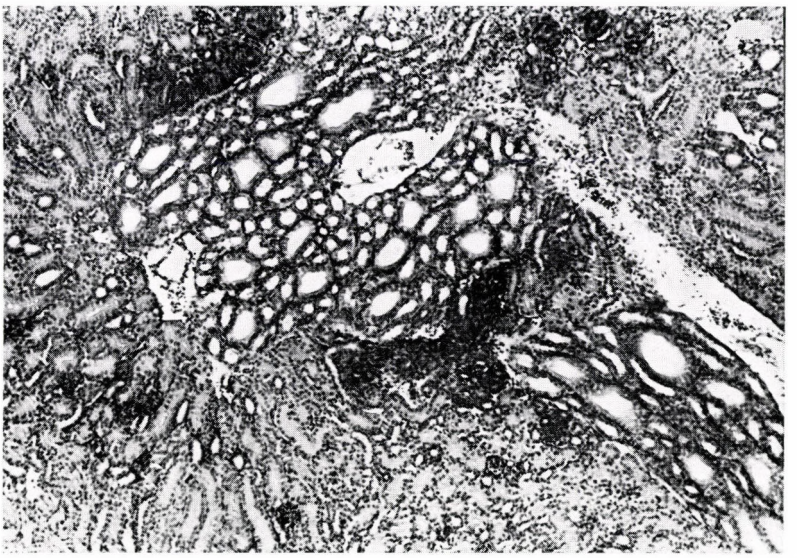

Fig. 11. Detail of kidney from a 69-day-old chicken exposed by contact to Marek's disease agent. A marked lymphoid cell infiltration can be seen in both renal cortex and parenchyma (Haemalaun and eosin, ×95)

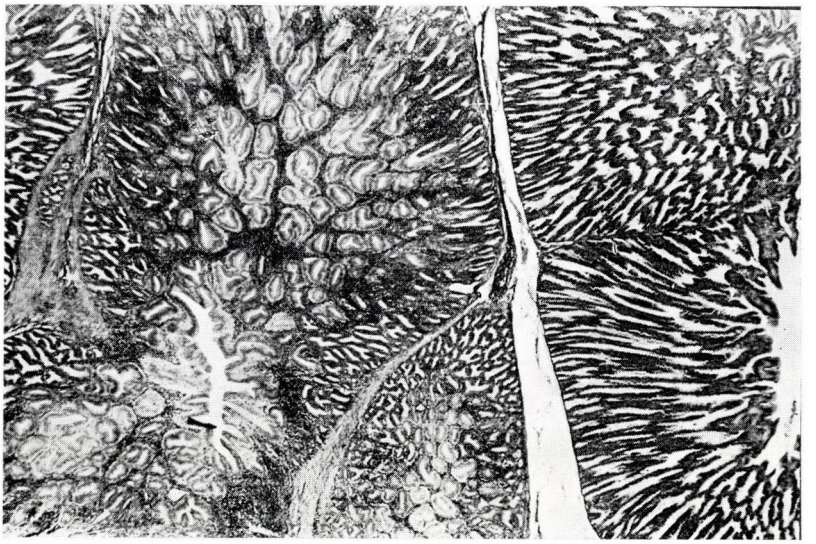

Fig. 12. Detail of proventriculus from a 67-day-old chicken exposed by contact to Marek's disease agent. Note the sporadic lymphoid cell infiltration in the deep glands of the propria (Haemalaun and eosin, ×45)

++++ affected tissue only recognizable in islets
+++++ affected tissue no longer recognizable.

Circles indicate oedema of certain peripheral nerves.

Lesions of the central nervous system usually became apparent from 41 days and always consisted of a lymphocyte cell infiltration around the vascular wall (Figs 1—4). The infiltrating cells formed a perivascular lymphocyte cuffing, usually comprised of two or more rows of cells, but sometimes a single row or focal in distribution and as a rule sharply demarcated against the ectodermal brain tissue. Vascular wall infiltrations were seen in both white and grey brain matter and in about half of the cases in the cerebellum as well. Cerebral lesions were located in the optic lobe (Fig. 1) and within or close by the nuclei of the medulla oblongata while cerebellar lesions were usually found in the granular layer (stratum granulosum), molecular layer (stratum moleculare) as well as in the white matter of the cerebellar lamellae (Fig. 2). In the cervical and lumbar intumescences most lesions were observed in the white matter and they were generally asymmetrically distributed (Fig. 3). Occasionally cell infiltrations were encountered in circumscribed areas of the cerebral and spinal meninges.

In the overwhelming majority of cases the infiltrating cells were mature lymphocytes. Infiltration of the vascular wall was not accompanied by degenerative neuron changes or glial proliferation.

Atypical, undifferentiated cells indicative of neoplasia occurred in a circumscribed area of the medulla oblongata of two birds, aged 69 and 60 days (Fig. 5); in the lumbar intumescence of these and two additional birds, aged 55 and 48 days (Fig. 6); as well as in the cervical intumescence of the former 55-day-old bird and another 25-day-old one. These diffusely infiltrating, lymphoblast-like cells contained an increased amount of ribonucleic acid, as demonstrated by staining with Mayor's acridine orange. They formed massive perivascular cuffing in the affected areas and the surrounding nerve cells were severely degenerated.

In peripheral nerves cell-infiltration was endoneural; the cells usually formed rows or less often were distributed focally between the myelinated neurofibres of a nerve bundle. In most cases only a single bundle was affected; adjacent bundles contained very few infiltrating cells if any (Fig. 7). Most infiltrating cells were again mature lymphocytes (Fig. 8); but other inflammatory cells, such as histiocytes, plasma cells and quite rarely heterophilic cells were present in variable, generally low numbers. In the case of more pronounced infiltrations a large number of immature, lymphoblast-like cells with a high ribonucleic acid content were present. Three birds, aged 69, 60 and 48 days with lesions qualified +++ and ++++ displayed neoplastic-like cell infiltrations also in the central nervous system. In the changed area the myelin sheath of the nerve fibres underwent different degrees of degeneration, depending on the extent of infiltration, but the axon usually remained intact. A distinct oedema without cellular infiltration was noticed in the peripheral nervous system of 12 birds.

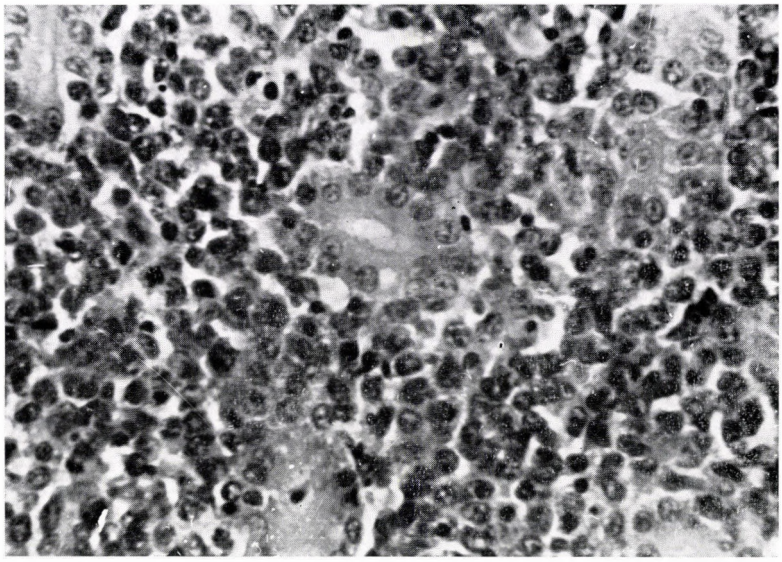

Fig. 13. Detail of kidney from a 55-day-old chicken exposed by contact to Marek's disease agent. Many immature blast-type cells, rich in chromatin and with a large cytoplasm, are present among the lymphoid cells surrounding the tubules (Haemalaun and eosin, ×600)

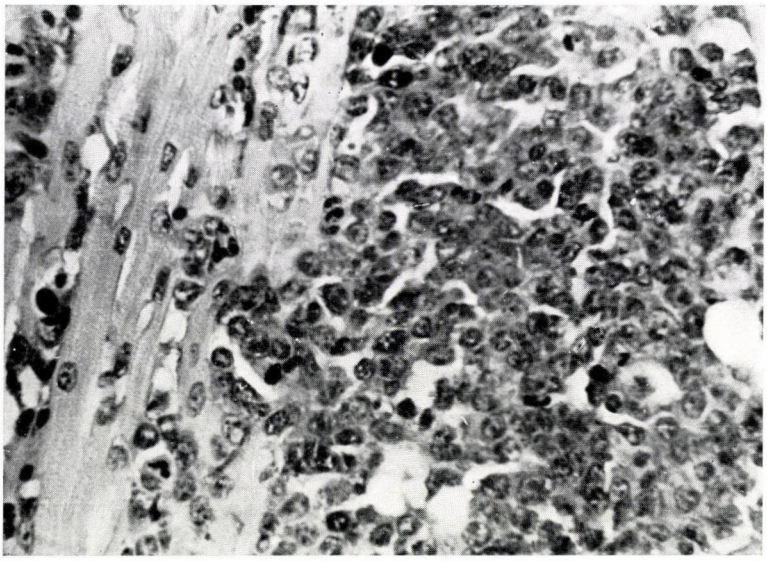

Fig. 14. Detail of heart from a 69-day-old chicken exposed by contact to Marek's disease agent. Many large, chromatin-rich, neoplastic-type cells are visible among the lymphoid cells infiltrating the muscle fibres (Haemalaun and eosin, ×600)

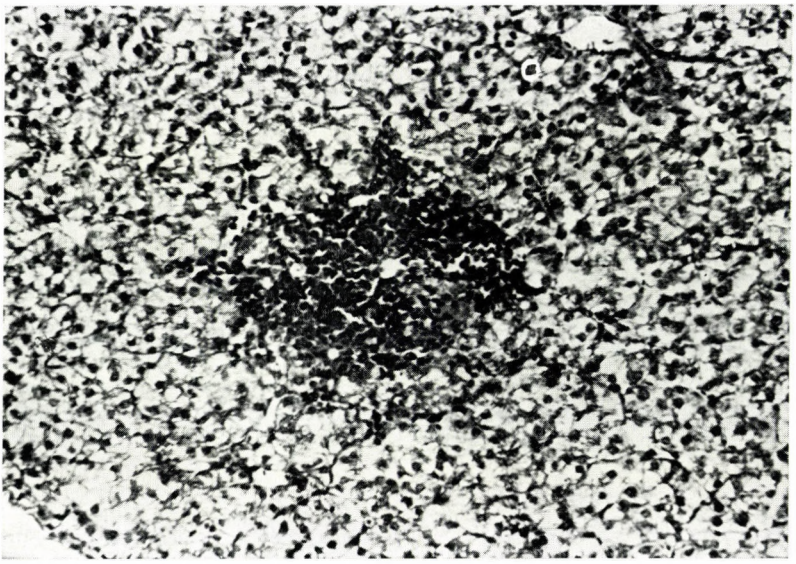

Fig. 15. Detail of liver from 2-day-old chicken exposed by contact to Marek's disease agent. The focal infiltration around the central vein consists of differentiated lymphocytes (Haemalaun and eosin, ×240)

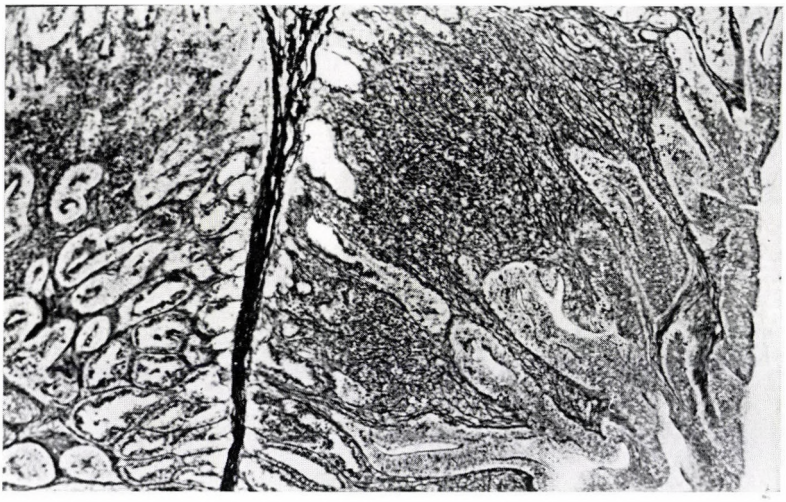

Fig. 16. Detail of proventriculus from a 76-day-old chicken exposed by contact to Marek's disease agent. Note the structure of the reticular fibres in infiltrated areas of the deep proprial glands (Gömöri's silver impregnation technique, ×45)

Of the extraneural organs, the heart frequently showed lesions at the borderline between the myocardium and epicardium; the infiltrating cells were chiefly arranged in rows, less often focally (Fig. 9). In the liver, the infiltrating

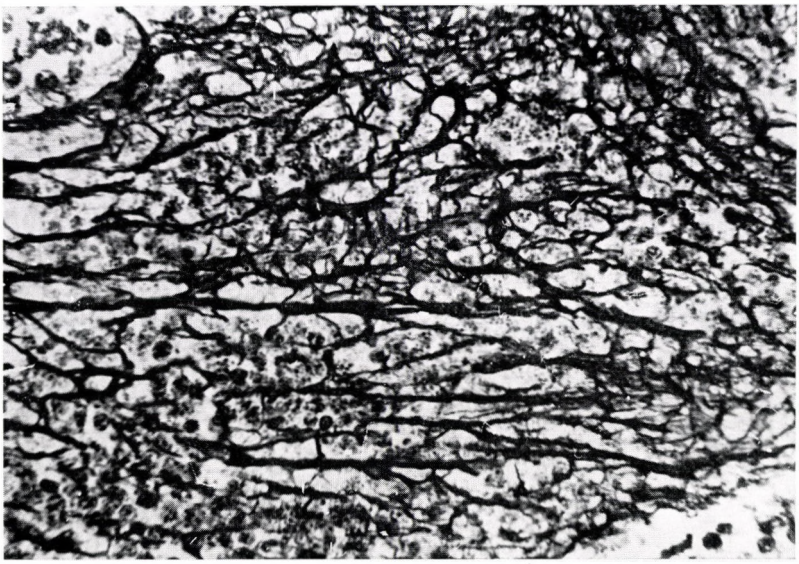

Fig. 17. Detail of Fig. 16 at higher magnification (×600). The proliferated reticulum fibres are seen disseminating in coarse structure

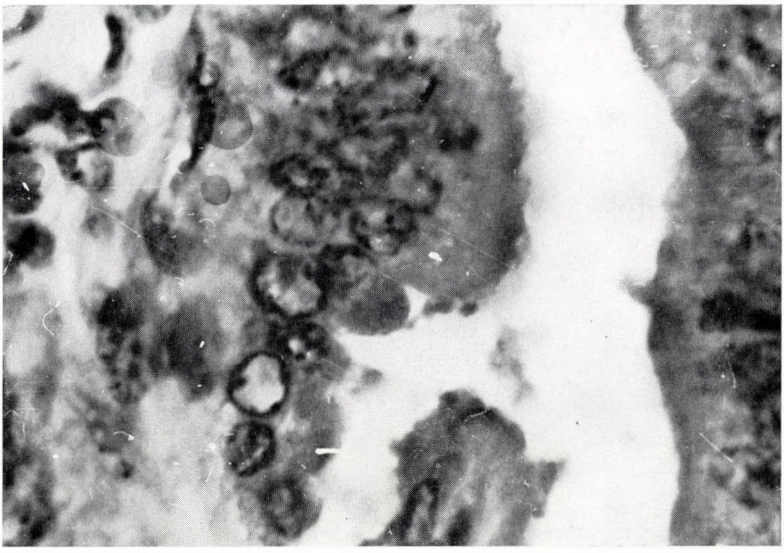

Fig. 18. Detail of proventriculus from a 76-day-old chicken exposed by contact to Marek's disease agent. Note the nuclear hypertrophy and perichromasia in some of the cylindrical epithelial cells lining the central duct of the deep proprial glands. (Haemalaun and eosin, ×1400)

cells formed circumscribed foci in the parenchyma and/or along the blood vascular walls (Fig. 10). Similar foci with, however, less distinct margina occurred in the kidney between the tubules and sometimes around the glome-

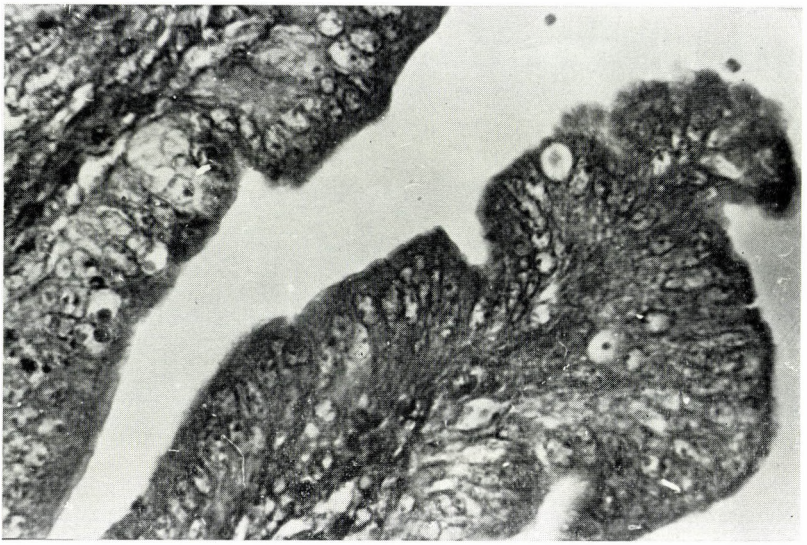

Fig. 19. Detail of proventriculus from a 76-day-old chicken exposed by contact to Marek's disease agent. Nuclear inclusions are distinguishable in some of the cylindrical epithelial cells lining the central duct of the deep proprial glands (Haemalaun and eosin, ×240)

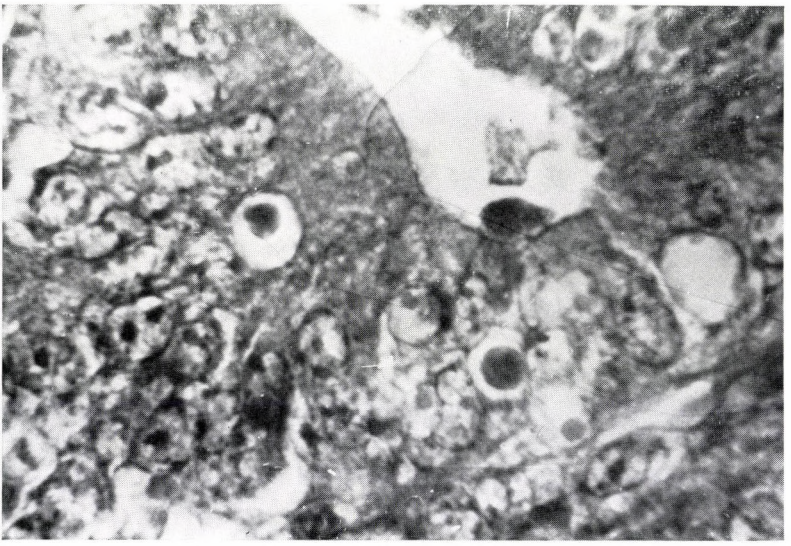

Fig. 20. Detail of proventriculus from a 69-day-old chicken exposed by contact to Marek's disease agent again showing nuclear inclusions in some of the cylindrical epithelial cells lining the central duct of the deep proprial glands (Farkas—Mallory stain, ×1400)

ruli (Fig. 11) while in the bone marrow, infiltrating cells were scattered in small groups.

