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Varia

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STUDIES ON ROUGHNESS AND ENDURANCE OF FLOOR SURFACES IN ANIMAL HOUSES

By

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(Received April 2, 1973)

It is known from practical observations that management failures are often responsible for diseases of the extremities in swine.

Concrete flooring, inadequate attendance (Behrens, 1961; Domán, 1972; Maclean, 1968; Nilsson, 1964; Osborne, 1950; Penny et al., 1963) and the lack of exercise associated with closed systems of management (A. B. Kovács, 1972) were shown to be causal or predisposing factors and, apart from them, feeding errors and congenital abnormalities were also found to play a part (Claus, 1962; Hámori, 1972; Meyer, 1963; Rieck, 1961).

Animals maintained in large-scale units require management systems apt to ensure optimal environmental conditions to eliminate or at least minimize deleterious factors, including those responsible for surgical diseases.

Investigations of optimal flooring in animal houses have been centered on the wearing surface, the quality of which is decisive in respect of the health state of the foot. As yet no systematic studies have been reported on the smoothness or roughness and abrasive resistance of stable floors, but certain erroneous views are gaining ground in respect of the desired quality of surface finish. Among others Gábor (1968) has advocated the roughening of floor surfaces for rendering them skid-proof.

Apart from the main physical properties of the floor (thermic properties and bearing strength), wear resistance and surface finish are those main quality parameters which should be precisely established and taken into consideration at construction in order to prevent the foot diseases related to inappropriate flooring.

In the machine industry, the roughness values of metal surfaces are prescribed by well-defined criteria (Beer et al., 1968) which can, however, by no means be adapted for the evaluation of floor surfaces. As long as no mechanical, pneumatic, electric or optical instrument is available for direct determination of the roughness value of floor surfaces, the investigator is resorted to estimations based on comparison to certain commonly used objects.

Materials and methods

The roughness (smoothness) of floor surfaces was assessed indirectly by measuring the degree of attrition of the horny part of the foot on the ground that it is exposed to a much greater tear-and-wear on rough than on smooth surfaces.

The abrasive resistance of the floor was measured by applying friction of a known value directly to its surface. Both examinations were carried out with a slit-phase electric equipment of 130 W output, operated from the mains (220 V), and constructed specially for the purpose. The circular movement of the electric motor was transformed to a linear alternating motion by means of

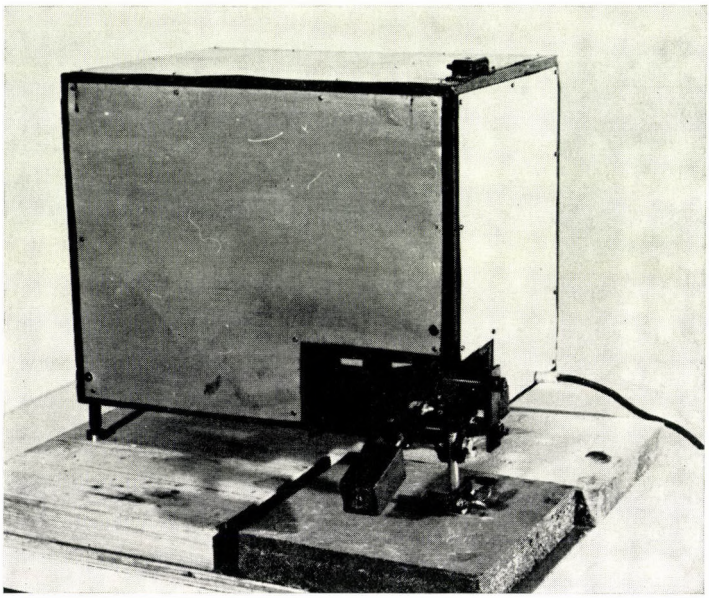

Fig. 1. Equipment for testing of surface roughness and wear resistance of flooring

a driving rod. The path of motion was 8 cm long and motion frequency was 80/min (Fig. 1).

In both experiments, specimens of the nail of 8—12-month- old Large White pigs were used as test pieces. One cm long, 0.3 cm thick specimens were cut from the anterior and lateral parts of the wall in line with the horny leaves (laminae) and were dried at room temperature before use. The average moisture content of the horn specimens was 7.5%. The specimens were so located in the equipment that their longitudinal axis was square with the floor surface, whereas their width with the direction of movement. Thus at a single movement (friction) the specimen made contact with a 8 cm long by 1 cm wide area of the floor surface. A load of 2.5 kp was put on the horn specimens by means of a lead mouse, weighing 1 kp, and an arm.

The greater part of the floor types examined were commonly used varieties, the rests were constructed specially for the purpose of the experiments. Floors made of wood, brick, concrete, asphalt, plastics and rubber were tested. The composition of the asphalt floor designated in Table I with "x" differed

considerably from the traditional formula, consisting of cold-processed bitumen, cement and furnace cinders, a blend giving a rough surface. Of the two synthetic floors Dynafarm was made in the GFR, Graboplast and the two rubber floors in Hungary.

Depending on the roughness or smoothness of the floor surface, mechanical rubbing with the horn specimen was carried out for 1—5 minutes. If the surface is extremely rough, the horn becomes so heavily worn in one minute that it can no longer be used. The wear surface of the horn specimens was rendered smooth for the experiment by preliminary abrasion for a few seconds. Five specimens, each from the anterior and lateral part of the wall, were rubbed to the floor under examination, the degree of wear was assessed from the mean weight loss of the specimens and the roughness value of the floor was estimated from the degree of wear. The specimens were weighed with an accuracy of ± 0.1 mg before and after the the rubbing test.

The wear resistance of the floor was assessed by the same procedure, except that in this instance the attrition of the floor surface and not that of the horn was measured.

The depth of the attrition groove was carefully measured with a gauge rule and read under a hand magnifying lens. The rubbing test was performed in five different areas of the floor, using fresh horn specimens for each, and a mean value was calculated from the five readings. According to the degree of wear, the floor was qualified as "good", "satisfactory" or "bad".

Results

Roughness values of floor surfaces, as estimated from the abrasion of the horny part of the foot and the wear resistance of floorings as estimated from the depth of the attrition groove produced in the rubbing test, are shown in Tables I and II. The data of both Tables are suitable for realistic estimates of floor quality from the surgical point of view.

Discussion

It is clear from the data of Table I that attrition of the horny part of the foot varied with the degree of roughness of the floor surface. As the conditions of the rubbing test were the same for each horn specimen and each type of floor, the experimental data enable a fairly accurate ranking of floor types. The horn was most heavily worn on rough concrete and on the rough asphalt designated with "x" and least worn on hardwood and hard asphalt floors. The endurance of horn specimens from the lateral wall was distinct from that

Table I

Roughness values of floor surfaces as assessed from attrition of horn

Serial no.	Type of flooring	Time of abrasion (min)	Weight (g) of horn specimens			Weight of abraded horn (g)	Qualification of floor		
				before abrasion	after abrasion		good	satis-fact.	bad
1.	Hardwood	5	A	0.5529	0.5516	0.0013	+	—	—
			L	0.4336	0.4280	0.0056	+	—	—
2.	Brick	5	A	0.5334	0.5306	0.0028	+	—	—
			L	0.4545	0.4446	0.0099	+	—	—
3.	Smooth-surfaced concrete	5	A	0.5039	0.4579	0.0460	—	—	+
		4	L	0.3880	0.3589	0.0291	—	—	+
4.	Rough-surfaced concrete	1	A	0.5227	0.4266	0.0961	—	—	+
		1	L	0.3619	0.2516	0.1103	—	—	+
5.	Asphalt (smooth surfaced)	5	A	0.6375	0.6342	0.0033	+	—	—
			L	0 3254	0.3200	0.0054	+	—	—
6.	Asphalt "x" (rough surfaced)	2	A	0.5740	0.5133	0.0607	—	—	+
		1	L	0.3327	0.2877	0.0350	—	—	+

Symbols used: A, specimen from anterior part of the wall of foot horn;
L, specimen from lateral part of the wall of foot horn

of the anterior part throughout, which can be attributed to differences in strength and thickness of horn.