Of the lesions in organs not examined regularly unless grossly changed, those in the proventriculus were most worthy of note: here infiltrating cells

always appeared between the deep glands of the propria arranging either focally or in rows (Fig. 12).

In all extraneural organs the infiltration consisted of lymphoid cells among which maturing blast-type cells, with a high ribonucleic acid content and often in the stage of mitosis, appeared in quite high numbers, even in relatively mild (++) lesions (Figs 13, 14). In birds younger than 27 days most infiltrating cells were mature lymphocytes (Fig. 15). Proliferation of reticular fibres which occurred in the proventriculus irrespective of age could be demonstrated in the involved areas of the heart, liver and kidney only at 76 days (Figs 16 and 17).

Depending on the degree of infiltration, the damaged tissue, above all that of the proventriculus, heart and liver, occasionally showed signs of nuclear degeneration, viz. karyomegaly and perichromasia (Fig. 18). In a few cases a roundish, pale-staining acidophilic aggregate with slightly indistinct margins was seen in the centre of the degenerated nucleus (Figs 19, 20).

Discussion

Of the 30 chickens examined betweeen the ages of 41—76 days, 28 (83.3%) exhibited infiltrative lesions both in parenchymatous organs and in the nervous system. Changes in the central nervous system without involvement of the peripheral nerves were encountered in only five cases, but once the peripheral nerves had been affected, the infiltrations were generally present in all of the four cases. An association of organic lesions with infiltrative neural changes could be demonstrated in 12 of the 21 birds examined between the ages of 18—39 days (57.1%). Since birds less than 18 days old never showed neural lesions, no specimens of nerve tissue were examined from chicks younger than 10 days.

We conclude, in accordance with MALIK (1969), that the neural lesions found by us did not differ from those encountered in the classical form of Marek's disease. Likewise, the presence of oedema of peripheral nerves without cell infiltration, which has been considered a characteristic change of acute Marek's disease (PAYNE and BIGGS, 1967; MALIK, 1969), also occurs in the classical form. Furthermore, it seems likely that the low incidence of paralytic symptoms in outbreaks of the acute form is due to the fact that the neural axon remains intact for a relatively long time (GRATZL and KÖHLER, 1968).

The presence of immature, neoplastic cells among the infiltrating lymphocytes was observed in both central and peripheral nervous systems of five birds and in the peripheral nerves alone of three more. Blast-like cells of a neoplastic character according to their mitotic activity as well as their increased ribonucleic acid content commonly appeared in various extraneural organs of birds older than 27 days. This finding was verified in conformity with those of

PAYNE and BIGGS (1967). In birds less than 27 days old, the overwhelming majority of the infiltrating cells were mature lymphocytes in which no increase of the cytoplasmic ribonucleic acid component could be demonstrated, either with methyl green-pyronine or with Mayor's acridine orange technique. Lymphocytic cell infiltration is probably due to viral action on the reticulo-endothelial system. FLETCHER et al. (1971) cite in support of it CARLSON and ALLEN's finding that lymphoid cells appeared already 6 hours after the viral inoculation into the wing web and the affected tissues were predominantly infiltrated by such cells after 36 hours. In our material lymphoid cell infiltrations occurred as early as 2 days of age (Fig. 15).

Lymphoid cell infiltrations in visceral organs were not usually manifested by any typical macroscopic lesions. Since the white pulp of the spleen normally contains some elements of lymphoid cells, depending on the immunobiological status of the involved bird, in accordance with FLETCHER's observation this organ was not exactly examined. In a similar way, the lymphoid cell infiltrations or lymph follicles in the wall of proventriculus are not regarded as pathological, as they are often found in healthy birds between the deep glands of the proventricular lamina propria. On the other hand, the nuclear degeneration of proventricular epithelial cells corresponds to the Cowdry type A nuclear inclusion bodies characteristic of the herpesvirus group; similar inclusions were reported by FLETCHER et al. (1971) in the epithelial cells of feather follicles.

SUMMARY

On a poultry farm with a history of the acute Marek's disease, 87 birds from a large broiler flock were exposed by contact to Marek's disease agent and later on examined for clinical signs, gross and microscopic lesions over the period from hatching to marketing (1—76 days of age). Detailed histological examinations of the central and peripheral nervous systems and certain extraneural organs revealed that 83.3% of birds aged 41—76 days and 57.1% of those aged 18—39 days displayed lymphoid cell infiltrations in both intra- and extraneural localizations. No neural lesions were recognized in birds younger than 18 days old, but lesions of parenchymatous organs occurred as early as two days of age and infiltrations showing a neoplastic character from 27 days. Neoplastic neural lesions were found in only a few instances. Cowdry type A nuclear inclusion bodies were seen in several cases, principally in epithelial cells of the proventriculus. The microscopic changes were not usually manifested by macroscopic examination.

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IMMUNOLOGICAL STUDY OF RABBIT CYSTICERCOSIS

IV. LOCALIZATION OF PRECIPITATING ANTIBODIES BY IMMUNOELECTROPHORESIS IN THE SERUM OF RABBITS EXPERIMENTALLY INFECTED WITH CYSTICERCUS PISIFORMIS (BLOCH, 1780)

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The production of humoral antibodies by rabbits in response to infections with *Cysticercus pisiformis* (Bloch, 1780), the larval form of *Taenia pisiformis* (Bloch, 1780) Gmelin, 1790 (Cestoda: Taeniidae), has repeatedly been confirmed by passive serum-transfer experiments (KERR, 1934, 1935; SIMA, 1937; CAMPBELL, 1938; LEONARD, 1940; LEONARD and LEONARD, 1941; LIEBMANN and BOCH, 1960; BACHMANN, 1964; NÉMETH, 1970). Antibodies to this parasite have also been demonstrated in the serum of infected rabbits by various serological methods with the use of sundry antigenic preparations of the tapeworm or the cysticerci (CAMPBELL, 1938; SILVERMAN, 1955; SHUL'TS and ISMAGILOVA, 1965; NÉMETH, 1965).

In a previous experiment, the serum of rabbits experimentally infected with the eggs of *T. pisiformis* produced precipitation lines in agar gel against whole saline extracts of both tapeworms and cysticerci (NÉMETH, 1965). Subsequent analyses by agar gel diffusion precipitation tests revealed that the invasion of the parasite induces a complex precipitin response in the rabbits. Four precipitating antibodies of different antigenic specificity were detected in serum samples collected at spaced intervals after single or double infections. The antigenic components reacting with these antibodies were found to be contained in the acid-soluble fraction of the whole saline extracts of tapeworms and cysticerci (NÉMETH, 1971).

No information is available in the literature on the class of immunoglobulins with which these antibodies are associated, however, so that their types has still to be defined. To further characterize the precipitin response evoked by invasions of oncosphaeres of *T. pisiformis*, experiments were carried out in which the relationship between immunoglobulins and precipitins was studied. By submitting original or concentrated serum to immunoelectrophoresis we have been able to localize these antibodies in the serum proteins of infected rabbits with the aid of various antigenic preparations of the parasite. The results of these studies are presented in this paper.

Materials and methods

Antigens. Whole saline extracts of *T. pisiformis* (TpE) and *C. pisiformis* (CpE) as well as "secretions and excretions" of the two stages (TpSE and CpSE) were obtained as previously described (NÉMETH, 1971).

Sera. The preparation of rabbit anti-*T. pisiformis* immune serum (anti-Tp) and anti-*C. pisiformis* immune serum (anti-Cp) was also as before (NÉMETH, 1971). The following additional sera were used:

Serum from infected rabbits. Twenty white New Zealand rabbits, aged 6—7 weeks and weighing 700—900 g, were each infected with 500 *T. pisiformis* eggs. The eggs were isolated from the gravid segments of tapeworms recovered from artificially infected dogs (NÉMETH, 1970) and introduced into the stomach of the animals down a tube fitted to a syringe. Sixty days after the primary infection, half of the rabbits were reinfected with 10,000 eggs. All animals were bled and dissected on the 100th day. Blood was drawn from the marginal ear vein at 10-day intervals and the serum samples were stored without preservative at —20 °C.

Preparation of polyspecific goat immune serum against whole rabbit serum (goat anti-rabbit immune serum). Three adult female goats with body weights of 50—60 kg were sensitized with pooled normal serum of adult rabbits that seemed to be healthy on clinical and anatomical evidence. Two ml of whole serum, emulsified in an equal volume of complete Freund's adjuvant,* was inoculated intramuscularly into each animal at multiple sites; the inoculation was repeated with the same dosage four weeks later. After another four weeks, the animals were injected subcutaneously six times at 5-day intervals with 5 ml serum diluted 1 : 3 with isotonic saline. If the antibody titre was high enough in a serum sample tested one week after the last injection, and if the serum proved to contain antibodies to an adequate number of serum components, as judged by a trial immunoelectrophoretic run, blood was drawn from the goat via the jugular vein. If the sample was of low titre, inoculations were continued. Immune serum was preserved by adding 0.01% merthiolate and 0.25% phenol, and stored at 4 °C.

Preparation of sheep immune serum against rabbit-serum globulins (sheep anti-rabbit globulin immune serum). Young sheep of 20—25 kg body weight were sensitized with rabbit serum globulins obtained from pooled normal rabbit serum by precipitation with one-third saturated ammonium sulphate as described by STELOS (1967). Aliquots of globulin solution containing 90 mg protein were emulsified in equal portions of complete Freund's adjuvant and injected intramuscularly into each animal at multiple sites in a total volume of 4 ml. Four weeks later, inoculation was repeated with the same dosage. After another four weeks, the animals were injected subcutaneously six times at 5-day intervals with a dose of globulin solution containing 50 mg protein. Blood was withdrawn from the sheep 8—10 days after the last inoculation provided the immune titre was adequate; if not, inoculations were continued. Immune serum was preserved with 0.01% merthiolate and 0.25% phenol, and stored at 4 °C.

* Difco Laboratories, Detroit, Michigan, U.S.A.

Immunoelectrophoresis

Immunoelectrophoretic analyses were performed in 1.5% agar gel* dissolved in barbital sodium-sodium acetate-hydrochloric acid buffer (pH 8.6, $\mu = 0.05$) containing 0.01% merthiolate. The agar solution was poured onto 6.5×9.0 cm glass plates to a depth of 1.5 mm and left to congeal, the plates being kept in a moist chamber at 4 °C until used the next day. Electrophoretic runs were carried out at room temperature under a potential gradient of 4 V/cm. Whatman No. 1 filter paper strips connected the agar plates with the buffer tanks, which contained the same buffer as the agar gel. Immunodiffusion following electrophoresis took place in a humid chamber at room temperature; its duration varied according to the reagents employed. After the precipitin bands had been formed, the immunoelectrophoretic plates were washed dried and then stained with amidoblack 10 B or acid fuchsin following BACKHAUSZ (1967).

Agar gel diffusion precipitation technique

The agar gel diffusion precipitation test was effected by the radial double diffusion method in plates of 1.5% Difco Noble-Agar in phosphate-buffered saline (PBS: 0.01 M, pH 7.1) containing 0.01% merthiolate and 0.003% methyl orange, following the previously adopted technique (NÉMETH, 1971).

Concentration of serum

In order to enhance precipitin activity, serum samples found to produce too weak precipitation were concentrated 5- to 6-fold by salting out with ammonium sulphate (NÉMETH, 1971).

Protein determinations

These were performed by the biuret method as described by CHASE and WILLIAMS (1968).

Results

Post-mortem examination carried out 100 days after the first administration of *T. pisiformis* eggs showed both inoculations to have been successful. Rabbits with a single infection harboured, on average, 228.6 (range: 82—391) encapsulated, fully mature cysticerci on the omentum and under the pelvic peritoneum. An average of 394 (range: 128—427) cysticerci were recovered from the rabbits reinfected on day 60 of the experiment. Besides the mature

* Special Agar-Noble, Difco Laboratories, Detroit, Michigan, U.S.A.

cysticerci, still migrating young larvae, which had obviously developed from the oncosphaeres of the reinfecting dose, were also found in the abdominal cavity of rabbits in this group.

Serum samples collected before the 12th—15th day of infection produced no precipitate with CpE and TpE antigens in the agar gel diffusion precipitation test. Up to the 30th—40th day the serum of some rabbits developed mostly weak precipitation lines, but thereafter samples from all animals precipitated up to the very end of the experiment, the most marked precipitin

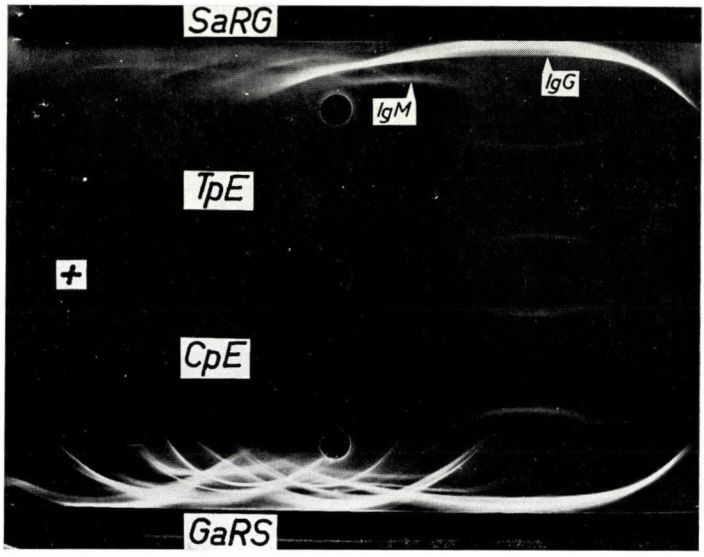

Fig. 1. Precipitins to "somatic" antigens of *T. pisiformis* and *C. pisiformis* localized by immunoelectrophoresis in the serum of rabbits infected with *C. pisiformis*. After electrophoresis of a serum sample (wells) collected 30 days after the infection, the horizontal troughs were filled as follows: SaRG, Sheep antiserum to rabbit globulins; GaRS, Goat antiserum to rabbit whole serum; TpE, *T. pisiformis* whole saline extract (3 mg protein/ml); CpE, *C. pisiformis* whole saline extract (4 mg protein/ml). Precipitin lines were formed against both TpE and CpE in the position of IgG immunoglobulins

bands being observed between the 30th and 70th days of infection. The precipitin activity of serum samples varied not only during the course of infection, however, but also markedly between individuals at the same stage of infection. This was evidenced by the differences in both the number and intensity of precipitation bands produced. Most of the serum samples from singly infected rabbits formed only a single band to CpE and TpE, though two clear lines developed with the serum collected from some animals 30—70 days after infection.

Superinfection with 10,000 eggs conspicuously augmented the precipitin activity above that of singly infected animals and after the 20th day of rein-

fection the serum of the majority of rabbits formed three, in some cases even four, bands against CpE or TpE.

In serum samples shown to contain a demonstrable amount of precipitins by the gel diffusion test, the electrophoretic mobility and the type of serum components carrying the antibodies were determined by immunoelectrophoresis as follows. The serum was first electrophoretized in agar gel. Two longitudinal troughs were then cut out parallel to the direction of electrophoresis; one of the troughs was filled with different dilutions of CpE, TpE,

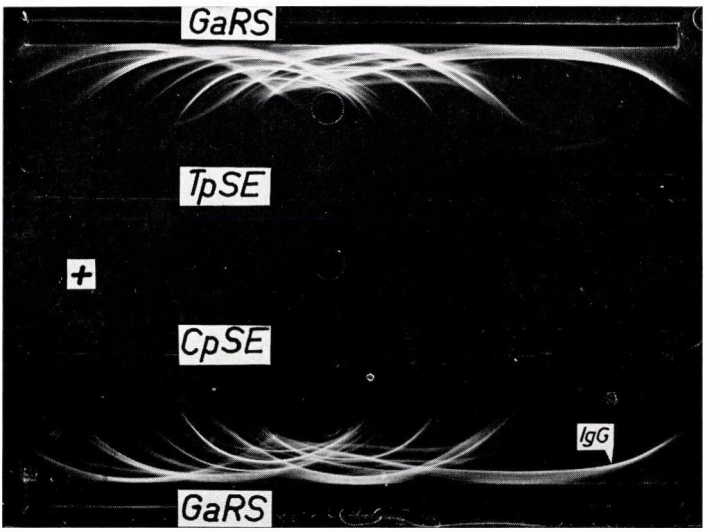

Fig. 2. Precipitins to "secretions and excretions" antigens of *T. pisiformis* and *C. pisiformis* localized by immunoelectrophoresis in the serum of rabbits infected with *C. pisiformis*. After electrophoresis of a serum sample (wells) collected 30 days after the infection, the horizontal troughs were filled as follows: GaRS, Goat antiserum to rabbit whole serum; TpSE, "Secretions and excretions" antigen of *T. pisiformis* (3 mg protein/ml); CpSE, "Secretions and excretions" antigen of *C. pisiformis* (2.5 mg protein/ml). Precipitin lines were formed against both TpSE and CpSE in the position of IgG immunoglobulins

CpSE or TpSE, the other with goat anti-rabbit immune serum or sheep anti-rabbit globulin immune serum, and these were allowed to diffuse at right angles to the separated components of the serum sample.

Serum samples of diverse precipitating activity, collected at different stages of infection, were examined individually with this method. Samples with the same reactivity obtained in the same stage of infection were pooled in equal proportions; sera of low precipitating activity were concentrated beforehand by ammonium sulphate precipitation. The most characteristic results are presented in Figs 1—4.

As can be seen, the precipitation bands against the various concentrations of CpE, TpE, CpSE and TpSE coincided with the maximum of the IgG

precipitation arc. There was no variation in the localization of precipitating antibodies between serum samples obtained at different stages of the infection. By comparing the precipitation bands developed against the different antigen solutions with the immunoelectrophoretic picture of the serum it was found that the precipitation lines produced by the worm antigens and the corresponding antibodies differed only with respect to the activity of the serum samples but never in their position. Every serum sample consistently produced precipitation bands against the antigen solutions corresponding to the maximum

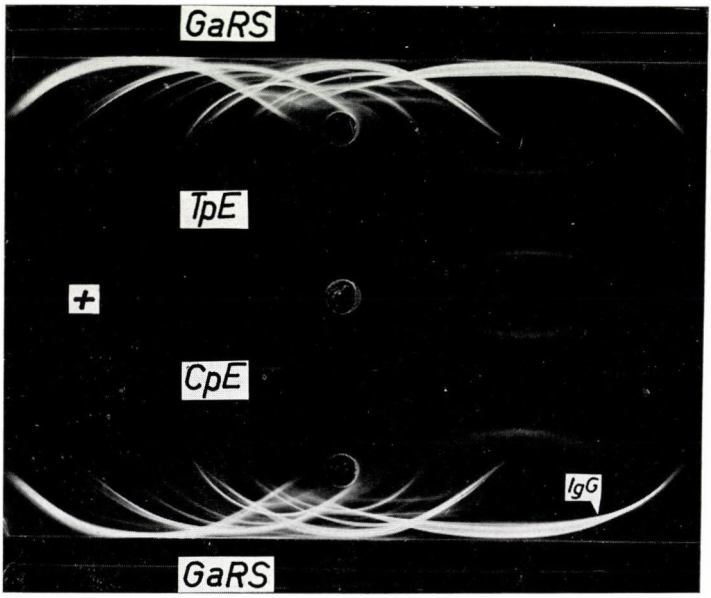

Fig. 3. Immunoelectrophoretogram of rabbit serum showing the localization of precipitins to "somatic" antigens of *T. pisiformis* and *C. pisiformis* 40 days after reinfection. After electrophoresis of the serum sample (wells) the horizontal troughs were filled as follows: GaRS, Goat antiserum to rabbit whole serum; TpE, *T. pisiformis* whole saline extract (4 mg protein/ml); CpE, *C. pisiformis* whole saline extract (5 mg protein/ml)

of the IgG arc but these bands never extended to the entire area of the electrophoretic mobility of the IgG globulins. The result was the same whether the serum was collected after the first or the second infection, and it made no difference whether the "somatic" or the "metabolic" antigens of the worms or the cysticerci were used to demonstrate the precipitins.

It should, however, be stressed that, owing to the strongly variable serum precipitin content, the immunoelectrophoretic analysis of every serum sample required diverse antigen concentrations and various diffusion times for the optimal formation of precipitation lines. It was thus impossible to demonstrate

in a single run on the same plate all the precipitation lines observed in gel diffusion tests together with the immunoelectrophoretic picture of the serum.

As is apparent from the figures, only a single precipitin line was produced when 10—15 μl serum was analysed, even if the serum under test formed two or more bands in reaction with exactly the same antigens in the agar gel diffusion precipitin test. This was presumably mainly because the amount of antibodies lay below the threshold for producing perceptible precipitation against all antigenic components.

The precipitins responsible for all lines in the gel diffusion precipitation could be localized by simultaneous analyses of 20—30 μl of the same serum

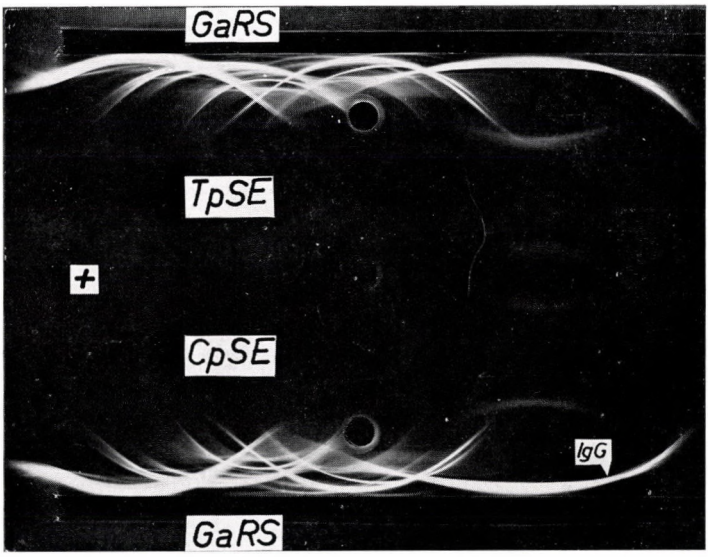

Fig. 4. Immunoelectrophoretogram of rabbit serum showing the localization of precipitins to "secretions and excretions" antigens of *T. pisiformis* and *C. pisiformis* 40 days after reinfection. After electrophoresis of a serum sample (wells) the horizontal troughs were filled as follows: GaRS, Goat antiserum to rabbit whole serum; TpSE, "Secretions and excretions" antigen of *T. pisiformis* (4 mg protein/ml); CpSE, "Secretions and excretions" antigen of *C. pisiformis* (mg protein/ml)

on several plates. Different concentrations of antigen solutions were then diffused against the separated serum components on one plate, and the immune sera to rabbit serum proteins on another plate. The plates were thereafter incubated until the optimal formation of the precipitation bands. The number of bands so formed was equal to that observed in the preliminary gel diffusion analysis. These bands lay parallel to each other between the antigen troughs; the strongest line was next to the antigen trough, while the other ones, which were considerably weaker and shorter, located further away from the antigen-containing troughs. Comparison of the arrangement of the precipitation lines

with the immunoelectrophoretic pattern showed the bands to coincide with the maximum of the IgG precipitation arc.

Localization of precipitins was particularly difficult in serum displaying weak precipitin activity, as in these cases it needed several attempts to observe even an occasional faint line. Preliminary concentration by salting out with ammonium sulphate made it clear, however, that the precipitins migrated with the IgG globulins.

Precipitating antibodies in the anti-Tp or anti-Cp immune serum of hypersensitized rabbits showed the same electrophoretic mobility as those in the serum of the infected rabbits. Those sera with fairly high antibody titre (i.e. with precipitin titres between $1 : 2^{11}$ and $1 : 2^{14}$ on titration with stock TpE or CpE antigen containing 15 mg protein per ml) also produced precipitation against the different concentrations of TpE and CpE only at the maximum of the IgG arc. The most intense and longest precipitin band was found next to the antigen trough and even with immune sera with the highest precipitin titre did not extend over the entire area of IgG mobility. Away from the antigen trough, 4—5 much shorter, parallely arranged bands of variable intensity were sometimes formed.

Discussion

CAMPBELL (1938) was the first to demonstrate that the infection of rabbits with *Cysticercus pisiformis* evokes humoral antibodies capable of producing precipitation with the antigenic components of the parasite. The presence of precipitating antibodies was subsequently confirmed by several serological methods using different antigenic preparations (SILVERMAN, 1955, 1956; SHUL'TS and ISMAGILOVA, 1965; NÉMETH, 1965), and detailed investigations have been carried out concerning the dynamics of humoral antibody production (SHUL'TS and ISMAGILOVA, 1965; NÉMETH, 1971) as well as the number of antigen-antibody systems demonstrable with "somatic" and "metabolic" antigens of the parasite in the agar gel diffusion precipitation test (NÉMETH, 1971). Information has been lacking, however, on the immunoglobulin classes to which the precipitins produced in response to the invasion of *T. pisiformis* oncosphaeres belong. Antibodies belonging to different immunoglobulin classes are known to have dissimilar biological properties and to play different roles in the various immune reactions (COHEN and PORTER, 1964; COHEN and MILSTEIN, 1967; STANWORTH and PARDOE, 1967). Consequently, a more exact definition of immune phenomena elicited by the parasite and the role played by the antibodies in the defence mechanism of the host against the invading oncosphaeres requires the determination of the immunoglobulin class(es) to which the antibodies in question belong.

Three immunoglobulin classes are known in the rabbit: IgG, IgM and IgA. These possess a number of common physico-chemical, structural and antigenic features but also certain specifically individual properties, and may be distinguished by the structural, and hence antigenic, differences of their heavy polypeptide chains (COHEN and PORTER, 1964; CEBRA and ROBBINS, 1966; COHEN and MILSTEIN, 1967; STANWORTH and PARDOE, 1967).

There are numerous reports showing that the antibodies produced by rabbits in response to different antigens may belong to any of the three immunoglobulin classes, although in any particular situation one or the other class may be predominant in the serum, depending on the chemistry, physical state, dosage and mode of administration of the antigens, as well as on several not clearly defined factors (see for example BAUER and STAVITSKY, 1961; SVEHAG and MANDEL, 1962, 1964; BAUER et al., 1963; UHR and FINKELSTEIN, 1963; BOREL et al., 1964; GREY, 1964; ONOUE et al., 1964; SHULMAN et al., 1964; SVEHAG, 1964; WEIDANZ et al., 1964; BENEDICT, 1965; FREEMAN and STAVITSKY, 1965; HOCKER and BAUER, 1965; ROBBINS et al., 1965; SCHOENBERG et al., 1965; ALTEMEIER et al., 1966; OSLER et al., 1966; PIKE et al., 1966; SANDBERG and STOLLAR, 1966; ARMSTRONG and SWORD, 1967; CARTER and HARRIS, 1967; PIKE, 1967; SAMLOFF and BARNETT, 1967; WEI and STAVITSKY, 1967).

A search of the available literature has turned up no investigation comparable with the results of the present study, and to our best knowledge there is no report concerning the immunoglobulin classes with which the antibodies produced by rabbits in response to *Taenia* antigens are associated. Even with other helminthic antigens the relationships between immunoglobulins and the antibodies induced by the parasite have so far been studied in only a few instances. MAUSS (1941), on fractionating the serum of rabbits infected with *Trichinella spiralis* by ammonium sulphate precipitation and by ultracentrifugal separations, detected complement-fixing, precipitating and protective antibodies in the euglobulin fraction, although some antibody activity was also present in the pseudoglobulin fraction. Precipitins to *Trichinella* antigens have been demonstrated in the gamma globulin (WRIGHT and OLIVER-GONZÁLEZ, 1943), beta-2 and gamma globulin (BIGUET et al., 1965), IgG globulin (LUPASCO et al., 1964; CRANDALL and MOORE, 1968), and also in the IgG and IgM globulins (KWAN, 1971) fractions, while the fluorescent antibodies have been found to be associated with the IgG and IgM globulins (CRANDALL et al., 1967; SADUN et al., 1968). More recently, CRANDALL and MOORE (1968) demonstrated the presence of anti-*Trichinella* antibodies of all three immunoglobulin classes in the sera of infected rabbits. KWAN (1971) suggested that the bulk of precipitins are carried by the IgG globulins, whereas protective antibodies are of the IgM type.

Fluorescent antibodies in rabbits infected with *Dirofilaria uniformis* are

associated with both the IgG and the IgM globulins (SADUN et al., 1967), while the flocculating antibodies evoked by infection with *Schistosoma mansoni* with IgG alone (ZVAIFLER et al., 1967). GEYER (1965) demonstrated the presence of precipitins in both the beta and gamma globulin fractions of serum from rabbits injected with the filariform larvae of *Strongyloides papillosus*. Some authors suggest that homocytotropic antibodies produced by rabbits in response to helminthic infections belong to an immunoglobulin class other than IgG and IgM (SADUN et al., 1967, 1968; ZVAIFLER et al., 1967).

On the basis of the findings presented in this paper, the conclusion seems to be justified that the rabbit produces only precipitins of the IgG type during the immune response to invasion by *T. pisiformis* oncosphaeres. The precipitin activity of the serum and the number of detected antigen-antibody systems showed considerable individual variations, a phenomenon presumably due to disparities in the animals' reactivity and possibly other factors. However, irrespective of such discrepancies, the serum protein carrying precipitins proved to be of the IgG globulin class at all stages of primary and secondary infection, whether an isotonic saline extract of adult worms and infective cysticerci or their "metabolic" antigens were used to demonstrate the antibodies.

Our results are consistent with the findings of LUPASCO et al. (1964) who, likewise by immunoelectrophoretic method, localized the anti-*Trichinella* precipitins in the IgG globulin fraction, GEYER (1965) in contrast, as noted above, demonstrated precipitins with the same technique also in the beta-globulin zone of serum from rabbits artificially sensitized with *Strongyloides papillosus* larvae.

Although, according to the literature, antibodies of both the IgG and IgM classes can produce precipitation, those of the IgG type are much more potent (GREY, 1964; SHULMAN et al., 1964; WEIDANZ et al., 1964; BENEDICT, 1965; MULHOLLAND et al., 1965; ONOUE et al., 1965; ROBBINS et al., 1965; LINDQVIST and BAUER, 1966; PIKE et al., 1966; TADA and ISHIZAKA, 1966; PIKE, 1967). Had demonstrable amounts of IgM-type precipitins been present in the sera tested, the immunoelectrophoretic analyses against the various antigen preparations would have yielded double-humped precipitation bands in cases of identical, and crossing bands in cases of different, antigenic specificity. However, no such bands were observed, even when the strongly precipitating serum of artificially sensitized rabbits was analysed. Presumably IgM antibodies were either not produced at all or produced only in amounts below the minimum quantity demonstrable by the method employed. Clarification of this point requires further investigations.

Acknowledgement. The author is much indebted to Miss Ilona HALASI for valuable technical assistance.

SUMMARY

Rabbits, 6—7 weeks old, weighing 700—900 g, were infected perorally with 500 eggs of *Taenia pisiformis*, and a group of them was reinfected with 10,000 eggs 60 days later. Serum samples collected at frequent intervals were tested for the presence of precipitins by agar gel double diffusion technique using as antigen "secretions and excretions" and whole saline extracts of both *Taenia pisiformis* and *Cysticercus pisiformis*.

Sera of known precipitin activity were submitted to electrophoresis in agar and subsequently diffused against worm antigens, goat antiserum to rabbit whole serum or sheep antiserum to rabbit globulins. With all sera precipitin lines developed against the antigenic preparations at the position of IgG globulins. It appears therefore, that the precipitating antibodies produced by rabbits in response to invasion by *Taenia pisiformis* oncosphaeres are associated with the IgG immunoglobulin class.

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IMMUNOLOGICAL STUDY OF RABBIT CYSTICERCOSIS

V. CHARACTERIZATION OF THE ANTIBODY RESPONSE TO EXPERIMENTAL INFECTION WITH CYSTICERCUS PISIFORMIS (BLOCH, 1780)

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Rabbits infected with *Cysticercus pisiformis* (Bloch, 1780), the larval form of *Taenia pisiformis* (Bloch, 1780) Gmelin, 1790 (Cestoda: Taeniidae), produce antibodies to the antigenic components of both the adult and larval forms of the parasite. The presence of antibodies in the serum of infected rabbits has so far been demonstrated by ring precipitation (CAMPBELL, 1938; SHUL'TS and ISMAGILOVA, 1965), larval precipitation (SILVERMAN, 1955, 1956), agar gel diffusion precipitation (NÉMETH, 1965, 1971), complement fixation (SHUL'TS and ISMAGILOVA, 1965 and indirect haemagglutination (SHUL'TS and ISMAGILOVA, 1965; NÉMETH, 1965).

The characteristics of the humoral antibody response to invasions of the parasite are, however, largely unknown, and no information is yet available on the class of antibodies produced in the course of the infection. Previous investigations have revealed that the oncosphaeres of *T. pisiformis* induce a complex precipitin response in rabbits. In serum samples collected at spaced intervals after the experimental infections, four types of antibodies of different antigenic specificity were demonstrated by the agar gel diffusion precipitin test using antigenic preparations from *T. pisiformis* and *C. pisiformis* (NÉMETH, 1971). The immunoelectrophoretic analysis of such precipitating rabbit sera with polyspecific anti-rabbit immune serum as well as both "metabolic" and "somatic" antigens of *T. pisiformis* and *C. pisiformis* revealed that precipitins belonging to the IgG immunoglobulin class were produced (NÉMETH, 1972).

In attempts to further characterize the humoral antibody response, additional experiments were carried out in which the heterogeneity and sequence of antibody productions as also the relationship between immunoglobulins and antibodies were studied during both primary and secondary infections with *C. pisiformis*. For this purpose (i) serum samples were fractionated by salt precipitation, by preparative zone electrophoresis in agar and by ion exchange chromatography, and the serum fractions were then examined for the presence of antibodies by larval precipitin, agar gel diffusion precipitation and indirect haemagglutinations tests; (ii) mercaptoethanol sensitivity of the antibodies was assayed; (iii) immune sera were produced to antigen-antibody precipitates made with serum of infected rabbits and the antigens of *T. pisiformis* and *C. pisiformis*, and the types of the serum protein components precipitated by these anti-complex antisera were then identified through immunoelectrophoresis of pooled normal rabbit serum.

The results of these studies are reported in the present paper.

Materials and methods

Rabbits

White New Zealand rabbits aged 5—7 weeks and weighing 500—1000 g were used, kept under the conditions described earlier (NÉMETH, 1971).

Antigens

The preparation of the extracts of *T. pisiformis* (TpE) and *C. pisiformis* (CpE) used as antigens in the agar gel diffusion precipitin test has already been described (NÉMETH, 1971). The antigen employed for the indirect haemagglutination test was prepared from *T. pisiformis* according to LANCEFIELD's technique (1928) with the modification of NÉMETH (1965).

Taenia pisiformis eggs

Eggs were isolated from gravid proglottids collected from artificially infected dogs (NÉMETH, 1970). The rabbits were infected by introducing the required doses of eggs in 5 ml saline into the stomach through a rubber tube fitted to a syringe.

Migrating larvae

Migrating larvae used for the *in vitro* larval precipitin test were harvested from rabbits infected with 500 *T. pisiformis* eggs. The animals were sacrificed between days 24 and 30 following infection. Freely moving young cysticerci were washed away from the abdominal cavity of the skinned animals with isotonic saline, collected in a measuring cylinder and cleaned by 5 or 6 subsequent decantations in isotonic saline. After removal of the last washing fluid, the larvae were placed into Hanks' solution containing 300 I.U./ml crystalline penicillin and 0.2 mg/ml streptomycin, and utilized within 24 hrs. Strictest sterility was not observed, but sterile materials and equipments were used throughout the procedure.

Mature cysticerci

Rabbits artificially infected with *T. pisiformis* eggs were killed after the 8th week of infection. Bladderworms encysted on the peritoneum were taken out of the abdominal cavity together with the adjacent tissues, and the larvae were released by careful disruption of the connective tissue capsules in isotonic saline. Damaged cysticerci were picked out under a stereoscopic microscope at a 4- to 5-fold magnification, and discarded. Apparently intact larvae were washed by decantation in four or five changes of 37 to 38 °C isotonic

saline and then incubated for 60 minutes at 38 °C in saline containing 300 I.U. crystalline penicillin and 0.2 mg streptomycin per ml. After three subsequent washings with sterile saline the larvae were used on the day of preparation.

Sera

The preparation of anti-*T. pisiformis* rabbit immune serum (anti-Tp), anti-*C. pisiformis* rabbit immune serum (anti-Cp), and polyspecific goat immune serum to rabbit whole serum (goat anti-rabbit serum) has already been described (NÉMETH, 1971, 1972). The following additional sera were applied.

Normal rabbit serum

Apparently healthy adult rabbits of 3—4 kg were bled through the carotid artery into separate dishes and then dissected. The sera of pathologically normal animals not infected with *C. pisiformis* were pooled and stored at —20 °C until use.

Serum of infected rabbits

Serum was collected from rabbits artificially infected with *T. pisiformis* eggs. Forty animals were each infected with 500 eggs, 10 animals served as uninfected controls. Ten rabbits in the infected group, the serum of which produced marked precipitation with CpE in the agar gel diffusion precipitin test, were bled on the 30th day following infection, while 15 animals were reinfected with 10,000 eggs on day 60. All the experimental rabbits were dissected and carefully examined to evaluate the success of the infections and to determine the cysticercus burden of the infected group, as well as to identify the occasional spontaneous infections in the control group. Blood samples were drawn from the marginal ear vein of all animals at 7—10 day intervals following the first infection. Sera were stored without preservative at —20 °C until use. Serum samples collected at the same stage of infection were pooled in equal proportions for salt fractionation, preparative electrophoresis and anion exchange chromatography, whereas individual samples were used for all other analyses. The serum of uninfected animals served as controls.

Production of anti-bovine serum albumin (anti-BSA) and anti-human serum albumin (anti-HSA)

Rabbits weighing 3 to 4 kg were sensitized with BSA* or HSA** in the following manner.

* Phylaxia, Veterinary Biologicals and Feedstuffs, Budapest, Hungary.
** "Human" Institute for Serobacteriological Production and Research, Budapest, Hungary.

Aliquots of a solution containing 10 mg albumin were emulsified in equal volumes of complete Freund's adjuvant* and injected subcutaneously into each animal at multiple sites in a total volume of 2 ml. The same dose of antigen was administered a month later in incomplete Freund's adjuvant. After another four weeks 20 mg albumin in solution were given subcutaneously, and on the next two days a further 5 and 10 mg, respectively, were injected into the marginal ear vein. The last series of inoculations was repeated after a week, and the animals were bled 9 or 10 days following the last intravenous injection. The sera were stored at —20 °C without preservative until use.

Production of anti-rabbit IgG immune serum

Sheep were sensitized with pure rabbit IgG. Aliquots of solution containing 60 mg IgG were emulsified with equal volumes of complete Freund's adjuvant, and administered subcutaneously to 25—30 kg sheep on three occasions at one-month intervals. Four weeks after the third inoculation 50 mg IgG were injected subcutaneously three times at five-day intervals. Blood was drawn from the jugular vein 8 — 12 days after the last inoculation. If necessary, the sheep were resensitized by subcutaneous administration of IgG. The immune sera were preserved by 0.25% phenol and 0.01% merthiolate, and stored at 4 °C.