The degree of wear of the horny part as assessed from the test results cannot be directly applied to the living animal. The rubbing force employed in the experiments scarcely abraded part of the specimens, whereas from others a 2—3 mm long portion, well measurable also in terms of weight (mp), was worn away. Another reason of the inconclusiveness of numerical data (time of rubbing and thickness of the horny part) for the living animal is the fact that in the latter the wear resistance of the foot horn is greatly influenced by the physiological state and by the moisture content of the horn, which greatly depend on the conditions of management (dry or moist floor, soiled or contaminated floor). In the case of pigs, the wear of the foot horn and floor surface are both subject to the influence of static and dynamic forces. The test results unequivocally show that apart from the body weight and movement velocity of the pig, the roughness of the floor surface plays a decisive role in the abrasion of foot horn. Since the movements of pigs are characteristically slow, it would be difficult to estimate the proportions at which the static and dynamic forces come into display. It is known that during walking, the foot slips forward (friction !) when it descends on the ground.

Table II

Data of wear resistance tests on different types of flooring

Serial	Type of flooring	Time of abrasion (min)	Degree of floor abrasion		Depth of attrition groove (mm)	Qualification of floor		
			on visual inspection	on manual inspection		good	satis-factory	bad
1	Firewood (softwood)	5	scarcely visible	scarcely tangible	0.1	+	—	—
2	Beechwood (hardwood)	5	scarcely visible	scarcely tangible	0.1	+	—	—
3	Brick (solid)	5	scarcely visible	scarcely tangible	—	+	—	—
4	Brick (soft)	2	well visible	tangible groove	3—5	—	—	+
5	Concrete with smooth surface finish	5	well visible	roughened	0.2	—	—	+
6	Rough-surfaced concrete	1	well visible	tangible groove	1—2	—	—	+
7	Asphalt	5	scarcely visible	scarcely tangible	0.1	+	—	—
8	Asphalt	2	well visible	deep groove	5—6	—	—	—
9	PVC	5	conspicuous wear	tangible groove	0.2	—	—	+
10	Graboplast	2	conspicuous wear	tangible groove	3	—	—	+
11	Dynafarm	2	conspicuous wear	tangible groove	3	—	—	+
12	Rubber (trefoil pattern)	5	well visible	well tangible	0.5	—	+	—
13	Rubber (pyramid pattern)	5	conspicuous wear	pyramid pattern worn off, smooth	3.5	—	+	—
14	Rubber (rough-surfaced)	5	well visible	well tangible	1	—	+	—

The experimental results accord well with the practical observation that pigs maintained without bedding on a rough-surfaced floor (rough concrete and asphalt "x" in the experiment) show, apart from a marked abrasion of horn from the bearing surface (lower edge), sole and external lateral wall, a partial or complete attrition of the horny part of the plantar cushion already within 2—4 months. The horn of the lateral wall becomes straightened by wear in the

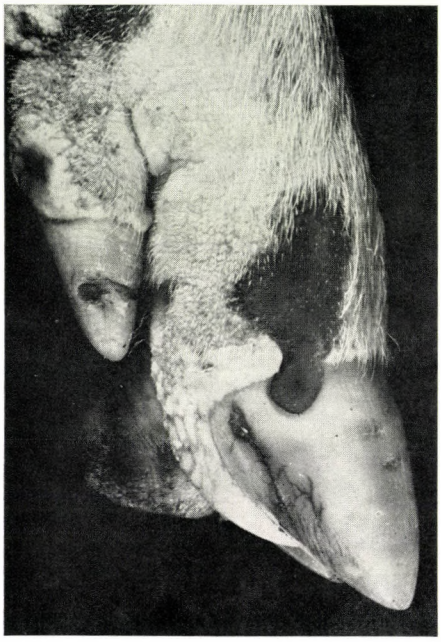

Fig. 2. Excessive abrasion of the lateral wall of foot horn and skin injuries in the regions of coronet and fetlock joint

lying animal which is inclined to rub the external lateral part of the nail to the floor (Figs 2 and 3). Heavily worn nails predispose for inflammation of the corium, lameness, loss of appetite and frequently even to infection aggravated by complications.

It can be seen from the data on the wear resistance of floors (Table II) that the mechanical friction of horn specimens caused different degrees of abrasion or pitting, depending on the type of flooring. Certain floors originally regarded as strong became heavily worn in a short time. In general, the degree of abrasiveness was greater than expected from the material, quality and processing technology of the floor types examined.

The wear resistance of softwood and hardwood floors proved to be equal.

Test results with brick floors differed considerably, depending on the application of friction to the surface or to the sides. The surface was easily worn

away, whereas the side and edges, apparently harder in texture, resisted wear. Smooth concrete behaved in every respect similar to rough concrete as soon as the finishing layer was worn away. It was found that, whether smooth or rough, the wear resistance of concrete floors depends exclusively on processing, thus both may be resistant to wear, *i.e.* "good", or show pitting on wear and prove a "bad" floor which predisposes for foot injuries.

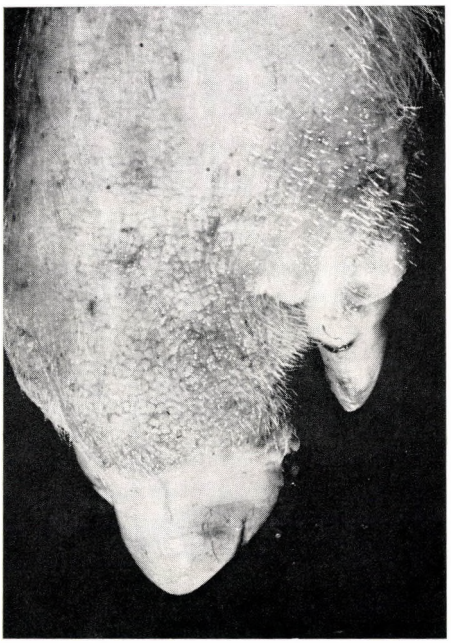

Fig. 3. Stumplike appearance of foot horn owing to excessive wear of anterior wall

As already mentioned, hard asphalt and hardwood floors were found to be the best in respect of wear resistance.

The asphalt floor designated with "x" was more heavily worn than expected (Fig. 4) and the synthetic (Graboplast and Dynafarm) and rubber floors also showed little resistance to wear (Figs 5, 6). This accords well with the practical observation that Dynafarm was markedly worn away and peeled off after 1.5 years of use so that the pigs began to chew it. Also, Dynafarm proved to be markedly slippery in both dry and moist state, preventing the pigs from safely moving around.

All three rubber floors were found to be havily worn after rubbing for 5 minutes. The floor with the pyramid pattern (Fig. 7) and the coarse-surfaced floor were both worn smooth. Thus the qualification of rubber floors as "satisfactory" (Table II) is greatly conditional.