Production of anti-rabbit IgG + IgM immune serum

Sheep were injected with a BSA — rabbit anti-BSA antigen-antibody precipitate which was prepared as follows. The anti-BSA rabbit serum, previously decomplemented with HSA — rabbit anti-HSA antigen-antibody precipitates, was mixed with BSA to equivalence. The mixture was incubated for 2 hours at 37 °C and left overnight at 4 °C. The precipitate formed was centrifuged for 15 min. at 3000 revolutions per minute (r.p.m.) and thoroughly washed five or six times with 2—4 °C isotonic saline in a total amount four times the initial volume of anti-BSA immune serum. Each washing was followed by a 15-min. centrifugation at 3000 r.p.m. After the last centrifugation the precipitate was suspended in saline and stored at 4 °C until use. Aliquots of the precipitate with a total protein content of 60 mg were then emulsified in complete Freund's adjuvant and administered subcutaneously to sheep at multiple sites. A month later inoculation was repeated with the same dose. After another four weeks the animals were injected intramuscularly three times at 7-day intervals with an aliquot of the precipitate in a suspension containing 20 mg protein. Blood was drawn from the jugular vein 8—12 days after the last inoculation. If necessary, the sheep were resensitized. Immune

* Difco Laboratories, Detroit, Michigan, U.S.A.

sera were preserved with 0.25% phenol and 0.01% merthiolate, and stored at 4 °C. Immunoelectrophoretic analysis confirmed that they precipitated only IgG and IgM globulins.

Preparation of anti-rabbit IgM immune serum

This antiserum was obtained from the anti-rabbit IgG + IgM sheep immune serum by absorption of anti-IgG antibodies with immunochemically pure rabbit IgG insolubilized with glutaraldehyde* according to the procedure of AVRAMEAS and TERNYNCK (1969). Absorption with fresh doses of insolubilized IgG was repeated until the disappearance of all anti-IgG antibodies. Completeness of absorption was verified by the agar gel diffusion precipitin test; the serum reacted only with the IgM immunoglobulin.

Preparation of anti-complex antisera

White Leghorn cocks were sensitized with antigen-antibody precipitates consisting of *Taenia* and *Cysticercus pisiformis* antigens and the corresponding serum antibodies.

Three different serum pools were used for the preparation of the antigen-antibody complexes:

Pool I: Pooled serum of rabbits killed on day 30 of the primary infection. The haemagglutination (HA) titre was 1 : 128 and the antibody protein content, as determined by the quantitative precipitation test, amounted to 0.19 mg/ml.

Pool II: Pooled serum from rabbits killed 40 days after the reinfection: HA titre 1 : 2 048, antibody protein content 0.96 mg/ml.

Pool III: Anti-Cp and anti-Tp rabbit immune serum mixed in equal volumes: HA titre 1 : 16,384, antibody protein content 4.9 mg/ml.

The serum pools were defatted and decomplemented prior to preparation of the antigen-antibody complexes. Two different ways were used for the preparation: (a) Two hundred live, infective cysticerci were incubated in 50 ml of each serum pool for 4 hours at 37 °C and overnight at 4 °C. The cysticerci were removed next day and the precipitate was centrifuged for 15 min. at 3000 r.p.m. then suspended in isotonic saline. After adding fresh cysticerci to the serum, the procedure was repeated. The precipitates obtained in this way were collected, suspended in four times the original serum volume of cold isotonic saline and centrifuged for 15 minutes at 3000 r.p.m. This washing procedure was repeated five or six times, discarding the supernatant each time. After the last centrifugation, the precipitate was suspended in isotonic saline containing 0.01% merthiolate, and stored at 4 °C until use. (b) Live

* Reanal, Budapest.

cysticerci were incubated in the serum and the precipitates formed were collected in the same way described in the foregoing. TpE and CpE antigenic solutions pooled in equal proportions were then added to the serum at equivalence, and the mixture was kept for 2 hours at 37 °C and overnight at 4 °C. The antigen-antibody precipitates obtained with the two procedures were pooled, thoroughly washed and stored as described in the previous paragraph.

Antigen-antibody precipitates were prepared from each serum pool with both the above techniques. Three cocks were sensitized with each type of the complexes, as follows: A suspension of precipitates containing 10 mg total protein in complete Freund's adjuvant was injected subcutaneously at multiple sites twice at a one-month interval. Four weeks after the second inoculation, aliquots of the precipitates representing 5 mg protein were injected intramuscularly in isotonic saline three times at five-day intervals. The cocks were exsanguinated 9—12 days after the last injection. The anti-complex antisera were preserved with 0.01% merthiolate and 0.25% phenol, and stored at 4 °C.

Serological methods

Indirect haemagglutination, agar gel diffusion precipitin and *in vitro* larval precipitation tests were employed to detect the antibodies to the antigens of the parasite.

Indirect haemagglutination test (IHA) was carried out with tanned erythrocytes as described earlier (NÉMETH, 1965). Before each examination the haemagglutination system was checked by testing four standard sera with titres of 1 : 16, 1 : 256, 1 : 1024 and 1 : 4096. Results were not accepted unless the standard sera reacted with the antigen-treated red blood cells according to their titre and the requisite controls were clear. From each test sample serial two-fold dilutions were made, beginning at 1 : 2, 1 : 3 and 1 : 5 initial dilutions, and the highest dilution still yielding full haemagglutination was regarded as the titre of reaction.

Agar gel diffusion precipitation test was carried out by the radial double diffusion method in 1.5% agar gel* dissolved in phosphate-buffered saline (PBS: 0.01 M, pH 7.2) containing 0.01% merthiolate and 0.003% methyl orange (NÉMETH, 1971).

In vitro larval precipitation test was performed with young cysticerci in agglutination tubes. Three or four larvae were placed into 0.3—0.4 ml of the test sample and the tubes were kept at 37 °C for two hours then overnight at 4 °C. Results were read under a microscope at about 20- to 25-fold magnification. As controls, in every test larvae were incubated under the same conditions in PBS and in known positive and negative sera. The test was considered

* Special Agar-Noble, Difco Laboratories, Detroit, Michigan, U.S.A.

as positive if clearly visible flocculation could be seen in the sample and the controls were clear.

The mean titre values for the various experimental groups were calculated after KÜHN (1959) from the two-base logarithms of the individual titres.

Mercaptoethanol treatment

The test samples were dialysed at 4 °C against two changes of 0.1 M 2-mercaptoethanol (2-ME) for 48 hours, 0.01 M iodoacetamide for 24 hrs and several changes of PBS (0.02 M, pH 7.5) for 48 hrs. The controls were treated similarly, with the only difference that isotonic saline was employed instead of 0.1 M 2-ME.

Immunoelectrophoresis

Immunoelectrophoretic analyses were carried out in an 1.5% Difco Noble Agar gel buffered with veronal sodium-sodium acetate-hydrochloric acid (pH 8.6, ionic strength 0.05) (NÉMETH, 1972).

Preparative zone electrophoresis in agar gel

One per cent Difco Noble Agar, dissolved in veronal sodium-sodium acetate-HCl buffer (pH 8.6, ionic strength 0.05), was poured onto a $0.7 \times 13 \times 28$ cm plexiglass plate to give a 6 mm thick layer. The agar was left to congeal at room temperature and the agar block was used for electrophoresis after being kept in a humid chamber at 4 °C for 20 hours. Three ml serum, previously dialysed with the above buffer, was applied in 1% agar into a 0.5×15 cm transversal trough cut out of the agar gel, 14 cm away from the cathodal end of the block. The buffer tanks contained the same buffer that was used for preparation of the gel. Saturated potassium chloride solution was layered over the platinum electrodes. Contact between outer and inner buffer tanks was ensured by U-shaped glass tubes of 1.5 cm I.D., filled with a mixture of one part 3% agar and nine parts saturated potassium chloride solutions in distilled water. Whatman-3MM filter paper strips connected the inner tank to the agar block. Electrophoresis was carried out under a potential gradient of 3.0—3.5 V/cm, at room temperature, for 20 hours. After electrophoretic separation, the agar block was cut into 1 cm transversal slices and each segment placed in a separate tube. The agar was then crushed with a glass rod, frozen at —20 °C, thawed at room temperature and spun at 10,000 r.p.m. for 20 minutes at 4 °C. The separated fluid was finally concentrated to the original volume of the sample by pervaporation, dialysed against PBS (0.02 M, pH 7.5) containing 0.01% merthiolate, and stored at 4 °C until use. The protein concentrations of the fractions were determined by Folin-Ciocalteu phenol reagent

Ion exchange chromatography

The column chromatographic procedure with DEAE-Sephadex A-50* anion exchanger was employed. The anion exchanger, swollen in Tris-HCl buffer (0.1 M, pH 7.0) containing 0.5 M NaCl and equilibrated with 0.1 M Tris-HCl buffer (pH 7.0), was packed into a 2×20 cm column. After running two bed volumes of starting buffer (0.1 M Tris-HCl, pH 7.0) through the anion exchanger bed, 5 ml serum previously mixed in equal proportions and dialysed with the same buffer for 24 hours was applied onto the bed surface. Elution of the bound proteins was achieved with a salt gradient linearly increasing to 0.5 M NaCl in 0.1 M Tris-HCl buffer (pH 7.0). Flow rate was 0.3—0.4 ml per min. The effluent was collected in 5-ml fractions and the protein distribution was determined by measuring the O.D. of the fractions at 280 nm with a Unicam SP-500 spectrophotometer. The collected fractions were analysed with anti-rabbit IgG and anti-rabbit IgM immune sera by micro-immunodiffusion test, and the eluates were pooled into four fractions according to the location of IgG and IgM globulins and to the peaks of the elution curve, as shown in Figs 5 and 6. The pooled fractions were then concentrated by ultrafiltration to the original volume of the sample, dialysed against several changes of PBS (0.02 M, pH 7.5), analysed immunoelectrophoretically and tested for the presence of antibodies.

Salt fractionation

The serum sample was diluted to 1 : 5 with isotonic saline, and precipitated under continuous stirring by dropwise addition of saturated ammonium sulphate in half the total volume of the diluted sample. The mixture was incubated at room temperature for 4 hours and then centrifuged at 3000 r. p. m. for 15 minutes. The precipitate was washed three times in 40% saturated ammonium sulphate, followed each time by a centrifugation at 3000 r.p.m for 15 minutes. After the last washing the sediment was dissolved in two-thirds the original sample volume with isotonic saline. The ammonium sulphate having been completely removed by dialysis against several changes of saline, the material was dialysed against PBS (0.02 M, pH 7.5) containing 0.01% merthiolate, and finally brought to the original volume of the sample with the same buffer.

The supernatant obtained following the salting out and centrifugation of euglobulins was dialysed against several changes of isotonic saline to remove ammonium sulphate, concentrated by ultrafiltration to the original volume of the serum, and finally dialysed against PBS (0.02 M, pH 7.5) containing 0.01% merthiolate.

* Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Purification of rabbit IgG globulin

Rabbit IgG was prepared from pooled normal rabbit serum by batch separation on DEAE-Sephadex A-50 anion exchanger. The dry anion exchanger was swollen in Tris-HCl buffer (0.1 M, pH 7.0) containing 0.5 M NaCl, equilibrated by decantation in Tris-HCl buffer (0.1 M, pH 7.0), then the buffer was removed from the ion exchanger by suction on a Büchner funnel. Two hundred g of the wet anion exchanger was suspended in 500 ml serum diluted with an equal volume of Tris-HCl buffer (0.1 M, pH 7.0), and the mixture was incubated at 4 °C for 60 minutes with occasional agitations. The slurry was then filtered on a Büchner funnel and the cake was washed four times in 130-ml portions of buffer, sucking off the fluid each time. The filtrates were pooled, and the whole procedure was repeated with a new portion of fresh ion exchanger. Finally the filtrate was concentrated by ultrafiltration to a protein content of 30—40 mg per ml, dialysed against several changes of PBS (0.02 M, pH 7.5) containing 0.01% merthiolate, and stored at 4 °C. As revealed by immunoelectrophoretic analysis with polyspecific anti-rabbit goat immune serum, the material obtained was pure IgG globulin.

Proteins bound by the ion exchanger were desorbed with 2 M NaCl solution; the ion exchanger was re-used after equilibration with 0.1 M Tris-HCl buffer (pH 7.0).

Purification of rabbit IgM globulin

Thirty ml rabbit serum was diluted to 300 ml and dialysed against phosphate buffer (0.02 M, pH 6.0) at room temperature for 2 hours under continuous magnetic stirring. The buffer was then changed and the dialysis continued for another 4 hours. The white precipitate formed was centrifuged for 15 min. at 3000 r.p.m., dissolved in 5 ml of 5% salt solution and centrifuged once more to remove any insoluble material. The clear supernatant was layered on top of a 2×100 cm Sephadex G-200 gel column prepared and equilibrated in 0.1 M Tris-HCl (pH 8.0) + 0.2 M NaCl buffer according to Borsos and Rapp (1965).

Fractionation was carried out at room temperature with an eluant flow rate of 15 ml/hour. The eluate was collected in 3-ml fractions, and the protein distribution was determined by measuring the O.D. of the fractions at 280 nm with a Unicam SP-500 spectrophotometer. The eluates were tested by immunodiffusion using anti-IgG and anti-IgM immune sera, and the fractions containing IgM globulin in the ascending half of the first elution peak were pooled and concentrated by ultrafiltration. Purity of the isolated IgM was verified by immunoelectrophoresis with polyspecific anti-rabbit goat immune serum. Occasional contaminating alpha macroglobulins were removed through agar block zone electrophoresis.

Defatting of sera

Serum used for ion exchange chromatography and for the production of antigen-antibody complexes and also all immune sera employed in the immunodiffusion analyses were defatted as follows: 0.2 to 0.4 g Aerosil 380 powder* was suspended in 10 ml serum, stirred continuously for 4 hours at room temperature and centrifuged thereafter at 10,000 r.p.m. for 20 minutes at 4 °C.

Decomplementation of serum

Serum employed for the preparation of antigen-antibody complexes was decomplemented by means of a HSA — anti-HSA antigen-antibody system. Eight ml anti-HSA rabbit serum (60 mg antibody protein) and an equivalent amount of HSA (6.7 mg) were added to 100 ml of previously defatted serum, kept for 2 hours at 37 °C and then overnight at 4 °C. The precipitate was centrifuged and discarded next day. The procedure was repeated in the same way.

Determination of antibody content

The antibody protein content of serum samples was determined by the quantitative precipitation method of DÉVÉNYI and GERGELY (1963), using the biuret reaction to measure the protein content of the immune precipitates.

Ultrafiltration

This was carried out by negative pressure dialysis at about 50—100 mm Hg, using 8 DC dialysis tubing** (CRAIG, 1968).

Determination of proteins

Protein determinations were made with the Folin-Ciocalteu phenol reagent*** and the biuret reaction, as described by CHASE and WILLIAMS (1968).

Results

Autopsy

As revealed by P.M. examination 100 days after the first infection, neither cysticerci nor any signs of a cysticercal infection were detected in the control animals, while all rabbits infected with *T. pisiformis* eggs harboured

* Degussa, Baden, West Germany.
** Union Carbide Corporation, Chicago, U.S.A.
*** Fisher Scientific Co., Fair Lawn, New Yersey, U.S.A.

a great number of cysticerci. An average of 211 cysticerci (range: 78—377) were recovered from the rabbits receiving a single infection and 397 (range: 142—438) from those which were reinfected on day 60 of the experiment. In the first group of animals mature cysticerci with fully developed scolices were encapsulated at their ultimate site, mostly on the omentum and under the pelvic peritoneum. Besides the encysted bladderworms, still migrating young larvae were, however, also found in the abdominal cavity of the animals in the reinfected group. These larvae had obviously developed from the oncosphaeres introduced at reinfection, as they were considerably smaller in size and apparently less mature than the encapsulated cysticerci.

The nature of the gross hepatic lesions verified this observation. Namely, in the liver of animals which had undergone a single infection, only signs of the finished reparation of tissue damage caused by preceding larval migration were found, in the form of tortuous, threadlike cicatrices, linear patterns and more or less extensive patches of fibrous thickening in the liver capsule, whereas the liver of rabbits reinfected on day 60 displayed, in addition, yellowish-white or yellowish-grey tunnels of 2—3 mm width, criss-crossing the liver parenchyma and still in the stage of reparation. The occurrence of these tunnels could be attributed unequivocally to the parasites deriving from the oncosphaeres introduced at the second infection.

Antibody responses to experimental infection with C. pisiformis

Dynamics of the haemagglutinin response

Haemagglutinating (HA) antibodies appeared in the serum during the first week of infection: already on day 7 the serum of all rabbits contained, though only in small amounts, HA antibodies to an average titre of 1 : 5.9 (range: 1 : 2—1 : 16). From day 7 onwards all serum samples tested contained a detectable amount of HA antibodies. The dynamics of HA antibody production in response to primary infection is shown in Fig. 1, where mean titres individually assayed in the serum of 10 animals at spaced intervals are presented. The amount of HA antibodies increased steadily from the first week of the infection and reached a peak titre of 1 : 512 (range: 1 : 128—1 : 1024) by day 50. This was followed by a slight decline so that by the end of the experiment, 100 days after the first infection, the sera haemagglutinated to a mean titre of 1 : 208 (range: 1 : 64—1 : 512).

The haemagglutinin response to the secondary infection is demonstrated in Fig. 2, which represents the average of HA titres in the serum of 15 rabbits. As can be seen from the figure, the HA titre increased remarkably following the challenge infection with 10,000 eggs on day 60, the mean titre amounting

to 1 : 3743 (range: 1 : 1024—1 : 8192) by the 100th day of the experiment (40 days after reinfection). This is roughly eight times higher than the greatest mean titre observed in response to primary infection, and about eighteen times as much as the average titre found on the same day in the serum of rabbits infected once only.

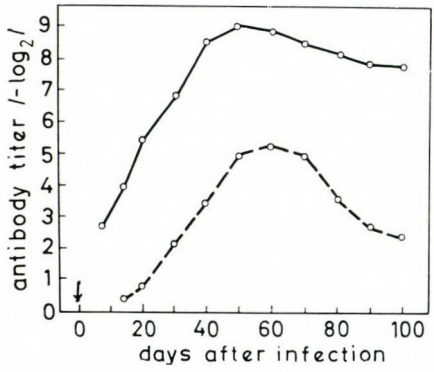


Fig. 1. Antibody pattern in serum of rabbits after a single infection with 500 *T. pisiformis* eggs. ——— mean HA titre; ..... mean precipitin titre. Infection at day 0.

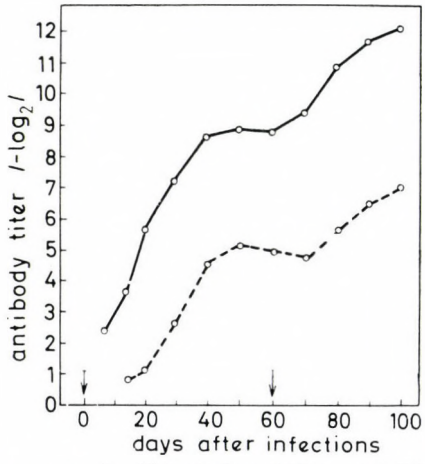


Fig. 2. Antibody pattern in serum of rabbits infected first with 500 and later with 10,000 *T. pisiformis* eggs. ——— mean HA titre; ..... mean precipitin titre. Arrows indicate time of infections

Dynamics of the precipitin response

Precipitating antibodies were first demonstrated on the second week of the primary infection. On day 14, samples from 11 of the 25 rabbits produced precipitin lines with CpE in agar gel; on day 20, samples from 16; and from day 30 onwards, the samples of all animals.