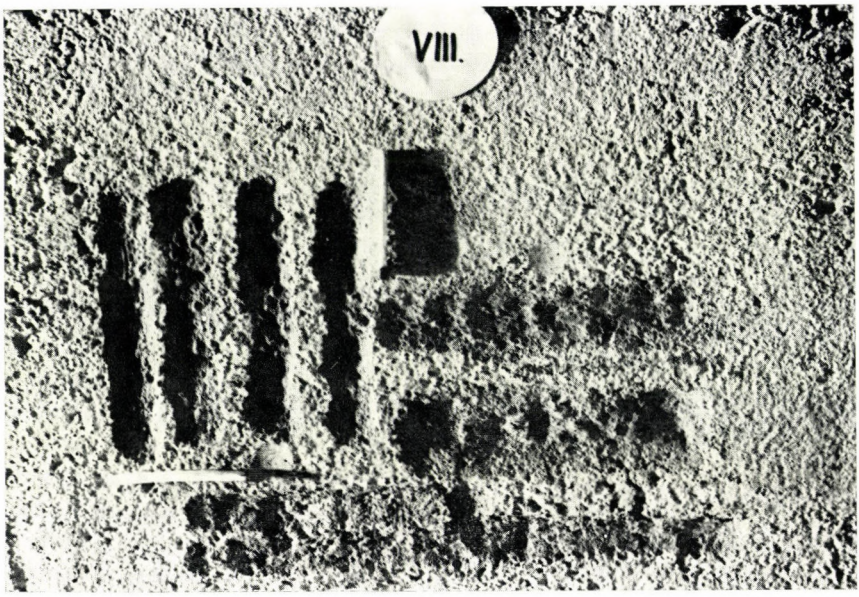

Fig. 4. Surface of Graboplast floor after mechanical rubbing (friction) for two minutes

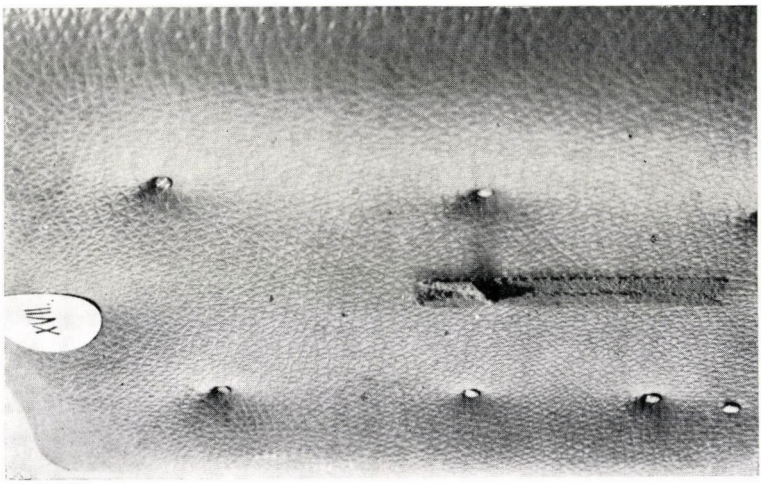

Fig. 5. Surface of Dynafarm floor after mechanical rubbing (friction) for two minutes

The surface layers of the floors behaved differently in the rubbing tests. It may be stated on the basis of the test results (Table II) that the wear resistance of "good" stable floors should be equal to that of hard asphalt or hardwood.

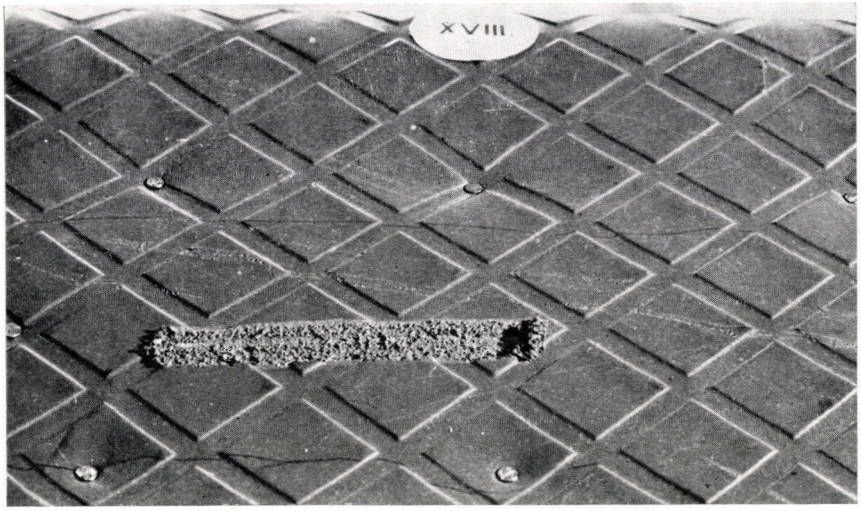

Fig. 6. Rubber floor (pyramid pattern) after mechanical rubbing (friction) for five minutes

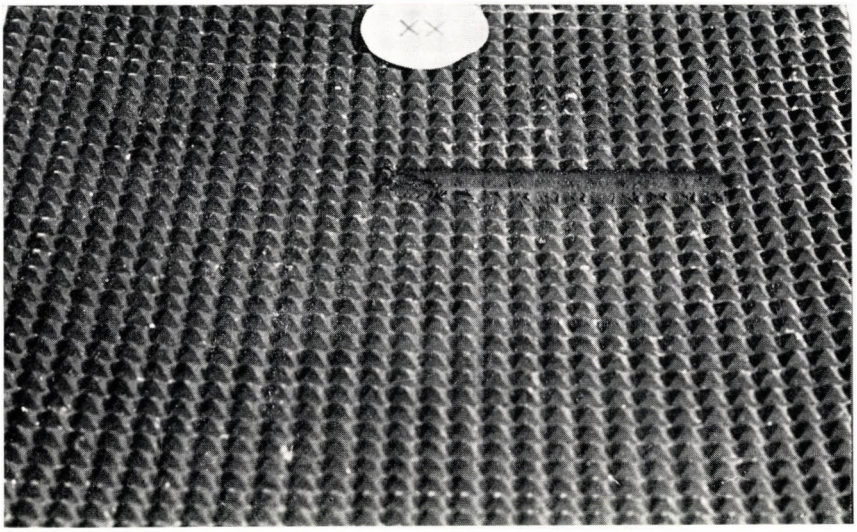

Fig. 7. Surface of asphalt "x" floor after mechanical rubbing (friction) for five minutes

The conditions of the present experiments can serve as a model for establishing floor parameters by means of a technically improved equipment and a test material (possibly synthetic) equal in hardness to the horn of the foot.

The need for such technical facilities seems still more urgent in view of the practical observation that floors of low strength and low wear resistance are excessively worn away in a very short time, especially in the surroundings of the feeders and drinkers, where the animals stand and patter about with the

forelegs. *E.g.*, the asphalt "x" floor showed in the most exposed areas pits fit to manage a walnut or an egg and general unevenness over the entire surface within a few months (Fig. 8).

In view of the results of the wear resistance tests, the question is justly put whether any of the known floor materials would possess all criteria of a good floor, *viz.* optimal thermic properties, elasticity and, simultaneously,

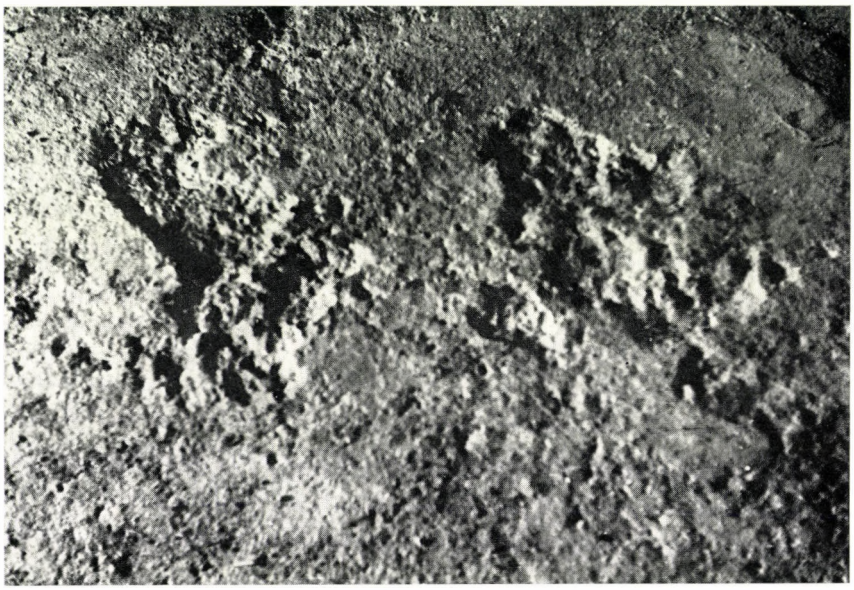

Fig. 8. Heavily worn, pitted asphalt "x" floor after use for a few months in a pig house

endurance. Ideal elasticity should be of such a degree that alterations caused by the descension of the foot on the floor surface should disappear immediately after the foot is lifted. Impressions and footprints left in a soft plastical floor interfere with cleaning and disinfection and, after a certain time, may become the source of foot injuries, distortion and fracture. Soft floors are easily worn away, which is another reason of their unsuitability. It follows that floors with ideal elastic properties are not at present available.

Recent attempts for introduction of rubber and synthetic floorings into animal houses proved to be a failure for the above reasons, as shown also by the present tests. Newer formulas of synthetic floor material require thorough testing in the laboratory and a minimal period of one year in an animal house for the evaluation of their practical value.

Another problem awaiting clarification by experimental evidence is whether the prevention of foot diseases in pigs does in fact require the biological elasticity of flooring. Future investigations should therefore be extended

to interrelationships of the extremities with hard or soft (elastic) flooring. Taking into consideration the biological requirements of the animal, the related investigations should be centered on the following two main points: (1) the load imposed upon the joints, tendons and muscles of the extremity during walking (slow or fast movement) and (2) the conditions of comfortability in lying position (at rest).

It is clear without special investigations that in the case of swine, the hardness of the floor does in itself not nearly as much predispose for foot disease as the roughness of floor surface. This follows on the one hand from the fact that the swine neither performs, nor is obliged to perform, rapid movements, whence injuries arising from hard ground, large body weight and velocity are negligible; on the other hand the mechanism of movement and the anatomical structure of swine foot greatly compensate the large body weight against hard ground. It is known that the plantar cushion of swine is very well developed, occupying more than two thirds of the sole.

In the light of the above speculations it seems highly possible that the disadvantages of hard flooring chiefly take effect in the lying position. Increase of tissue and decubitus in the area of the joints are frequent injuries and also painful, which is in itself sufficient to cause reduction of feed comsumption and weight gain as well as neurohormonal disorders. Rough floor surfaces easily cause erosions and injuries which predispose for infections, various complications, arthritis and ulceration. The investigator exploring the possibilities of foot disease prevention should always strictly distinguish between damages related to hard flooring and those related to the conditions of closed management systems.

The numerical data of the experiments as well as the inspection and manual examination of the floors indicate that at present acid-fast, hard asphalt is the flooring of choice for adult swine kept without bedding.

Hardwood floors are in principle as good as hard asphalt, but their disinfectability, bacterium contents and endurance (towards chewing by the pigs) require further study.

The present studies on pig house floors have unequivocally clarified those surface quality criteria which prevent excess attrition of the horn of the foot in pig houses without bedding. An appropriate instrument for the numerical recording of roughness values of the various floor surfaces is, however, still lacking and until it is made available, estimations based on comparison to dressed hardwood might probably be helpful.

Summary

Inadequacy of flooring is frequently responsible for foot diseases in swine. The roughness of surface and the abrasive resistance of various types of flooring (wood, concrete, asphalt, plastics, rubber) were tested by a mechanical equipment specially constructed for the purpose. Specimens of the horny part of swine foot, of given size and weight, were located in the equipment and rubbed to the floor for 1—5 minutes. The roughness of the floor surface was estimated from the degree of attrition of horn, whereas resistance to wear from the depth of the attrition groove or defect produced by friction. The horn was found to be worn heavily by rough floor surfaces (*e.g.*, rough concrete, rough asphalt), but the floors became worn themselves to different degrees as a result of rubbing. Among the floors tested, hardwood and hard asphalt showed the greatest resistance to friction. It is proposed that until an appropriate method of floor testing is evolved, surface roughness should be estimated by comparison to dressed hardwood.

References

Beer, Gy., Kiss, T. and Párkány, M.: Fémismeret és gépgyártástechnológia III. Budapest, 1968. p. 198. Behrens, H.: Schweinezucht und Schweinemast, 1961, 137. Claus, A.: Schweinezucht und Schweinemast 1962, 124. Domán, I.: Magyar Állatorvosok Lapja (MÁL), 1972, 209. Gábor, L.: Hőfizikai igénnyel fellépő mezőgazdasági épületek padlószerkezetei. Budapest, 1968. Hámori, D.: Csontgyengeségi syndroma sertéseken. In: Áldásy, P. and Hámori, D.: Iparszerű állattartás állategészségügyi követelményei, Information Centre of the Ministry of Agriculture and Food, Budapest, 1972. B. Kovács, A.: Állattenyésztés **27** (1972), 159. Maclean, C. W.: Vet. Rec. **83** (1968), 71. Meyer, H.: Dtsch. tierärztl. Wschr., 1963, 176. Nilsson, S. A.: Nord. Vet. Med. **16** (1964), 128. Osborne, H. G.: Aust. vet. J. **26** (1950), 316. Penny, R. H. C., Osborne, A. D. and Wright, A. I.: Vet. Rec. **75** (1963), 1225. Rieck, G. W.: Mitt. Dt. Landwirtschaftsges. **76** (1961), 1061.

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STUDIES ON H_2S PRODUCTION BY *BRUCELLA OVIS* STRAINS

By

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(Received February 5, 1974)

The production of H_2S has generally been used as an important differentiating feature of *Brucella* species.

Brucella ovis, the species responsible for epididymitis in rams was found to lack H_2S-producing capacitiy by its investigators (SIMMONS and HALL, 1953; BUDDLE and BOYES, 1953; GDOVIN et al., 1955; MEYER and CAMERON, 1956; KLAHN, 1958; SZABÓ and NYIREDY, 1967). MORGAN and GOWER (1966) also confirmed that like the *Br. melitensis* reference strain "M 16", *Br. ovis* 63/290 is lacking the H_2S-producing activity when measured by HUDDLESON's method (1928).

In contrast, BUDDLE (1956) in a later publication as well as TOPLEY and WILSON (1966) and TRILENKO (1970) reported that certain strains of *Br. ovis* are indeed capable of producing a moderate amount of H_2S applying HUDDLESON's method.