As is shown in Figs 1 and 2, the overall pattern of the mean precipitin titre was similar to that of mean HA titre. The average precipitin titres in both infection groups, however, were 2.8 to 5.3 exponents lower throughout the experiment than the average HA titres for the same samples.

The mean precipitin titre also showed an increase following reinfection, and by the end of the experiment (40 days after reinfection) the antibody levels had risen gradually to a titre 3 to 3.5 times higher than the maximum mean titre found in response to primary infection. On day 100 serum samples from reinfected rabbits precipitated to an average titre of 1 : 117 (range: 1 : 32—1 : 512), while those from once-infected rabbits gave an average titre as low as 1 : 5.3 (range: 1 : 2—1 : 16). Moreover, serum collected after reinfection developed denser precipitin lines and contained precipitins to a wider spectrum of antigenic components than samples from the primary infection. Thus, in the agar gel diffusion precipitin test with CpE as antigen, usually three lines, and occasionally even four, were formed by serum from reinfected rabbits, whereas with samples obtained from rabbits after the primary infection a single line prevailed, and at most two developed.

Antibody activity of sera from hypersensitized rabbits

Rabbits artificially sensitized with homogenates and whole extracts of *T. pisiformis* or *C. pisiformis* produced a large quantity of antibodies. The HA titres of both anti-Tp and anti-Cp sera varied between 1 : 2^{13} and 1 : 2^{18}. These sera formed 6 to 9 marked precipitin bands on reaction with TpE or CpE in the agar gel diffusion precipitin test.

Characterization of immunoglobulins in serum from primary and secondary infections and in serum of artificially sensitized rabbits

Salt fractionation

Serum samples were separated into two fractions by salting out the euglobulins with "one-third" saturated ammonium sulphate. The separated fractions were analysed by immunodiffusion and immunoelectrophoresis, using a polyspecific goat antiserum to rabbit serum proteins and monospecific anti-IgG and anti-IgM immune sera, and then tested for antibodies. The precipitated euglobulin fraction contained, in addition to immunoglobulins, 2—4 serum protein components with beta and alpha mobility. In most of the supernatants neither IgG nor IgM globulins were detected, though IgG globulins were sometimes present in trace amounts.

Results obtained with the various serological tests are presented in Table I. Unfractionated sera gave positive reactions in both the agar gel dif-

fusion precipitin and IHA tests and also produced clearly visible precipitates with live cysticerci. With only slight differences, the euglobulin fractions produced the same reactions as the original whole sera, whereas supernatants were negative in each test on all occasions.

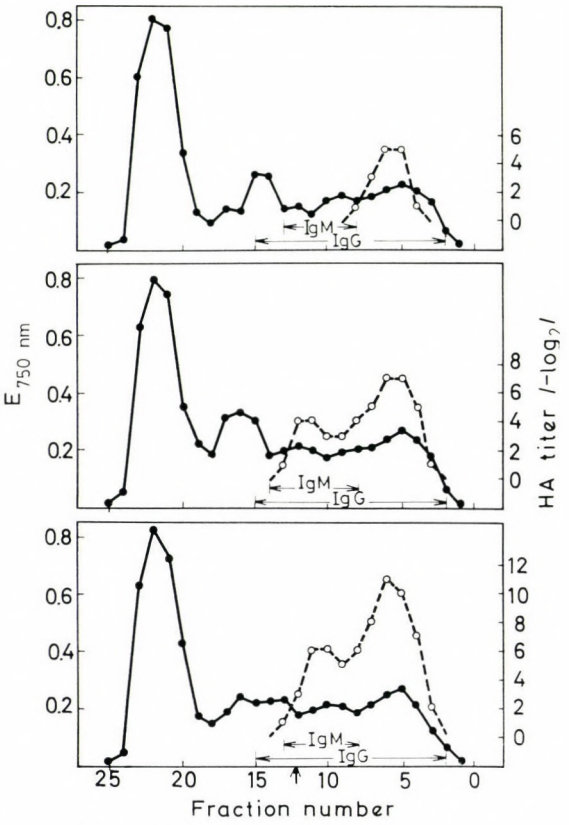


Fig. 3. Preparative zone electrophoresis in agar gel of rabbit serum containing antibodies to *Taenia* and *Cysticercus pisiformis* antigens. Top: serum collected on day 40 of primary infection (HA titre 1 : 256). Middle: serum collected 40 days after reinfection (HA titre 1 : 2048). Bottom: anti-Tp hyperimmune rabbit serum (HA titre 1 : 16,384). ——— protein distribution; - - - - - HA titre. Arrow indicates point of sample application

Fractionation of serum by preparative zone electrophoresis in agar

Serum samples from the rabbits were separated into 25 fractions. The IgG and IgM globulins were located in the electrophoretogram by checking the individual fractions against monospecific anti-IgG and anti-IgM immune sera using the micro-immunodiffusion technique. Each fraction was then tested by IHA test for the presence of antibodies. The results of such investigations with three different pooled sera are illustrated in Fig. 3.

Serum collected from the primary infection exhibited a single peak of antibody activity located in the region of slow-migrating IgG. The maximum levels of HA antibodies coincided with the peak of IgG globulin. Antibody activity was never detected in the region of IgM globulins (see Fig. 3, top). The same results were achieved whether serum from early or late stages of the primary infection was examined.

In the serum samples collected on days 20, 30 and 40 after reinfection, on the other hand, antibodies were demonstrated over almost the entire range

Table I

Results of serological tests with serum fractions separated by ammonium sulphate precipitation

Days after primary infection	Days after secondary infection	Whole serum			Euglobulin			Supernatant		
		HA titre	precipitin titre+	lppt*	HA titre	precipitin titre+	lppt*	HA titre	precipitin titre+	lppt*
20		64	4	+	64	4	+	0	0	—
40		384	24	+	256	16	+	0	0	—
60		512	32	+	512	32	+	0	0	—
80		256	16	+	192	8	+	0	0	—
100		192	8	+	128	6	+	0	0	—
	10	512	32	+	256	16	+	0	0	—
	20	1024	64	+	1024	64	+	0	0	—
	30	2048	128	+	2048	96	+	0	0	—
	40	2560	128	+	2048	128	+	0	0	—

+ Determined by titration in agar gel diffusion precipitation test
* In vitro larval precipitation test: + = positive; — = negative reaction

of IgG mobility of the electrophoretograms. In these cases the antibody activity was represented by two peaks, the larger of which corresponded to the peak of the slow-migrating IgG, while the smaller one extended to the region of the fast-migrating IgG and coincided with the position of IgM globulin (see Fig. 3, middle). This distribution was especially pronounced when anti-Tp or anti-Cp immune serum of fairly high antibody titre was fractionated (see Fig. 3, bottom).

In additional experiments the 25 individual fractions were combined into three pooled fractions according to the distribution of IgG and IgM globulins among the individual fractions. Fraction pool 1 was obtained from fractions containing exclusively IgG globulin (fractions 1—7 or 8); fraction pool 2 was made from fractions that coincided with the region of IgM globulin

and the fast-moving IgG globulins (fractions 8 or 9—16); while fraction pool 3 contained the remaining fractions (fractions 17—25). The pooled materials were concentrated by pervaporation to two-thirds of the original serum volume, dialysed against PBS (0.02 M, pH 7.5) containing 0.01% merthiolate, then analysed immunoelectrophoretically and finally tested for antibodies.

Immunoelectrophoretic analyses against polyspecific goat anti-rabbit serum as well as anti-rabbit IgG + IgM immune serum (Fig. 4) showed that

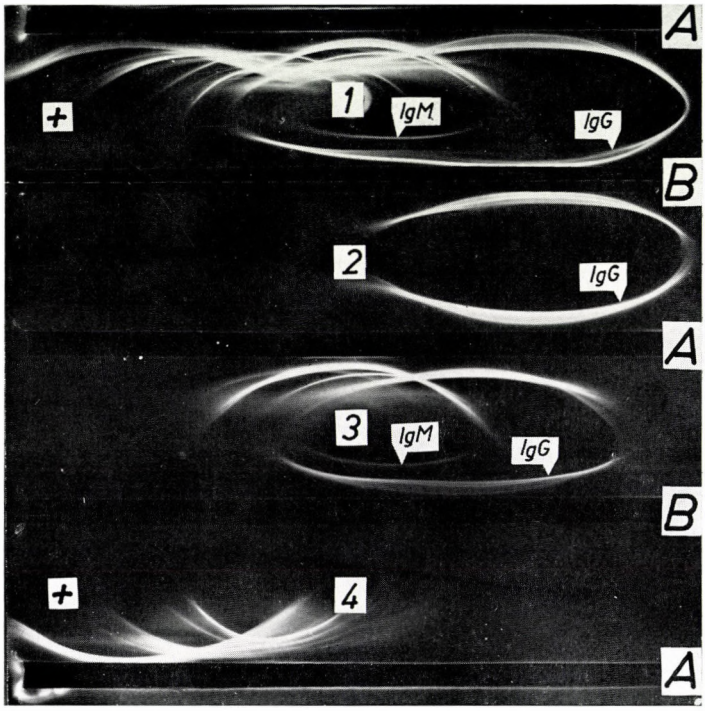


Fig. 4. Immunoelectrophoretic analysis of serum protein fractions separated by preparative zone electrophoresis in agar gel. 1, whole serum; 2, 3 and 4, separated fraction pools 1, 2 and 3, respectively. Precipitin bands developed with goat antiserum to rabbit whole serum (A) and with anti-rabbit IgG + IgM immune serum (B)

fraction pool 1 contained only slow-migrating IgG globulins; fraction pool 2 comprised fast-migrating IgG globulins, IgM globulins as well as other beta- and alpha-globulins; while fraction pool 3 consisted of albumin and alpha-globulins.

Antibodies were detected serologically only in fraction pools 1 and 2 (Table II). When serum samples from any stage of the primary infection were fractionated, fraction pool 1 contained the entire antibody activity recovered, whereas when serum samples obtained from the secondary infection were

investigated, antibodies were also demonstrated in fraction pool 2 by all of the serological methods used. In the latter cases, fraction pool 1, which was immunoelectrophoretically pure IgG globulin, contained 70—80% of the total antibody activity recovered. Antibodies present in fraction pool 1 were resistant to reduction with 2-mercaptoethanol, whereas the antibody activity of fraction pool 2 was considerably reduced by this treatment.

Table II

Results of serological tests with serum protein fractions separated by preparative zone electrophoresis in agar

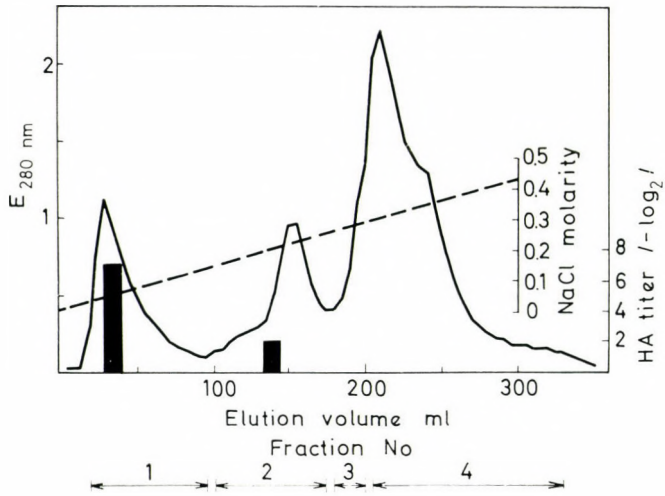
Days after primary infection	Days after secondary infection	Fraction pool 1			Fraction pool 2			Fraction pool 3		
		HA titre	precipitin titre+	lppt*	HA titre	precipitin titre+	lppt*	HA titre	precipitin titre+	lppt*
20		32	2	+	0	0	—	0	0	—
30		96	4	+	0	0	—	0	0	—
40		256	32	+	0	0	—	0	0	—
60		320	32	+	0	0	—	0	0	—
80		128	16	+	0	0	—	0	0	—
100		64	4	+	0	0	—	0	0	—
	20	256	32	+	196	8	±	0	0	0
	30	512	64	+	192	16	±	0	0	0
	40	512	64	+	128	32	±	0	0	0

+ Determined by titration in agar gel diffusion precipitation test
* In vitro larval precipitation test: + = positive; — = negative reaction

Fractionation of serum by DEAE-Sephadex A-50 anion exchange chromatography

Representative results for the chromatographic fractionation of sera on DEAE-Sephadex A-50 anion exchanger are presented in Figs 5 and 6. As revealed by immunodiffusion analyses with monospecific anti-IgG and anti-IgM immune serum, the IgG globulins were present through about 80—90% of the chromatogram effluent, while the IgM globulins were eluted in the fractions between 58—94% of the elution volume.

Gradient elution of pooled serum permitted the separation of serum components into four fractions. Immunoelectrophoretic analyses against goat anti-rabbit serum and anti-rabbit IgG + IgM immune serum demonstrated (Figs 5 and 6) that fraction 1 was pure IgG globulin. This fraction contained



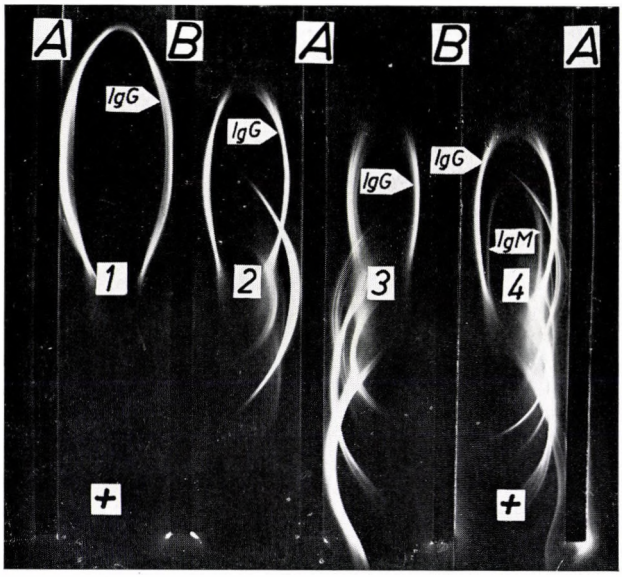


Fig. 5. Fractionation of serum from rabbits infected with *C. pisiformis* by anion exchange column chromatography on DEAE-Sephadex A-50.
Graph: column chromatogram showing distribution of HA antibodies in a serum sample (HA titre 1 : 256) collected on day 30 of primary infection. The HA titres of the fractions are represented by the colums. ——— protein distribution.
Immunoelectrophoresis: well numbers indicate fraction numbers. Precipitin bands developed with goat antiserum to rabbit whole serum (A) and anti-rabbit IgG + IgM immune serum (B)

most of the IgG globulins and was eluted in the first peak of the chromatogram. Fraction 2 comprised beta-globulins as well as the IgG globulins eluted in the second peak of the elution curve. Fraction 3 was composed of albumin, alpha- and beta-globulins and also included fast-migrating IgG globulins. Fraction 4 contained the total amount of the eluted IgM globulins as well as many other serum proteins and was also contaminated with fast-migrating IgG globulins.

Table III

Antibody activity of protein fractions separated from rabbit serum by anion exchange chromatography on DEAE-Sephadex A-50

Days after primary infection	Days after secondary infection	Fraction 1			Fraction 2			Fraction 4**		
		HA titre	precipitin titre+	lppt*	HA titre	precipitin titre+	lppt*	HA titre	precipitin titre+	lppt*
14		16	0	—	0	0	—	0	0	—
20		32	2	+	0	0	—	0	0	—
30		256	8	+	4	0	—	0	0	—
40		256	16	+	16	2	—	0	0	—
60		128	32	+	8	0	—	0	0	—
80		128	8	+	4	0	—	0	0	—
100		64	2	±	0	0	—	0	0	—
	10	128	16	+	8	0	—	0	0	—
	20	192	24	+	16	0	—	32	0	—
	30	256	32	+	16	0	—	32	0	—
	40	512	64	+	16	2	—	64	0	—

+ Determined by titration in agar gel diffusion precipitation test
* In vitro larval precipitation test: + = positive; — = negative reaction
** Absorbed with anti-rabbit IgG immune serum

The results of serological investigations are presented in Table III. Most of the antibody activity was recovered in fraction 1 on chromatographic fractionation of serum samples obtained from rabbits with a single infection. The amount of antibody activity present in fraction 2 could be attributed to contamination by IgG globulin which was eluted in the second peak of the chromatogram. This was confirmed by immunoelectrophoresis (see Figs 5 and 6). The antibody activity demonstrated in this fraction amounted to only 3—7% of the total antibody activity recovered. In fraction 3 and 4 antibody was never detected with either of the serological tests employed when serum samples obtained from rabbits receiving a single inoculation were chromatographed. In contrast, when serum samples collected after reinfection were

fractionated, about 10—15% of the total haemagglutinin activity was present in fraction 4, though contaminating IgG globulins had previously been absorbed with anti-IgG immune serum insolubilized with glutaraldehyde. Precipitins could be demonstrated in fraction 1 exclusively. This fraction developed the same precipitin line(s) as the original serum samples on reaction with CpE in agar double diffusion precipitation test, and also produced clearly visible precipitates with live cysticerci. Fraction 4 gave negative reactions with both tests.

Antibodies detected in fraction 1 were resistant to 2-mercaptoethanol, while the antibody activity present in fraction 4 was completely lost upon treatment (Table IV).

Table IV

Haemagglutination titres of chromatographic fractions 1 and 4 of serum from reinfected rabbits after treatment with mercaptoethanol

Days after re-infection	Fraction 1		Fraction 4*	
	Control	ME-treated	Control	ME-treated
10	128	128	0	0
	192	160	0	0
	256	256	4	0
20	128	128	12	0
	128	128	16	0
	256	256	24	0
30	256	256	32	0
	320	320	48	0
	384	320	64	0
40	512	512	64	0
	640	640	64	0
	640	512	80	0

* Absorbed with anti-rabbit IgG immune serum

2-Mercaptoethanol (2-ME) sensitivity of antibodies

The antibody activity of individual serum samples was studied by IHA test before and after treatment with 0.1 M 2-ME. Typical results obtained by comparing the HA titres of control samples with those of treated sera are presented in Table V. The results indicate that treatment with 2-ME caused, on the average, a consistent 1.9—3.1 exponent reduction in the HA titre of serum collected after reinfection, whereas it only minimally altered the HA titre of samples obtained from the primary infection.

Analysis of immune serum to antigen-antibody complexes

Antisera were produced to antigen-antibody precipitates made with pooled serum from infected or artificially hypersensitized rabbits and antigens of *T. pisiformis* and *C. pisiformis*. The serum protein components detected by the different anti-complex antisera were identified through immunoelectrophoresis with pooled normal rabbit serum.

Table V

Effect of mercaptoethanol treatment on haemagglutination titres of whole rabbit sera obtained after primary and secondary infection

Days after infection		N° of animals	Mean HA titre ($-\log_2$) ME treatment		ME sensitivity*
primary	secondary		before	after	
7		10	2.7	2.5	0.2
14		12	3.6	3.2	0.4
20		10	5.4	5.1	0.3
30		14	7.2	7.0	0.2
40		15	8.6	8.3	0.3
60		13	8.7	8.6	0.1
80		10	8.1	7.7	0.4
100		10	7.7	7.5	0.2
	10	15	9.1	7.2	1.9
	20	15	10.4	8.0	2.4
	30	15	11.3	8.4	2.9
	40	15	11.9	8.8	3.1

* Differences between mean HA titre before and after ME treatment

Representative results for immune serum to each type of the different antigen-antibody complexes are illustrated in Figs 7 and 8. Immunoelectrophoretic analyses revealed that all anti-complex antisera obtained reacted exclusively with the rabbit immunoglobulins and were consistently non-reactive towards all other serum protein components of the rabbit.