The present studies were carried out to reconcile the conflicting opinions.

Materials and methods

A) The following *Brucella* strains were used:

1. 17 *Br. ovis* strains freshly isolated from the seminal fluid of infected rams in the laboratory of Artificial Insemination Centre, Budapest
2. *Br. ovis* 63/290, reference strain (Weybridge)
3. *Br. melitensis* "M 16", reference strain (Weybridge)
4. *Br. suis* 1330, reference strain (Weybridge)
5. *Escherichia coli* Eck O_{141} (Prague)

B) *Determination of H_2S production.* Ten tube cultures were simultaneously prepared from each strain on serum-dextrose agar and were incubated at 37°C, the *Br. ovis* strains in the presence of 10% CO_2, the *Br. melitensis* and *Br. suis* strains under aerobic conditions. Two cultures of each strain were tested for H_2S production every other day, using two different methods:

1. lead-acetate indicator strip procedure proposed by HUDDLESON (1928) and employed by MORGAN and GOWER (1966).

2. The culture was rinsed from the medium with 3 to 4 ml Leibich broth, containing 20% brucella-negative serum. The germ count of the suspension thus obtained was 10^7—10^8/ml. Microcultures were then prepared from the suspensions of *Br. ovis* and control strains in the wells of Takátsy's Microtitrator plates as follows: 0.1 ml suspension was placed in each of 12 wells in one row. Two drops of the serum-broth, also containing THIRY's (1960) MI-DI reagent (Reanal, Budapest), were added to the first 6 wells, and serum-broth without reagent was added to the remaining 6 wells. Each plate was prepared in two replicas and were placed in a Petri dish lined with a disc of moist filter paper. They were incubated at 37.4°C, one in the presence of 10% CO_2, the other under aerobic conditions. The aerobic microcultures were read at 6, 12 and 24 hours, those incubated in 10% CO_2 atmosphere after 12 and 24 hours. The reaction was scored on the basis of colour shades, as follows:

Reaction	—	+	++	+++	++++
Colour of microculture	Light brownish--yelow	Brown "button"	Brown	Dark brown	Black

Prior to use, the Microtitrator plates were soaked in 5% sulphuric acid solution for 6 hours, washed several times in sterile distilled water, dried, and sterilized either with a mixture of ether and ethanol or by irradiation with a bactericidal lamp.

To determine the sensitivity of the two H_2S tests, comparative studies were performed with dilution series prepared from 0.13% aqueous solutions of H_2S.

The lead acetate strip-test was read after 30 minutes, the MI-DI test after an incubation of the reaction mixtures for 4 hours at 37°C, whereafter, according to preliminary experiments, the results do not change.

Results

As can be seen in Table I, the MI-DI test showed a medium degree of H_2S production by all examined *Br. ovis* strains from 48 hours of culturing to the end of the observation period. Reference strains *Br. melitensis* "M 16" and *Br. suis* 1330 began to show a moderate or a strong positive reaction already after 24 hours. The traditional lead acetate strip technique detected H_2S production exclusively with the *Br. suis* strain. The isolate *Br. ovis* 4160 caused a slight colour change at 96 hours, but neither before nor after that time. In both tests, the *Br. suis* strain showed a strong positive reaction, whereas the *E. coli* strain a negative reaction.

Table I

Production of H_2S by *Brucella ovis*, *Brucella melitensis* "M 16" and *Brucella suis* 1330 strains

Serial No.	Designation of *Brucella* strains	H_2S production as measured by the MI-DI test at				
		24	48	72	96	120
		hours				
1	*Brucella ovis:* 5250		++	++	++	++
2	*Brucella ovis:* 4160		++	++	++	++
3	614		+	+	+	+
4	4240		++	++	++	++
5	4150		++	++	++	++
6	1635		++	++	++	++
7	2030		++	++	++	++
8	5120		++	++	++	++
9	5130		++	++	++	++
10	4640		++	++	++	++
11	5310		++	++	++	++
12	5316		++	++	++	++
13	2166		+	+	+	+
14	2336		++	++	++	++
15	3350		++	++	++	++
16	3610		++	++	++	++
17	5020		++	++	++	++
18	*Br. ovis* 63/290 Weybridge		++	++	++	++
19	*Br. melitensis* "M 16" Weybridge	++	++	++	++	++
20	*Br. suis* 1330 Weybridge	+++	+++	+++	+++	+++
21	*E. coli* Eck O_{141} Prague	—	—	—	—	—

Serial No.	Designation of *Brucella* strains	H_2S production as measured by the method of Morgan et al. at				
		24	48	72	96	120
		hours				
1	*Brucella ovis:* 5250		—	—	—	—
2	4160		—	—	+	—
3	614		—	—	—	—
4	4240		—	—	—	—
5	4150		—	—	—	—
6	1635		—	—	—	—
7	2030		—	—	—	—
8	5120		—	—	—	—
9	5130		—	—	—	—
10	4640		—	—	—	—
11	5310		—	—	—	—
12	5316		—	—	—	—
13	2166		—	—	—	—
14	2336		—	—	—	—
15	3350		—	—	—	—
16	3610		—	—	—	—
17	5020		—	—	—	—
18	*Br. ovis* 63/290 Weybridge		—	—	—	—
19	*Br. melitensis* "M 16" Weybridge		—	—	—	—
20	*Br. suis* 1330 Weybridge	+++	+++	+++	+++	++
21	*E. coli* Eck O_{141} Prague		—	—	—	—

— negative; + slightly positive; ++ moderately positive; +++ strongly positive

According to the control examinations performed with serial dilutions of aqueous H_2S solution, the MI-DI test is about twenty times as sensitive as the lead acetate paper strip procedure. The sensitivity difference was especially conspicuous with those bacteria which are poor H_2S producers.

Discussion

Production of H_2S has generally been regarded as an important differentiating feature of certain bacteria, and the classification and species and type differentiation of *Brucellas* has largely been based on it. The present studies clearly show that both *Br. ovis* and *Br. melitensis* "M 16" produce H_2S, although less than demonstrated with the traditional lead acetate paper strip technique. The aforementioned strains produce H_2S continuously, at a low intensity and in roughly equal amounts. The presence of H_2S production in all strains previously regarded as non-producers could be unequivocally demonstrated with the MI-DI test, which is about 20 times as sensitive as the traditional method. Thus the value of H_2S production as a differentiating feature has been reduced from absolute to a merely quantitative level. In this light, the reliability of H_2S production as a criterion for *Brucella* type (species) differentiation might well be questioned. The same applies to the method of HUDDLESON because, conform to the observations of BUDDLE and others, one of our *Br. ovis* isolates showed a low positive reaction also with lead acetate paper technique at four days of culturing.

Summary

In attempt to reconcile the conflicting opinions on the H_2S production of *Br. ovis* strains, 17 *Br. ovis* isolates and the reference strains *Br. ovis* 63/290, *Br. melitensis* "M 16" and *Br. suis* 1330 were examined with the traditional lead acetate paper strip technique of HUDDLESON and with the MI-DI test. While the former procedure had negative results with all strains but a moderate amount of H_2S was clearly detected by MI-DI test at all examined *Br. ovis* and *Br. melitensis* strains. Production was found to take place continuously at a low intensity and the amounts produced by the different strains were roughly equal. Control examinations with aqueous solutions of H_2S showed that the MI-DI test is about 20 times as sensitive as HUDDLESON's procedure. It is concluded that H_2S production cannot be regarded as an absolute differentiating feature of *Brucella* strains, its significance being only quantitative. In this light, the value of H_2S production in species and type differentiation of *Brucellas* needs reconsideration.

*

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References

ALFÖLDY, Z., IVÁNOVICS, GY. and RAUSS, K.: Orvosi mikrobiológia, immunitástan és parazitológia. Budapest, 1973. BUDDLE, M. B. and BOYES, B. W.: Austr. vet. J. **29** (1953), 145. BUDDLE, M. B.: J. Hyg. (Camb.) **54** (1956), 351. GDOVIN, T., HRUDKA, Fr., CHLADECKY, Fr. and KOPPEL, Z.: Sborn. Česk. Akad. Zem. Věd, 1955, 617. HUDDLESON, I. F.: Differentiation of the species of the genus *Brucella*. In: Symposion on undulant fever. Annual Meeting of the American Public Health Association. Michigan, 1928. KLAHN, J.: Tierärztl. Umschau, 1958, 208. MEYER, M. E. and CAMERON, H. S.: Amer. J. Vet. Res. **17** (1956), 495. MORGAN, W. J. B. and GOWER, S. G. M.: Techniques in the Identification and Classification of Brucella. In: GIBBS, B. M. and SKINNER, F. M.: Identification Methods for Microbiologists. Part A. London, 1966. SIMMONS, G. C. and HALL, W. T. K.: Austr. Vet. J. **29** (1953), 33. SZABÓ, I. and NYIREDY, I.: Magyar Állatorvosok Lapja, 1967, 439. THIRY, L.: Honvédorvos **12** (1960), 229. TOPLEY, W. W. C. and WILSON, G. S.: Principles of Bacteriology and Immunity. London, 1966. TRILENKO P. A.: Veterinariya, 1970 (7), 51.

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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE ADRENAL GLANDS OF GEESE AND DUCKS

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(Received March 1, 1974)

The role of the adrenal gland is more difficult to be evaluated in birds than mammals. The difficulties are partly related to the intermingling of the cortical and medullary tissues.

It is generally accepted that the cortex of the avian adrenal gland cannot be divided into the three distinct zones of its mammalian counterpart. SAUER and LATIMER (1931) mention that the peripheral strands have a glomerulosa-like appearance, whereas the interrenal cells of the central part of the gland resemble those of the mammalian reticular zone. CHESTER et al. (1962) suggest that looping of the interrenal or cortical cell cords against the capsule indicates zoning. KAR (1947) reveales that the cortical cells are differentiated from certain mesenchymal capsular cells and migrate progressively towards the centre of the gland. Further, SIVARAM (1964, 1965) confirmed the presence of a higher metabolic rate, mitosis and nuclear pyknosis in the periphery of the gland which might be accounted for such a progression. KONDICS (1971) demonstrates that the outer zone of the pigeon's adrenal gland controls first of all the electrolytes while the inner zone is controlled by ACTH. Authors generally agree that stress affecting the entire body, or increased requirement for corticosteroids are characterized both in mammals and birds by adrenal hyperfunction manifesting itself by depletion of cholesterol and ascorbic acid in the gland.

It seems to be of interest to study the adrenal gland in geese and ducks to clarify the regional differences of the cortical tissue as well as to examine the histological and histochemical changes which occur during the sexually active period.

Materials and methods

In 1973, two males and two females each of Pekin ducks and Rhine geese, originating from the State Farm in Tata, were examined every month in our Department. The birds were killed by bleeding, the adrenal glands were removed immediately and processed for microscopic examination as follows. Each adrenal gland was divided into five parts, three of which were taken freshly, and stained directly for demonstration of ascorbic acid (Bacchus-silver method), chromaffin reaction (potassium chromate) and formalin-induced fluorescence (FIF) test. The remaining two parts were fixed in 10% formalin, and one part was used for frozen sections to detect neutral fats by the Sudan black method and cholesterol by Schultz's technique, the remaining part was embedded in

paraffin. The paraffin-embedded sections were stained with the following techniques: Mayer's haematoxylin and eosin, Masson's trichrome, Gomori's silver impregnation and orcein.

Results

I. Goose adrenal gland

The gland is surrounded by a thin capsule of connective tissue, containing blood vessels and nerves. The capsule mainly consists of collagen and reticular fibres, with very few elastic elements. Delicate septa arising from the capsule and ramifying between parenchymae tissues form the interstitial tissue

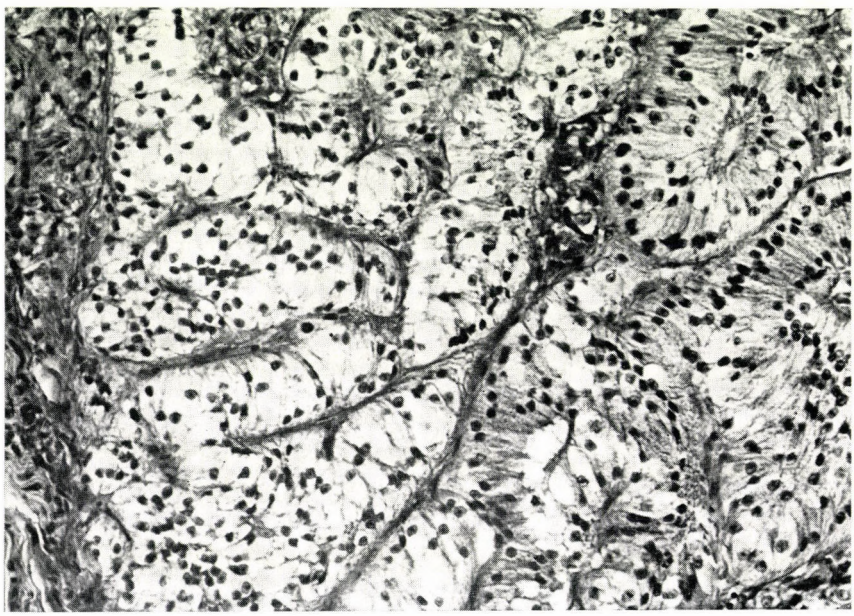

Fig. 1. Section of adrenal gland of goose. H and E. ×100

(Fig. 3). The septa are formed by collagen and reticular fibres. The interstitium is rich in blood vessels and sinusoids. Nerve fibres are also seen in the ramified septa. Groups of ganglionic cells are found both outside and inside the gland parenchyma. Foci of lymphocytes are seen among parenchymal cells.

The adrenal parenchyma consists of cortical and medullary tissues intermingling with each other (Fig. 1).