Immune serum produced against antigen-antibody precipitates made with only the "secretions and excretions" of mature cysticerci (CpSE) developed solely the characteristic precipitin arc of IgG globulin during immunoelectrophoresis, irrespective of whether serum collected after the primary or secondary infection or serum of hypersensitized rabbits was used for the preparation

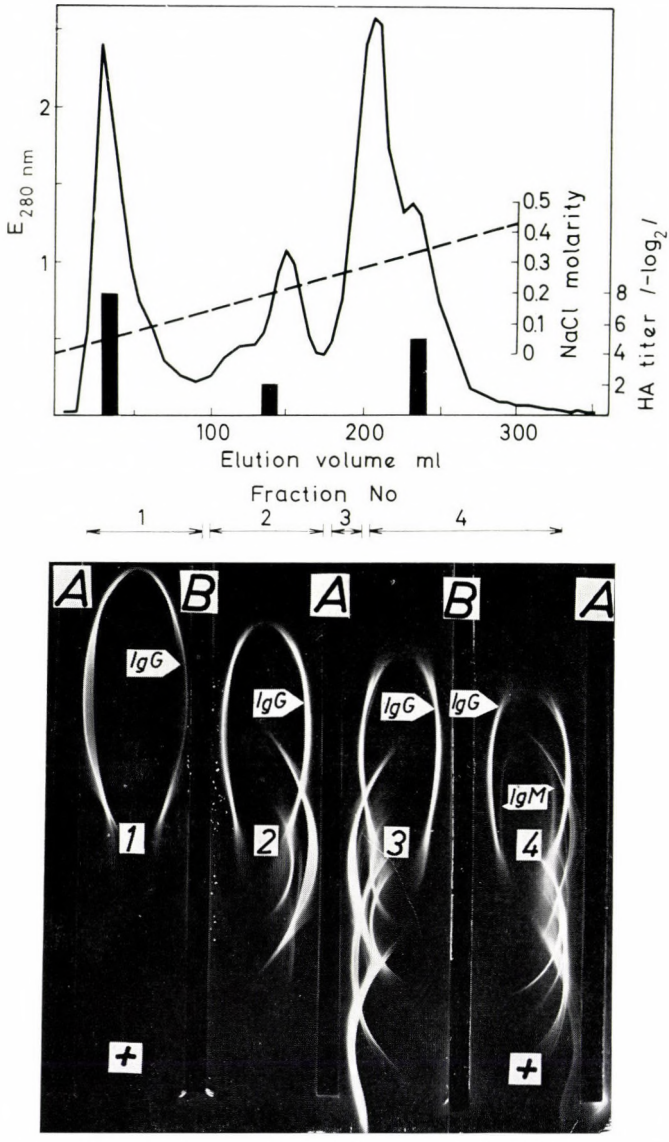


Fig. 6. Fractionation of serum from rabbits reinfected with *C. pisiformis* by anion exchange column chromatography on DEAE-Sephadex A-50.
Graph: column chromatogram showing distribution of HA antibodies in a serum sample (HA titre 1 : 2048) collected 30 days after reinfection. The HA titres are represented by the columns. ——— protein distribution.
Immunoelectrophoresis: well numbers indicate fraction numbers. Precipitin bands developed with goat antiserum to rabbit whole serum (A) and anti-rabbit IgG + IgM immune serum (B)

of complexes (see Fig. 8, G and F; Fig. 7, E). In contrast, immune sera to antigen-antibody complexes made with TpE, CpE and CpSE antigens and pooled serum of reinfected or artificially sensitized rabbits formed, besides

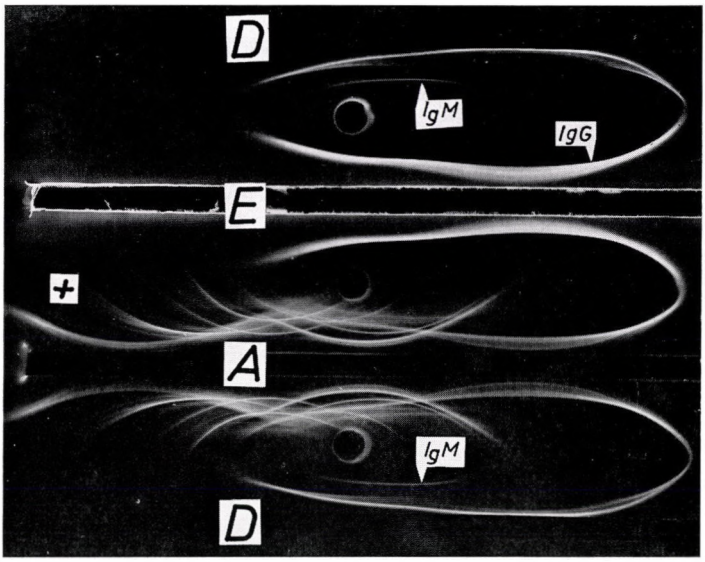


Fig. 7. Immunoelectrophoretic analysis of immune cock sera to antigen-antibody complexes prepared from serum of rabbits artificially immunized with *Taenia* and *Cysticercus pisiformis* antigens. After electrophoretic separation of pooled normal rabbit serum the troughs were filled with goat antiserum to rabbit whole serum (A), with cock antiserum to antigen-antibody precipitates made with TpE, CpE and CpSE (D), and with a cock antiserum to antigen-antibody precipitates made with only CpSE (E)

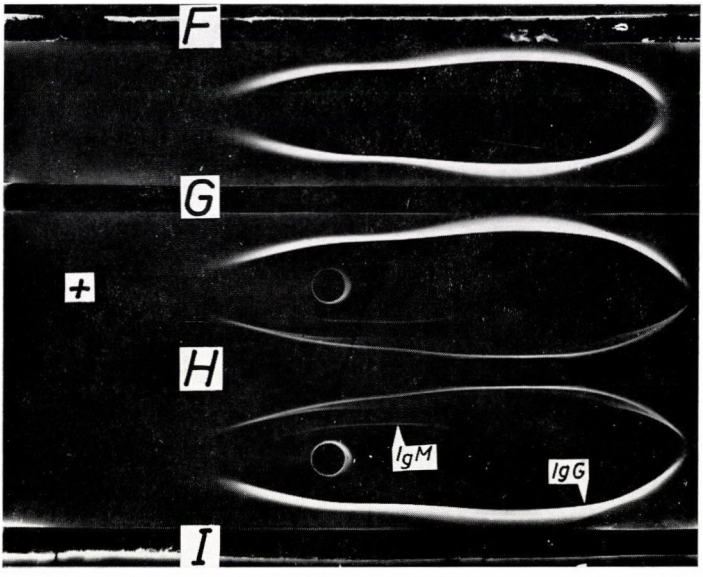


Fig. 8. Immunoelectrophoretic analysis of immune cock sera to antigen-antibody complexes made from serum of rabbits infected with *Cysticercus pisiformis*. After electrophoresis of pooled normal rabbit serum the troughs were filled with cock antisera to antigen-antibody preci tates made with TpE, CpE, and CpSE from serum pools obtained 30 days after the primary infection (I) and 40 days after reinfection (H), and with cock antiserum to antigen-antibody precipitates made with only the CpSE from serum pools obtained on day 30 of primary infection (G) and 40 days after reinfection (F)

the IgG arc, a further distinct precipitation line, which was tentatively demonstrated to correspond to the IgM globulin (see Fig. 7, D; Fig. 8, H). Thus, the anti-complex antisera giving rise to these precipitation lines during immunoelectrophoresis retained their reactivity towards IgM globulin after absorption with immunochemically pure rabbit IgG globulin insolubilized by glutaraldehyde, whereas those absorbed with purified rabbit IgM globulin failed to detect this component.

Immune serum obtained by immunization with antigen-antibody precipitates made with TpE, CpE and CpSE antigens and pooled rabbit serum from the primary infection developed a single precipitin line corresponding to IgG globulin (see Fig. 8, I).

Discussion

Dynamics of the antibody response in primary and secondary infection with C. pisiformis

It has long been known that many helminth infections stimulate the host to produce a considerable antibody response to the antigens of the invading parasites. But, despite the steadily increasing interest of recent years and the extensive work done to characterize the immunoglobulin classes which are produced, very little is known about the nature of the humoral antibody response of the rabbit to invasion by *T. pisiformis* oncosphaeres. No information on the pattern of antibody production is yet available in the literature except for the data reported by Shul'ts and Ismagilova (1965). In experiments performed on rabbits exposed to different doses of *T. pisiformis* eggs, these authors were able to demonstrate serum antibodies as early as a few days after the infection, by both IHA and ring precipitation tests, using the purified polysaccharide of Campbell (1937) and larval extract prepared according to the method of Trawinski (1936) as antigens, respectively. The ring precipitation test gave positive reaction as early as the second day of infection, occasionally in a titre of $1 : 10^7$. The dynamics of the serological reactions followed a very similar pattern during the course of infection. Both reactions reached a peak between days 10—30, became gradually less marked thereafter, and in most cases were negative by days 60—90 of the infection. The appearance and persistence of antibodies in individual rabbits showed a similar pattern, though the maximum titre of precipitating antibodies was considerably higher than that of haemagglutinating antibodies. No correlation was established between the size of inoculum and the appearance, persistence and titre of antibodies. Repeated infections caused only a transient rise in the antibody level, which was, however, unrelated to the dose of inoculum; both serological tests became negative by days 60—90, just as in the case of primary infection.

The results of the present study are inconsistent with several points of the above findings. Thus, haemagglutinins appeared during the first week of infection, and from day 7 onwards the serum of all animals contained a detectable amount of HA antibodies. In the agar gel diffusion precipitation test, however, demonstration of precipitins to CpE was completely unsuccessful before the 11th—14th days of the infection. Between days 15—30 serum from only a few rabbits brought about precipitation, whereas from day 30 onwards serum of all animals produced distinct precipitation line(s) in agar gel. The most marked precipitation lines occurred between days 30—60. Afterwards a decrease in the density of the precipitation lines ensued, though the serum of all animals still produced demonstrable precipitation at day 100 of the infection. The differences observable in the density of precipitation bands during the course of the infection parallelled the changes of serum precipitin titres: serum with the highest titre produced the most marked precipitation lines, and vice versa.

The time-course of precipitating and haemagglutinating antibody responses followed a similar pattern in both primary and secondary infection, the precipitin titre at any stage of infection was, however, 3—5 exponents lower than the HA titre. This difference, as also the observation that the IHA test detected antibodies earlier than the agar gel diffusion precipitin test, can be explained with the well-known disparate sensitivities of the two methods.

The pattern of antibody response following primary infection was characterized by a gradual increase in the antibody level from the second week of infection, with a peak between days 40—70. The maximum precipitin titre obtained in the agar gel diffusion precipitation test never exceeded a 1 : 128 dilution of the CpE stock antigen containing 10 mg protein per ml, while in the IHA test serum samples gave full haemagglutination at a maximum dilution of 1 : 1024. A decline of antibody titre ensued from day 70 of the infection, though antibodies were still readily demonstrable by both serological methods in all serum samples on day 100. As suggested earlier, the diminishing antibody titre is possibly associated with the development of the parasite (NÉMETH, 1971).

Again in contrast to the findings of SHUL'TS and ISMAGILOVA, the results obtained with serum from reinfected rabbits suggest an increased antibody production, which is obviously related to the antigenic stimuli of the newly invading oncosphaeres. During the "secondary" immune response evoked by challenge infection, the serum antibody level gradually rose by day 40 to a titre considerably higher than the maximum titre observed in response to primary infection. Furthermore, according to the immunodiffusion analyses, antibodies to a wider spectrum of antigenic components were produced as a result of secondary infection.

The discrepancy between the two sets of findings is not explicable by

individual variation of the animals' reactivity or the diversity of infective material, but is due rather to the differences in antigenic preparations and in the sensitivity of the serological methods used in the two laboratories. It should be stressed, however, that in the present experiment there was a considerable variation in the antibody activity of the serum samples, which was related to individual rabbits, to the number of inoculations, and to the time elapsed between infection and bleeding of the same rabbit. This variability may have been due to the individual variation of the animals' reactivity and, perhaps to a greater extent, to different "take" of larvae from the inoculated dose of oncosphaeres, as revealed by autopsy on day 100 of the infection. The various burdens of cysticerci may have yielded diverse amounts of antigenic substances and thereby quantitatively proportionate differences in antibody production.

From the results of the immunodiffusion analyses of serum samples obtained at different stages of infection the conclusion may be drawn that invasion of *T. pisiformis* oncosphaeres induces a complex antibody response in the rabbits, which verifies the previous observations (NÉMETH, 1971) and also is in harmony with the findings for other helminth infections (see *e.g.* BAISDEN and TROMBA, 1967; HILLYER and FRICK, 1967, 1971; HILLYER and RITCHIE, 1967; LEYKINA and BALLAD, 1970; SINCLAIR, 1970; WILLIAMS, 1970; WILLIAMS and SOULSBY, 1970; GUNDŁACH, 1971; MUNTYAN, 1971). Using CpE as antigen, at least four antibodies of different antigenic specificity were distinguishable in the serum of infected rabbits. Serum samples of each animal consistently detected at least one antigenic component. In addition, a maximum of two antigen-antibody systems were demonstrable with serum collected following the primary infection, while with samples obtained after the challenge infection in most cases three, and a maximum of four systems could be observed. These observations may reflect qualitative antigenic differences during development of the parasite. It may be that some antigens are not produced at all following a single infection and only appear as a result of disintegration of parasites in the partially immune host subsequent to a challenge infection. On the other hand, it is also conceivable that merely quantitative antigenic differences exist. That is, during the primary infection some antigens are produced in amounts below the threshold level required to induce an antibody response detectable by the agar gel diffusion precipitation test, but this level is surpassed by the new antigenic stimuli deriving from the oncosphaeres of the challenge infection. There is, however, no direct experimental evidence for either hypothesis, and clarification of the problem must await more ciritical studies.

Antibody classes in primary and secondary infections with C. pisiformis

In the rabbit three immunoglobulin classes — IgG, IgM and IgA — are known, which can be distinguished by structural, and hence antigenic, differences in their heavy γ, μ and α, polypeptide chains, respectively (COHEN and PORTER, 1964; CEBRA and ROBBINS, 1966; COHEN and MILSTEIN, 1967; STANWORTH and PARDOE, 1967). Antibodies have been demonstrated in each class (ONOUE et al., 1964, 1965, 1966; ROBBINS et al., 1965; ARMSTRONG and SWORD, 1967; CARTER and HARRIS, 1967; SAMLOFF and BARNETT, 1967; WEI and STAVITSKY, 1967).

Numerous reports indicate that rabbits may produce antibodies of all three immunoglobulin classes during the primary immune response to any antigenic material, although one or the other class may predominate in any particular situation (ONOUE et al., 1964; FREEMAN and STAVITSKY, 1965; ARMSTRONG and SWORD, 1967; PIKE, 1967; SAMLOFF and BARNETT, 1967; WEI and STAVITSKY, 1967). The secondary immune response of this species is usually characterized by the rapid synthesis of predominantly IgG antibody, though anamnestic responses involving IgM and IgA antibody production have also been reported (BAUER et al., 1963; UHR and FINKELSTEIN, 1963; BOREL et al., 1964; HOCKER and BAUER, 1965; ALTEMEIER et al., 1966; SAMLOFF and BARNETT, 1967). To date, though, no information has been available either on the heterogeneity and sequence of antibody production or on the classes of antibodies produced in rabbits to primary and secondary infections with *C. pisiformis*.

The results of the salt fractionation experiments with sera of infected rabbits clearly indicate that the antibodies detectable by IHA, agar gel diffusion precipitation and *in vitro* larval precipitation tests are associated with the euglobulin fraction. Serum samples completely lost their original antibody activity after euglobulins were salted out. As compared to the antibody activity of the original whole serum, there was only a light decrease in the antibody titre of the euglobulin fraction, attributable to the errors of the serological tests and loss of protein during the fractionation procedure.

According to the results of preparative agar gel zone electrophoresis, anion exchange chromatography and the 2-ME treatment, rabbits experimentally infected with *T. pisiformis* oncosphaeres did not exhibit the "early IgM" and "late IgG" antibody response which has been reported to be typical for many other antigens (STELOS, 1958; BAUER and STAVITSKY, 1961; BENEDICT et al., 1962; BAUER et al., 1963; SVEHAG, 1964; SVEHAG and MANDEL, 1964). Indeed, several data indicate that IgG and IgM antibodies are produced simultaneously rather than sequentially, and even that IgG antibodies can appear prior to, or concomitant with, the appearance of IgM antibodies

(BENEDICT, 1965; FREEMAN and STAVITSKY, 1965; ROBBINS et al., 1965; SCHOENBERG et al., 1965; ALTEMEIER et al., 1966; OSLER et al., 1966; ARMSTRONG and SWORD, 1967; WEI and STAVITSKY, 1967), whereas under particular circumstances, depending on the chemical nature, physical state, mode of administration and dosage of the antigen as well as several other unknown factors, a prolonged IgM antibody production can be observed without a shift to IgG antibody formation (SVEHAG and MANDEL, 1962, 1964; BAUER et al., 1963; UHR and FINKELSTEIN, 1963; WEIDANZ et al., 1964; SANDBERG and STOLLAR, 1966).

Attempts to characterize the antibody class(es) produced in response to experimental infection with *C. pisiformis* indicated that the earliest antibody demonstrable in the first week of infection was IgG globulin. Exclusively IgG antibodies were demonstrated late in the primary infection as well. Antibodies detectable by IHA, agar gel diffusion precipitation and by *in vitro* larval precipitation tests were found to migrate in the slow IgG globulin during agar gel zone electrophoresis, to elute in the first peak (pure IgG) on DEAE-Sephadex A-50, and to be resistant to reduction and alkylation. IgM antibodies were never detected by any of the methods employed after single infection.

IgG antibodies predominated also in the serum samples from secondary infection; from day 20 onwards, however, IgM antibodies too were demonstrable. The presence of IgM antibodies was verified by several pieces of evidence, first of all by the results of 2-ME treatment. The sensitivity of IgM globulin to 2-ME and the relative stability of IgG globulin has been widely employed as a method of differentiating the two immunoglobulin classes since the study of DEUTSCH and MORTON (1957). Comparison of serum titres before and after treatment showed that 2-ME only minimally altered the antibody activity of serum from primary infection, but considerably reduced that of serum obtained from reinfected animals.

The IgG and IgM antibodies were successfully separated from one another by column chromatography of pooled serum on DEAE-Sephadex. Antibodies eluted with the IgM globulin were sensitive to reduction with 2-ME and represented 10—15% of the total antibody activity recovered. The IgM fractions retained their original antibody activity even after absorption of contaminating IgG molecules with monospecific anti-IgG immune serum, whereas they lost this activity when exposed to 2-ME following absorption. In contrast, antibodies eluted with the IgG globulin were resistant to treatment with 2-ME and represented most of the recovered antibody activity. The low antibody activity present in the second peak of the chromatogram (about 3—7% of the total recovered activity) could be attributed to contamination by IgG globulin which was not eluted in the first peak, although the presence of antibodies of the IgA immunoglobulin class — which are known to occur in rabbit serum

(ONOUE et al., 1964, 1966; CARTER and HARRIS, 1967; SAMLOFF and BARNETT, 1967; WEI and STAVITSKY, 1967) — was not critically examined.

The results obtained through immunoelectrophoretical analysis of anti-complex antisera to antigen-antibody precipitates made with the serum of reinfected rabbits and the antigenic components of the parasite provide further evidence for the occurrence of IgM antibodies. It was shown that inoculation of such antigen-antibody precipitates into heterologous animals results in the production of antibodies forming, in addition to the characteristic IgG arc, a further distinct precipitation line upon immunoelectrophoresis with normal rabbit serum. The second precipitin line was tentatively identified as IgM globulin on the grounds of its location in the immunoelectrophoretogram and the following considerations. This precipitated serum component could not be any constituent of the complement, since all serum pools were decomplemented before being used for the preparation of antigen-antibody complexes. It has been found that adding 32 μg of ovalbumin and 288 μg of anti-ovalbumin to 1 ml of rabbit anti-BSA serum will remove the complement (TALIAFERRO and TALMAGE, 1955); in the present studies, about four times these quantities were applied from a HSA — rabbit anti-HSA antigen-antibody system. Furthermore, the second precipitin line could not derive from antibodies to the light polypeptide chains of IgG globulin present in the antigen-antibody complex, as the anti-complex antisera retained their reactivity towards the second component after absorption with immunochemically pure rabbit IgG globulin; on the other hand, when absorbed with purified rabbit IgM globulin, all anti-complex antisera failed to detect it. These findings clearly indicate that immunization by antigen-antibody precipitates made with serum of reinfected rabbits and the antigens of *T. pisiformis* and *C. pisiformis* stimulates the formation of antibodies specific for the IgG and for the IgM globulins.

Using the agar gel double diffusion technique, the IgG globulin fractions alone were able to form precipitation lines against CpE antigen. In no case was distinct precipitation observed with the IgM fractions, although a detectable amount of HA antibody was present according to the IHA test. This seems to contradict the results obtained by analysis of anti-complex antisera, which undoubtedly showed that IgM precipitins were also involved in the antigen-antibody precipitates. The failure to demonstrate IgM antibodies by the precipitation test is probably attributable to the disproportionate sensitivity of the serological methods for IgM antibody. A low level of antibody of the IgM class may have precluded the formation of visible precipitates and yet was sufficient to agglutinate the antigen-sensitized red blood cells in the IHA test. There are reports to suggest that both IgG and IgM antibodies are capable of causing precipitation; nevertheless the precipitation test is more efficient in detecting IgG antibody, in contrast to the IHA test, which tends to favour the detection of IgM (GREENBURY et al., 1963; GREY, 1964; SHUL-

MAN et al., 1964; WEIDANZ et al., 1964; BENEDICT, 1965; ONOUE et al., 1965; ROBBINS et al., 1965; ALTEMEIER et al., 1966; LINDQVIST and BAUER, 1966; OSLER et al., 1966; PIKE et al., 1966).

In previous experiments, it was observed that incubation of live *C. pisiformis* infective larvae in serum from infected rabbits gave rise to white flocculent precipitates. Similar observations have been made for anti-Tp and anti-Cp immune serum of artificially hypersensitized cocks (unpublished experiments). If the assumption is true that this precipitate formation results from direct interaction between the antigenic metabolites of the parasite and the antibodies present in the serum, the results obtained by electrophoretic analysis of immune serum produced against these precipitates in heterologous animals permit the conclusion that the "secretions and excretions" of the parasite stimulate the formation of IgG antibodies. The antigen-antibody precipitates prepared with the simultaneous application of both "metabolic" and "somatic" antigens of the parasite also induced the production of antibodies reacting primarily with rabbit IgG globulin. Even with this procedure antibodies of the IgM class could be demonstrated only in sera obtained from the secondary infection. This confirms the conclusion of previous studies on the localization of precipitins in the serum, that rabbits primarily produce precipitating antibodies of the IgG class in response to invasion of oncosphaeres of *T. pisiformis* (NÉMETH, 1972).

The observation that IgM antibodies were present during the secondary infection but not demonstrable in the course of primary infection is consistent with the finding of CRANDALL et al. (1967), who, using the fluorescent antibody test, demonstrated at first only IgG antibodies in the serum of *Trichinella*-infected rabbits; IgM antibodies appeared in the serum only late in the infection and after hyperinfection. In a subsequent study using the passive haemagglutination test (CRANDALL and MORE, 1968) they detected IgM anti body before IgG antibody following a single *Trichinella* infection. In serum from hyperinfected rabbits IgM antibody coexisted with IgG antibody.

The reason for the failure of the present experiments to detect IgM antibodies during primary infection with *T. pisiformis* eggs may be that this class of antibody was either not produced at all or produced in amounts below the threshold level for detection by the techniques employed. Summing up, it is evident that the invasion of *T. pisiformis* oncosphaeres induced a complex and heterogenous antibody response in rabbits. At least four antibodies, differing in antigenic specificity and belonging to two distinct immunoglobulin classes, were produced in the course of infection. Antibodies formed in response to primary infection belonged to the IgG immunoglobulin class. IgG antibodies predominated in the secondary infection, but IgM antibodies were also demonstrable.

It should be noted, lastly, that the investigations reported here cannot claim to cover all characteristics of the antibody response to infections with *C. pisiformis*, because of the nature of the antigenic preparations and the relative insensitivity of the methods used to detect antibodies. Further detailed studies are needed to learn more about the course of events.

*

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SUMMARY

The humoral antibody response of rabbits to primary and secondary infections with *Cysticercus pisiformis* (Bloch, 1780), the larval form of *Taenia pisiformis* (Bloch, 1780) Gmelin, 1790 (Cestoda: Taeniidae), has been studied.

Rabbits, aged 5—7 weeks, weighing 500—1000 g, were infected with 500 eggs of *T. pisiformis*, and a group of them were reinfected with 10,000 eggs 60 days later. On the 100th day of the experiment all animals were killed and examined for the presence of cysticerci. Blood samples were collected at frequent intervals and the serum tested for antibodies.

In response to infection all rabbits produced antibodies detectable by the agar gel diffusion precipitation, *in vitro* larval precipitation and indirect haemagglutination tests. Haemagglutinating antibodies appeared in all animals by the second week and persisted throughout the experiment. The dynamics of the haemagglutinin response was characterized by a gradual increase of the mean titre from the second week of infection to a peak on day 50, then a slight decline. The time-course of precipitating antibodies followed a similar pattern. Precipitin activity was first detected in the sera on the second week after infection, reached a peak between weeks 6—10, and fell considerably thereafter but was still present on the 100th day. Reinfection with 10,000 eggs on day 60 of the infection elicited an abrupt rise of both haemagglutinating and precipitating antibodies.

The classes of immunoglobulins carrying the antibodies produced in the course of infection were identified by salting out, by preparative zone electrophoresis in agar, by anion exchange column chromatography on DEAE-Sephadex A-50, by the alteration of antibody activity after 2-mercaptoethanol treatment, and by immunoelectrophoretical analysis of immune sera to antigen-antibody precipitates made with sera of infected rabbits and whole extract and/or "secretions and excretions" of *T. pisiformis* and *C. pisiformis*. It was found that the oncosphaeres of *T. pisiformis* induced a complex and heterogeneous antibody response in the rabbits. Antibodies to at least four different antigenic components were produced and were shown to be associated with two distinct immunoglobulin classes: antibodies formed in response to primary infections belonged to the IgG class exclusively, and while these were still predominant in secondary responses a small proportion (10—15%) of IgM antibodies was also detectable.

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ZUR RESISTENZ VON *EIMERIA STIEDAI*-OOCYSTEN GEGEN RÖNTGEN- UND GAMMA-BESTRAHLUNG

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Zur Resistenz von Kokzidienoocysten gegen Bestrahlung mit verschiedenen Strahlenarten liegen nur wenig und z. T. widersprüchliche Literaturangaben vor (SCHNEIDER, AYENI und DÜRR, 1972). Es erschien daher notwendig, erneut systematische Untersuchungen auf diesem Gebiet durchzuführen.

Material und Methodik

Für die Bestrahlungsversuche wurden unsporulierte *E. stiedai*-Oocysten unterschiedlichen Alters benutzt. Die Gewinnung und Reinigung der Oocysten wurde nach den bei AYENI (1969) angegebenen Verfahren vorgenommen. Bis zur Bestrahlung wurden die Oocysten in ca. 2%iger K_2CrO_7-Lösung im Kühlschrank bei ca. 4 °C aufbewahrt. Vor jeder Bestrahlung wurde das Kaliumbichromat ausgewaschen und die Oocystencharge auf etwa vorhandene unerwünschte Sporulationserscheinungen kontrolliert. Suspensionen, in denen die Sporulation bereits begonnen hatte, wurden nicht benutzt.

Die Bestrahlungen erfolgten mit unterschiedlichen Strahlenarten und Dosisleistungen (s. Tabelle I). Zur Dosimetrie wurde eine Fricke-Lösung (28 mg $FeSO_4$; 0,5 ml konz. H_2SO_4; 5 mg NaCl; A. bidest. ad 100 ml) verwendet.

Die Oocystendichte in den zu bestrahlenden Suspensionen betrug in der Regel etwa $5—10 \times 10^6$ Oocysten/ml. Die Bestrahlung erfolgte bei etwa 3—5 mm Schichtdicke in Petrischalen von 4 cm Durchmesser. Um bei Röntgenbestrahlung eine homogene Durchmischung und Bestrahlung zu gewährleisten, wurden die Suspensionen durch einen Magnetrührer während des Versuches ständig gerührt.

Während in den ersten Versuchen zur Überprüfung der Wirksamkeit der Gesamtdosis, der Dosisleistung und verschiedener Strahlenarten nur Oocysten des jeweils gleichen Alters benutzt wurden, wurde in einer zweiten Versuchsreihe der Einfluß des Alters von der 3. bis zur 11. Woche nach der Gewinnung der Oocysten durch Versuchsansätze in 14tägigen Zeitabständen untersucht.

Im Anschluß an die Bestrahlung wurden die Oocysten erneut in 2%iger K_2CrO_7-Lösung suspendiert und bei Zimmertemperatur (von 15—25 °C schwankend) in Petrischalen von 9 cm Durchmesser zur Sporulation angesetzt. Vom 1. bis 6. Tag wurden die Schalen durch tägliches Abnehmen des Deckels und Schwenken belüftet.

Zur Auswertung der Versuche wurde am 3., 6., 12., 24. und 30. Tag nach der Bestrahlung die Sporulationsrate bestimmt.

Tabelle I

Zusammenstellung der physikalischen Faktoren, die in den einzelnen Bestrahlungsversuchen Anwendung fanden

Strahlenart[1]	Dosisleistung[2]	Gesamtdosis[2]
Röntgen 50 kV	3, 15, 20, 30	30, 60, 120
Röntgen 100 kV	2,5, 5, 10	60, 120
	2,5, 15	60, 90, 210
	20	90, 210
	30	n × 30
		n = 1 bis 15 und 30
	35—40	30, 60
Röntgen 100 kV mit 0,2 mm Cu-Blech-Abschirmung	3	30, 60, 120
	5—7	30, 60
Gamma Co-60	2, 5, 7, 13	30, 60, 120
	13	40
	14—16	30, 60

[1] Röntgen 50 kV: Siemens Dermopan 2, 50 kV, 25 mA;
Röntgen 100 kV: X-Ray Generator PW [1140/00/60 mit Röntgenröhre FA 100/3 (Fa. CHF Müller) 100 kV, 27 mA];
Gamma Co-60: 20 000 Ci, Energie 1,17 und 1,33 MeV.

[2] Die Angaben wurden zur besseren tabellarischen Darstellung jeweils auf- bzw. abgerundet. In der Regel wurde die gewünschte Gesamtdosis nach einer Dosisleistungsmessung vor Versuchsbeginn durch Variation der Bestrahlungszeit erhalten. Nur vereinzelt erfolgte die Dosisleistungsbestimmung nach dem Versuchsende mit vorher festgelegten Bestrahlungszeiten.

Am 3. und 6. Tag wurden je 300 Oocysten differenziert in: a) degenerierte und unreife Oocysten; b) unsporulierte Oocysten; c) Oocysten, in denen die Sporonten 1. Kantenbildung, 2. Pyramidenbildung, 3. runde und ovale Sporoblasten zeigten; d) voll sporulierte Oocysten und e) Oocysten, in denen Anzeichen einer abnormen Sporulation zu sehen waren. Am 12., 24. und 30. Tag wurde nur noch zwischen 3 Sporulationsgruppen unterschieden: 1. unreife und nicht sporulierte Oocysten, 2. voll und teilweise sporulierte, aber degenerierte Oocysten sowie teilweise sporulierte Oocysten ohne Degenerationserscheinungen, 3. voll sporulierte Oocysten, in denen mindestens noch ein Sporozoit morphologisch unverändert (= nicht degeneriert) erschien.

Ergebnisse

1. Bei einer Auswertung der Versuchsansätze ergab sich eine in Abhängigkeit von dem Auswertungszeitpunkt unterschiedliche Abhängigkeit der Überlebensrate von der Bestrahlungsdosis (Abb. 1).

2. Ein großer Teil der Oocysten war nach der Bestrahlung nur noch bedingt lebensfähig. Sie begannen mit einer normalen Sporulation, konnten sie jedoch nicht mehr vollenden. Je höher die Bestrahlungsdosis war, in desto

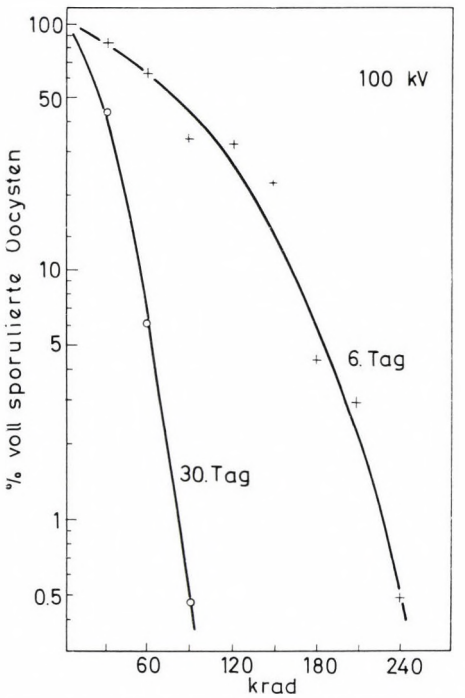

Abb. 1. Prozentsatz voll sporulierter *E. stiedai*-Oocysten (ca. 6 Wochen alt) am 6. und 30. Tag nach Röntgenbestrahlung (100 kV) in Abhängigkeit von der Bestrahlungsdosis. Anzahl der in der Kontrollprobe sporulierenden Oocysten = 100%

früherem Sporulationsstadium blieb die Entwicklung der Oocysten stehen (Abb. 2). Unterschiedliche Entwicklungsstadien in der gleichen Oocyste (z. B. voll entwickelte Sporozoiten neben nicht weiter entwicklungsfähigen Sporoblasten) wurden nur ganz selten gesehen und konnten nicht mit Sicherheit von Degenerationserscheinungen (s. auch Punkt 4) abgegrenzt werden.

3. Mit zunehmender Bestrahlungsdosis trat eine zunächst zu-, dann wieder abnehmende Anzahl abnorm sporulierender Oocysten (Ausbildung von 2 oder 3 Sporoblasten bzw. Sporozysten, aber auch 5—8 Sporoblasten gleicher und unterschiedlicher Größe) auf (Abb. 2).

4. Etwa vom 12. Tag nach der Bestrahlung an war bei jeweils gleicher Bestrahlungsdosis und Strahlenart eine Verminderung des Prozentsatzes vollsporulierter Oocysten durch Degenerationserscheinungen an den Sporozoiten festzustellen (Abb. 3 und 4). Die Sporozoiten einer Oocyste degenerierten dabei nicht alle gleichzeitig, sondern in unterschiedlichem Maße.

Es war z. B. möglich, daß in einer (in diesen Versuchen noch als voll sporuliert gewerteten) Oocyste nur noch ein morphologisch normal aussehender Sporozoit zu sehen war. Eine Differenzierung nach der Anzahl der noch

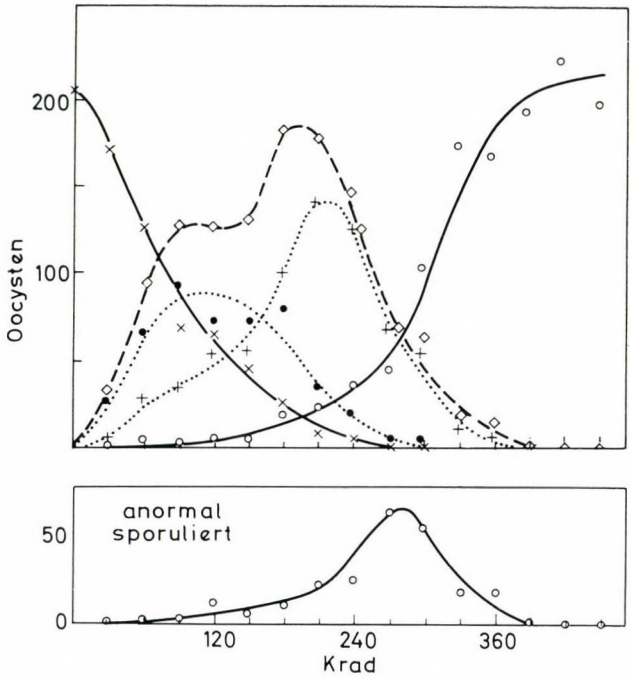

Abb. 2. Anzahl der am 6. Tag nach Röntgenbestrahlung (100 kV) vorhandenen einzelnen Sporulationsstadien von etwa 6 Wochen alten *E. stiedai*-Oocysten in Abhängigkeit von der Bestrahlungsdosis. Insgesamt wurden jeweils 300 Oocysten ausgezählt. Nicht eingezeichnet wurden degenerierte und unreife Oocysten sowie Oocysten mit Kanten- und Pyramidenbildung (letztere überwiegend = 0, maximal = 6 pro Probe). ×——× voll sporuliert; ◇——◇ teilweise sporuliert: +·····+ rund, ●·····● oval; ○——○ nicht sporuliert

morphologisch normal aussehenden Sporozoiten wurde im Rahmen der vorliegenden Arbeit nicht durchgeführt.