The cortical tissue is formed by anastomosing cell cords or strands looping toward the capsule. The cortical loops passing beneath the capsule form a clear, thin, peripheral or subcapsular zone (Fig. 1). The remaining cell cords

form a large broad inner zone in the centre of the gland. The subcapsular zone is composed of long columnar cells with small, ovoid compact nuclei and pale-staining highly-vacuolated cytoplasm. The cells of the inner zone are columnar in shape and have a cytoplasm more acidophilic and granular but less vacuolated than the cytoplasm of the subcapsular cells. The nuclei are round and contain one or more nucleoli. In both the subcapsular and the inner zones the nuclei tend to lie nearby the inner edge of the cell, *viz.*, in the central part of the cord, being pushed there by lipid accumulating at the sinusoidal edge. The cortical cells are in close contact within each cord, with no intervening connective tissue, blood vessels or lumen. On staining with Sudan black, much fewer sudanophilic droplets appeared in the cells of the subcapsular zone than

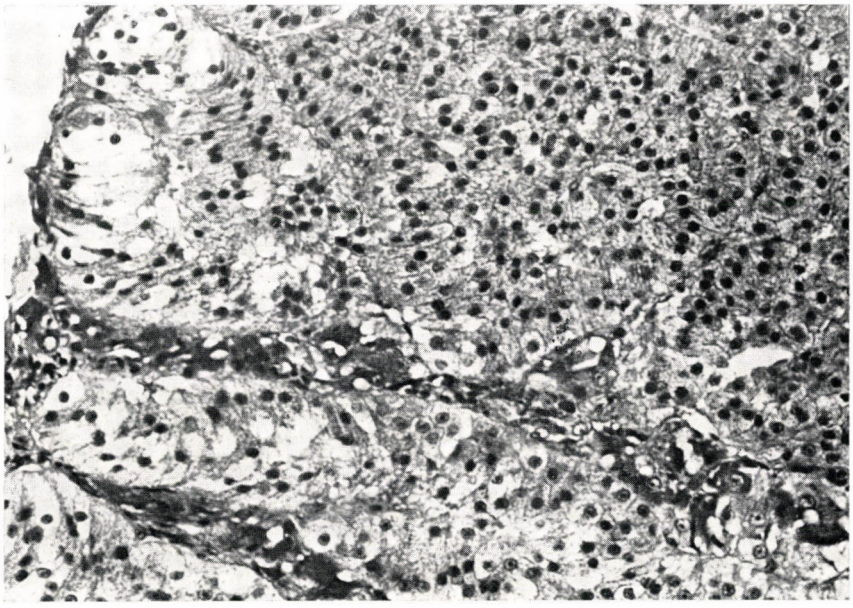

Fig. 2. Section of adrenal gland of duck. H and E. ×100

in those of the inner zone (Figs 5, 6). However, sections stained with Schultz's method showed that the subcapsular zone cells are richer in cholesterol and its esters (Fig. 8). The ascorbic acid content of the cortical cells appeared in the form of small black granules, equal in size and distribution in both the zones (Fig. 10).

In birds being in the sexually active period, the sudanophilic droplets were decreased in amount and appeared smaller in size in the cortical tissue cells. The cholesterol and ascorbic acid contents were decreased in both cortical zones, but cholesterol and its esters were still present in distinctive larger amounts in the subcapsular cells (Figs 7, 9).

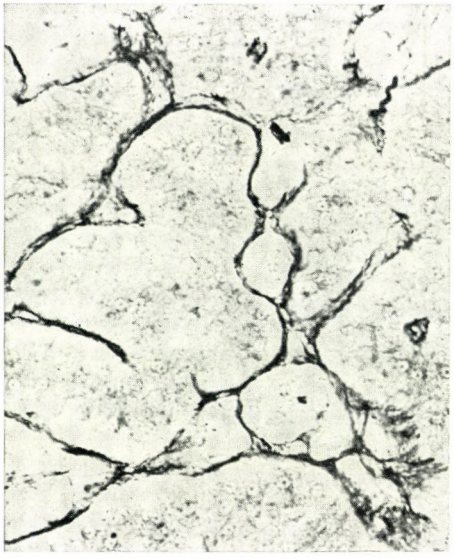

Fig. 3. Interstitial connective tissue in the adrenal gland of male goose. Silver impregnation. ×100

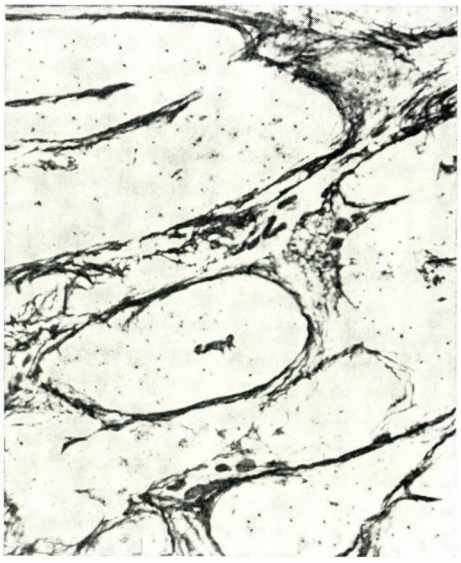

Fig. 4. Interstitial connective tissue in the adrenal gland of male duck. Silver impregnation. ×100

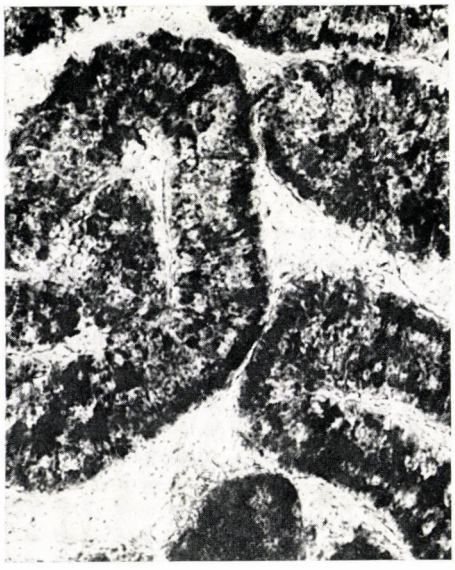

Fig. 5. Lipid content in the cortical cell cords of inactive female goose. Sudan black. ×100

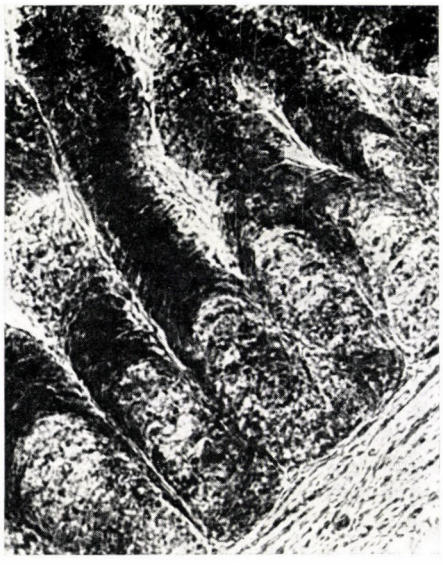

Fig. 6. Lipid content in the peripheral cell strands of the cortical tissue of female goose. Sudan black. ×100

The medullary tissue cells took on basic stain and generally appeared as bluish islets or scattered groups in between the eosinophilic cortical tissue (Fig. 1). A thin layer of medullary tissue formed by 2 to 3 rows of cells, was extending at the periphery of the gland, separating the connective tissue capsule from the subcapsular zone of the cortical tissue. The scattered groups of chromaffin tissue consist of at least two types of large polygonal cells (adrenalin- and noradrenalin-containing cells), with a basophilic granular cytoplasm and large round centrally located nuclei. The noradrenalin-containing cells are demonstrated by the dichromate reaction, assuming a dark brown hue (Fig. 11).

The granular contents of the catecholamine-containing cells (adrenalin and noradrenalin cells) do not notably change during the active period. Catecholamine-containing cells of the adrenal medulla showed a strong yellowish-green fluorescence in the FIF test. The medullary cells comprised a moderate amount of ascorbic acid granules distributed in cytoplasm (Fig. 9).

During sexual inactivity, the medullary tissue cells took on slightly more stain than during activity. This may have been due to the aggregation of many basophilic granules in the cytoplasm. The ascorbic acid granules are markedly increased in amount.