5. Niederenergetische Strahlung (50 und 100 kV) scheint bei gleicher Gesamtdosis eine geringfügig stärkere Wirksamkeit als höherenergetische Strahlung (100 kV, mit Kupferblech abgeschirmt, und Gamma-Strahlung [Co-60]) zu besitzen (Abb. 3 und 4). Die häufig niedriger liegenden Sporulationswerte nach der Bestrahlung mit 50 kV sind infolge der Unsicherheiten

der Dosisleistungsbestimmung in diesem Energiebereich nicht als 100%iger Beweis ansehbar. Die unter Punkt 4 erwähnte, bei diesen Versuchen noch nicht durchgeführte Differenzierung nach der Anzahl überlebender Sporozoi-

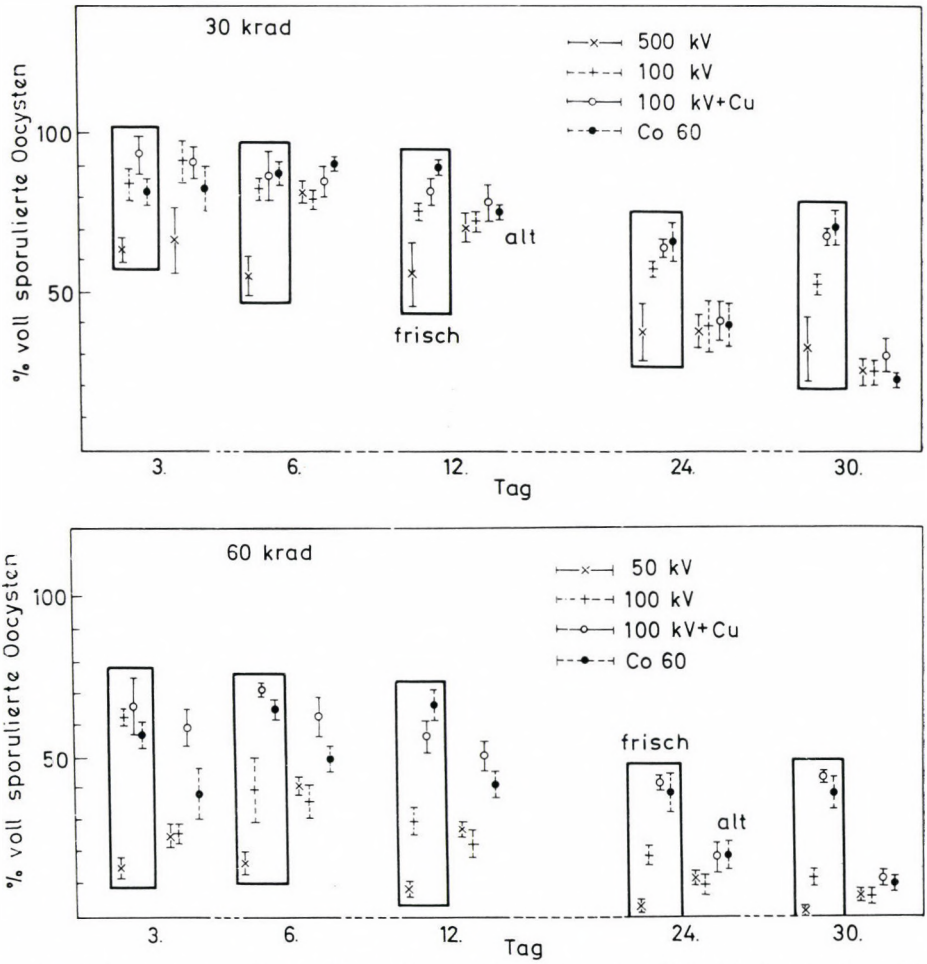

Abb. 3—4. Prozentsatz voll sporulierter *E. stiedai*-Oocysten in Abhängigkeit von dem Alter der Oocysten, dem Auszählungstag nach der Bestrahlung und der Bestrahlungsdosis (Abb. 3 = Gesamtdosis 30 krad; Abb. 4 = Gesamtdosis 60 krad). »Frische Oocysten« = 0—5 Wochen alt, »alte Oocysten« = 7—11 Wochen alt. Für die einzelnen Strahlenarten (Röntgenstrahlung 50 kV, 100 kV, 100 kV mit 0,2 mm Kupferblech abgeschirmt und Gammastrahlen von Co-60) wurde jeweils der Mittelwert sowie der mittlere Fehler des Mittelwertes eingetragen. Anzahl der in der Kontrollprobe sporulierenden Oocysten = 100%

ten im Verlaufe der auftretenden Degenerationserscheinungen könnte bei weiteren Untersuchungen möglicherweise ein eindeutigeres Bild ergeben.

6. Die Ergebnisse weisen darauf hin, daß auch das Alter der Oocysten die Wirksamkeit der Bestrahlung beeinflußt. Deutliche Unterschiede zeigen

sich am 24. und 30. Tag nach der Bestrahlung (Abb. 3 und 4). Eine Analyse der Ergebnisse unterschiedlich alter Oocysten am 30. Tag nach der Bestrahlung (Abb. 5) rechtfertigt dabei die in Abb. 3 und 4 vorgenommene Eingruppierung in »frische« und »alte« Oocysten, wobei die Grenze jeweils zwischen der 5. und 7. Woche gezogen wurde.

7. Ein Einfluß der Dosisleistung auf die Sporulationsrate war innerhalb der untersuchten Grenzen (s. Tab. I) nicht nachweisbar.

8. Bei Oocysten, die am 6. Tag nach einer Bestrahlung mit Gamma-Strahlen (Co-60) (ca. 40 krad) voll sporuliert waren, konnte bei in-vitro-Excystationsversuchen (nach KRIEG, 1971) an demselben Tag kein Unterschied

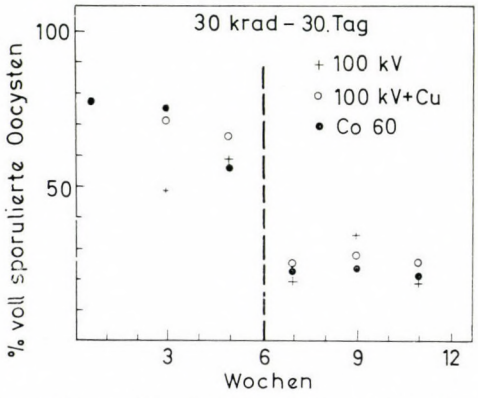

Abb. 5. Prozentsatz voll sporulierter *E. stiedai*-Oocysten am 30. Tag nach der Bestrahlung in Abhängigkeit von der Strahlenart (Röntgenstrahlung 100 kV mit und ohne 0,2 mm Kupferblech abgeschirmt und Gammastrahlen von Co-60) und dem Alter der Oocysten bei einer Bestrahlungsdosis von 30 krad. Es wurden für alle Untersuchungen Oocysten der gleichen Herkunft verwendet. Anzahl der in der Kontrollprobe sporulierenden Oocysten = 100%

in der Excystations- und Bewegungsrate zu unbestrahlten Oocysten festgestellt werden.

9. Eine sich andeutende geringfügige, bei höheren Bestrahlungsdosen zunehmende Sporulationsverzögerung wurde vorläufig nicht näher untersucht.

Diskussion

Die Abhängigkeit der Überlebensrate der Oocysten von der Bestrahlungsdosis wurde bereits mehrfach nachgewiesen (s. SCHNEIDER, AYENI und DÜRR, 1972). In den bisher vorliegenden Publikationen finden sich jedoch keine Hinweise auf die Abhängigkeit der Sporulationsrate von dem Untersuchungstag nach der Bestrahlung, die durch die oben erwähnte Sporulationsverzögerung (s. Punkt 9) bis zum 6. Tag nur geringgradig, vom 12. Tag nach der Bestrah-

lung an durch die auftretenden Degenerationserscheinungen (s. Punkt 4) in zunehmend stärkerem Ausmaß beeinflußt wird.

Die von FITZGERALD (1967) beschriebene Wirksamkeit von Gamma-Strahlen auf unsporulierte *E. stiedai*-Oocysten wurde bestätigt, jedoch konnte die von ihm angegebene z. T. verminderte Infektiosität der sporulierten Oocysten nicht überprüft werden.

Eine gewisse Abhängigkeit der Wirksamkeit von der Energie der Strahlen scheint vorhanden zu sein. Sie ist jedoch nicht in jedem Fall so deutlich, daß die Bemerkung von FITZGERALD (1968), die von verschiedenen Autoren benutzte unterschiedliche Strahlenart sei nicht die Ursache für deren stark voneinander abweichende Ergebnisse, vorläufig nicht widerlegt werden kann.

Eine signifikante Erhöhung der Anzahl anormal sporulierender Oocysten war im Dosisbereich von 120 bis 360 krad mit einem Maximum bei etwa 270 krad festzustellen (Abb. 2). Bei Bestrahlungen mit Dosen über 390 krad wurde im Gegensatz zu FITZGERALD (1967) nur noch ganz vereinzelt ($< 1\%$) anormale Sporulation festgestellt. Aus der von FITZGERALD (1967) für den Typ 1 seiner beobachteten abnormen Entwicklung gegebenen Beschreibung kann jedoch vermutet werden, daß es sich hierbei um keinen Entwicklungstyp, sondern um die nach einer letalen Bestrahlung sehr bald einsetzenden, auch in den eigenen Untersuchungen beobachteten Degenerationserscheinungen abgetöteter Sporonten gehandelt hat.

Die von FITZGERALD (1967, 1968) beobachtete höhere Excystationsrate bestrahlter Oocysten bei in-vitro-Versuchen konnte bei mit etwa 40 krad (Co-60) bestrahlten Oocysten nicht bestätigt werden.

Im Gegensatz zu den Untersuchungen von ALBANESE und SMETANA (1937), die bei 75 und 104 r/m und einer Gesamtdosis von 3000 r bei sporulierten Oocysten von *E. tenella* einen Einfluß der Dosisleistung auf die Vermehrungsrate feststellten, konnte in den eigenen Untersuchungen, allerdings in einem anderen Dosisleistungsbereich, kein derartiger Einfluß auf das Sporulationsergebnis beobachtet werden.

Weitere Untersuchungen müssen klären, ob die beobachtete Abhängigkeit der Absterberate noch sporulierender bestrahlter Oocysten von der Zeit mit einer eventuellen verminderten Resistenz gegen andere chemisch-physikalische Umweltfaktoren parallel läuft. Bei Sterilisationsverfahren (z. B. bei Abwasser und Klärschlamm, aber auch bei Käfigen und anderen Versuchsmaterialien), die mit Hilfe von Bestrahlung erfolgen, müßte dann nicht in jedem Fall die Letaldosis angewandt werden. In Abhängigkeit von den Umweltbedingungen (Selbsterhitzung, Fäulniserscheinungen, Austrocknung etc.) und Ablagerungszeiten könnten auch subletale Dosen bereits eine sichere Abtötung gewährleisten.

Der Angriffspunkt (Wirkungsmechanismus) der Strahlenschädigung ließe sich unter Umständen durch eine Kombination der Darstellung der

Sporulationskinetik (Abb. 2) nach verschiedenen Noxen (UV-, Röntgen-, radioaktive Bestrahlung, Ultrazentrifugation) mit anderen Untersuchungsmethoden (biochemische Analysen nach Ultraschallbehandlung, Untersuchung von Reversibilitätserscheinungen) ermitteln. Eine genauere Analyse der Ursachen (O_2-Mangel, Temperaturerhöhung, Alter, Bestrahlung, Ultrazentrifugation, Desinfektionsmittel) und Erscheinungsformen (inkomplette Sporulation, Ausbildung von Riesenformen, von Sporozoiten ohne Sporozystenhülle, Zwei- bis Achtfachteilung) abnormer Sporulationen könnte hierzu einen weiteren Beitrag liefern.

ZUSAMMENFASSUNG

Bestrahlungsschäden an *E. stiedai*-Oocysten äußern sich in vermindertem Sporulationsvermögen sowie bald nach vollendeter Sporulation eintretenden Degenerationserscheinungen. Es besteht eine Abhängigkeit der Abtötungsrate von dem Alter der Oocysten und vermutlich der Energie der Strahlung.

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РЕЗЮМЕ

БАКТЕРИАЛЬНАЯ ФЛОРА ЖЕЛУДКА И ОТДЕЛОВ КИШЕЧНИКА И ИХ ОПРЕДЕЛЯЮЩИЕ ФАКТОРЫ У РАНО ОТБИТЫХ ПОРОСЯТ

Ф. КОВАЧ, Б. НАДЬ и ДЬ. ШИНКОВИЧ

Докладывается о бактериологическом изучении желудка и кишечника 30 здоровых поросят до и после ранней отбивки. Поросята происходили от здоровых свиноматок и после отбивки в возрасте 14, 21 и 28 дней содержались или в оптимальных условиях, в климатизированных камерах (три помета), или в не хуже среднего зимних условиях. Изучена бактериальная флора и pH на 9 пунктах желудочно-кишечного тракта и данные обрабатывались статистически.

В значениях pH и бактериальной флоры пилорической и кардиальной частей желудка не было значимой разницы. В двенадцатиперстной кишке за исключением колиформных микробов наблюдалось сильное торможение, что особенно выраженным было в отношении клостридий. В последующих отделах тонкого кишечника зарегистрировано нарастание количества микробов и, таким образом, в последнем отделе подвздошной кишки уже получены показатели, характерные для толстых кишок. Бактериальные и условия pH слепой, ободочной и прямой кишок не отличались друг от друга.

При низких значениях pH (1,5—2,5) в желудке наблюдалось торможение роста колиформных микробов, но при наичаще регистрированных значениях pH — 2,5—4,5 — заметного торможения не наблюдали. В кишечнике не зарегистрировано изменений, связанных с pH. Между степенью наполненности тощей кишки и количеством микробов имеется прямая взаимосвязь, проявляющаяся в нарастании количества колиформных микробов. Не наблюдались изменения во флоре кишечника здоровых поросят, связанных только с фактом отбивки. Зато условия среды (содержания) консеквентно отражались на флоре кишечного тракта. Во всех отделах кишечника содержавшихся в традиционных условиях поросят колиформных микробов было всегда сигнификантно больше; у этих животных соотношение лактобациллов, стрептококков и колиформных микробов в подсосный период было тоже ненормальным.

КОШАЧЬИ ВИРУСНЫЕ ЗАБОЛЕВАНИЯ

I. Изучение вирусных штаммов, изолированных из пораженных верхних дыхательных путей кошек

Ф. САЛАИ, Ш. БЕРТА и М. КИШШ

За время последних двух лет изоличовано 12 вирусных штаммов из 12 кошек от 53 (22,6%), лечившихся из-за катара верхних дыхательных путей. Три среди этих штаммов (M_1, M_{11} и M_{12}) идентифицированы как герпетовирусы, тогда как остальные девять (M_2, M_3 . . . M_{10}) принадлежали к риновирусам группы пикорна. Штаммы M_3 и M_9 изучены детально.

Штамм M_9 отличался серологически не только от штамма M_3, но и от остальных семи штаммов пикорна. Штамм M_9 отличался от штамма M_3 еще и цитопатогенным эффектом и менее выраженной чувствительностью к трипсину, натрию деоксихолата и хлороформу.

Среди трех штаммов, изученных на патогенность, два — M_1 и M_9 — оказались сильно патогенными для кошки, тогда как M_3 был только слабо патогенным.

ДАННЫЕ К МОРФОЛОГИИ И ПАТОГЕНЕЗУ ОСТРОЙ ФОРМЫ БОЛЕЗНИ МАРЕКА

Ф. РАЦ, А. СЕКИ и А. ВАНИ

На одной птицеферме, неблагополучной в течение ряда лет по острой форме болезни Марека среди 11 800 животных от вылупления до 10-дневного возраста ежедневно, до 76-дневноо возраста (момента убоя) еженедельно три раза изучено клинически, патолого-анатомически и бактериологически всего 87 животных и 5 задохлых эмбрионов. Кроме этого гистологически изучались центральная и периферическая нервные системы и определенные экстранейральные органы. Последние исследования собраны в таблице.

Обнаружено, что в возрасте 41—76 дней у 83,3% животных, а в возрасте 18—39 дней у 57,1% животных имеются лимфоидные инфильтрации как в нервной системе, так и в экстранейральных органах. В животных моложе 18 дней инфильтрации в нервной системе не обнаружены. Зато в экстранейральных органах эти изменения обнаруживаются уже с 2 -дневного возраста, и с 27-дневного возраста они уже носят неопластический характер. Такие изменения в нервной системе имелись очень редко. Особенно в эпителиальных клетках железистого желудка обнаруживались включения типа Ковдри «А». Гистологические изменения обычно не сопровождались патологоанатомическими изменениями.

IV. ИЗУЧЕНИЕ ЛОКАЛИЗАЦИИ ПРЕЦИПИТИРУЮЩИХ АНТИТЕЛ ПРИ ПОМОЩИ ИММУНОЭЛЕКТРОФОРЕЗА В СЫВОРОТКЕ ЭКСПЕРИМЕНТАЛЬНО ИНВАЗИРОВАННЫХ CYSTICERCUS PISIFORMIS (BLOCH, 1780) КРОЛИКОВ

И. НЕМЕТ

Кролики возраста 6—7 недель и веса 700—900 г поголовно инвазировались 500 онкосферами Taenia pisiformis; у группы кроликов 60 дней позже произведена поголовная реинвазия 10 000 онкосферами. Образцы сыворотки, полученные в определенные сроки, изучались на наличие преципитинов путем двойной агар-жель-диффузии. В качестве антигенов использованы соматические и метаболические антигены, дальше, полные соляные экстракты как T. pisiformis, так и C. pisiformis.

Сыворотки с известной преципитирующей активностью подвергались электрофорезу на агаре и потом диффундировались в направлении антигенов гельминта, козьей антисыворотки против полной кроличьей сыворотки, и овчинной антисыворотки против глобулинов кролика. Линии преципитации получены со всеми сыворотками в локализации глобулинов IgG. Согласно этому нужно предполагать, что продуцируемие кроликом в ответ на инвазию онкосфер T. pisiformis преципитирующие антитела принадлежат к классу иммуноглобулинов IgG.

V. ХАРАКТЕРИСТИКА ИММУННОЙ РЕАКЦИИ ПРИ ИСКУССТВЕННОЙ ИНВАЗИИ CYSTICERCUS PISIFORMIS (BLOCH, 1780) У КРОЛИКОВ

И. НЕМЕТ

Изучалась иммунная реакция при первичной инвазии и реинвазии Cysticercus pisiformis у кроликов.

Кролики 5—7-недельного возраста, веса 500—1000 г поголовно инвазировались 500 онкосферами T. pisiformis; часть животных после 60 дней реинвазировалась поголовно 10 000 онкосферами. К 100-у дню эксперимента всех животных убили и изучили на инвазированность цистицерками. После инвазий по определенному плану от животных бралась кровь с целью изучения на антитела.

В ответ на инвазию все экспериментальные кролики продуцировали антитела, которые можно выявить пробами агар-жель-диффузионной преципитации, ларвальной преципитации и индиректной гемагглютинации.

Гемагглютинирующие антитела на второй неделе инвазии у всех инвазированных животных появились и были выявимыми до конца эксперимента. Характерным для динамики накопления гемагглютининов явилось то, что их средний титр со второй недели инвазии постепенно повышался и своего пика достиг к 50-у дню и потом немного понизился.

Динамика нарастания преципитирующих антител показывала ту же картину, как и динамика нарастания агглютининов. Преципитирующая активность наблюдалась первый раз на 2-ой неделе инвазии; своего пика она достигала между 6-ой и 10-ой неделями, потом стала заметно слабеть, но налицевствовала еще даже к 100-у дню инвазии.

Реинвазия животных 100 000 онкосферами и 60-у дню от первой инвазии вызывала быстрое повышение количества гемагглютинирующих и преципитирующих антител.

Типы появившихся иммуноглобулинов определялись пробами фракционированного высаливания, электрофорезом в агарных блоках, ионо-обменной хроматографией на ДЕАЕ Сефадекс-е А—50, чувствительностью к меркаптоэтанолу и элекрофоретическим анализом антисывороток против комплексов антиген-антитело, полученных из сывороток кроликов при помощи соматических и/или метаболических антигенов T. pisiformis и C. pisiformis. Эти наблюдения показали, что инвазия кроликов онхосферами T. pisiformis провоцирует образование как комплексного, так и гетерогенного, иммунологического ответа. За время инвазии образуются антитела не меньше чем против четырех антигенных компонентов паразита. Эти антитела принадлежат к двум различным иммуноглобулиновым классам: в ответ на первую инвазию образуются антитела типа IgG. После реинвазии доминируют антитела IgG, но имеются и антитела типа IgM.

ДАННЫЕ К УСТОЙЧИВОСТИ ООЦИСТ EIMERIA STIEDAI К ОБЛУЧЕНИЮ РЕНТГЕНОВЫМИ И ГАММА ЛУЧАМИ

Й. ДЮРР и В. РАЙЗЕР

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