II. Duck adrenal gland

The gland is covered by a relatively thick connective tissue capsule formed by collagen and reticular fibres and a few elastic elements. Thick septa originating from the capsule and branching in the gland parenchyma to form an abundant interstitial tissue (Fig. 4), are rich in blood vessels and nerves. The adrenal parenchyma consists of intermingled cortical and medullary tissues (Fig. 2).

Like in the goose the cortical tissue is composed of anastomosing cell cords, forming loops beneath the capsule, which constitute the subcapsular zone (Fig. 2). The subcapsular zone resembles that of the goose in the histological and histochemical features.

The medullary tissue appears in the form of a meshwork traversing the entire gland, covering an appreciable amount of the cut surface. The medullary cells (adrenalin- and noradrenalin-containing cells) are polyhedral, with coarse basophilic granular cytoplasm and large round, centrally placed nuclei (Fig. 12). The catecholamine cells show a yellowish-green fluorescence in the FIF test.

The histological and histochemical changes related to sexually active and inactive periods were exactly the same in duck as in goose.

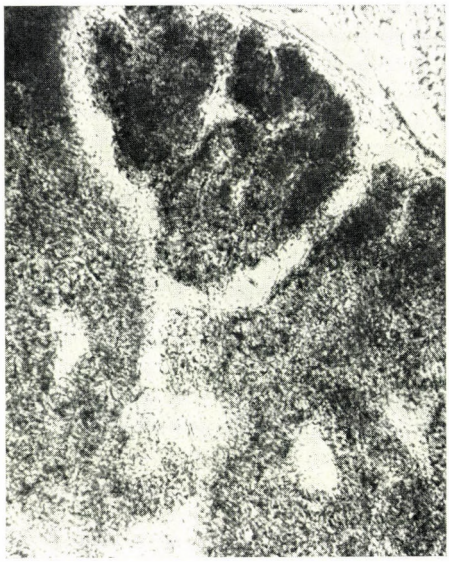

Fig. 7. Cholesterol content in the adrenal cortical tissue of active female goose. Schultz's method. ×100

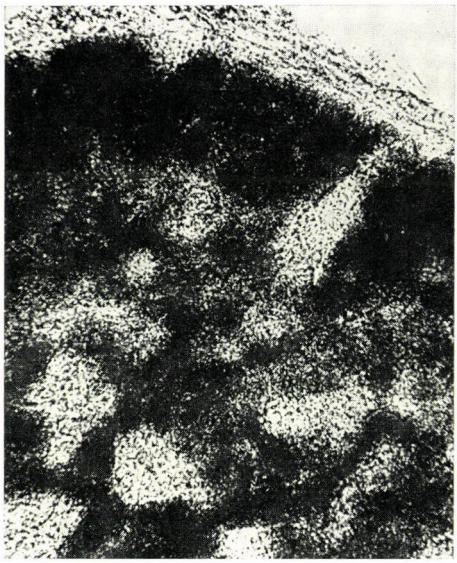

Fig. 8. Cholesterol content in the cortical tissue of inactive female goose. Schultz's method. ×100

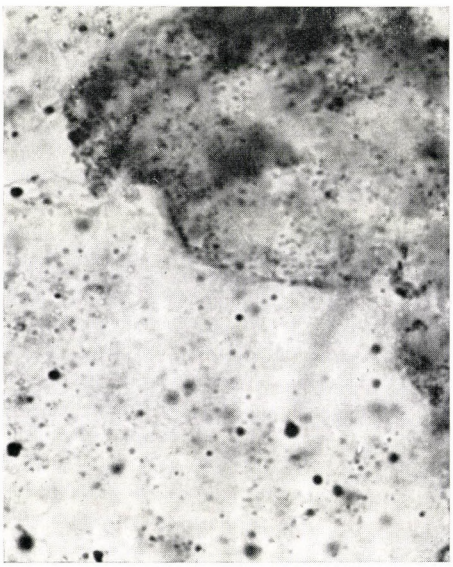

Fig. 9. Ascorbic acid granules in the adrenal tissue of active male goose. Silver stain. ×250

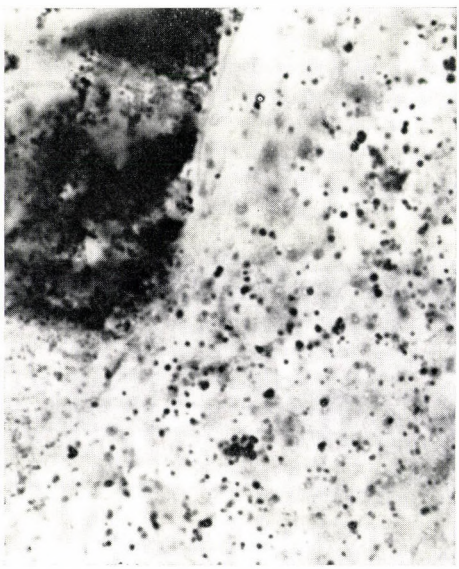

Fig. 10. Ascorbic acid granules in the adrenal tissue of inactive male goose. Silver method. ×250

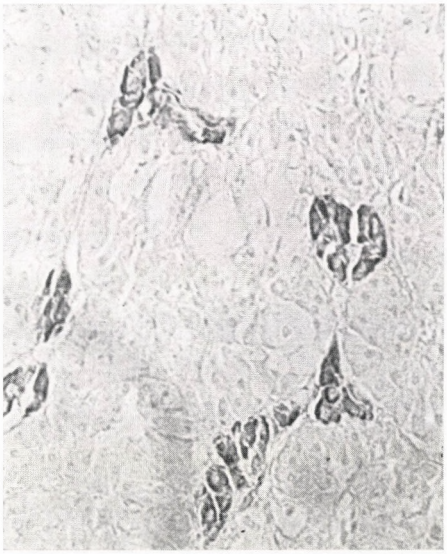

Fig. 11. Distribution of the chromaffin cells in the adrenal gland of male goose. Chromaffin reaction. ×100

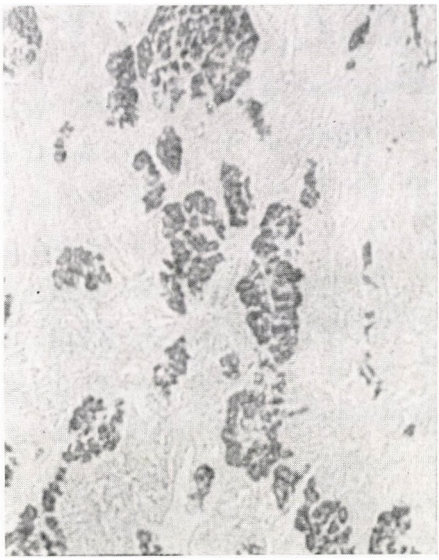

Fig. 12. Distribution of the chromaffin cells in the adrenal gland of male duck. Chromaffin reaction. ×100

Discussion

The present studies were performed to obtain more information on regional zonal differences in adrenal cortical tissue of geese and ducks, and on histological and histochemical changes taking place during the sexually active period. Several workers, studying the histological structure of the avian adrenal gland (UOTILA, 1939; MILLER and RIDDLE, 1942; KAR, 1947; BENOIT, 1950) found it to consist of intermingled cortical and medullary tissues. The cortical tissue is composed of anastomosing cell cords or strands, forming loops beneath the connective tissue capsule.

The present examinations showed that the subcapsular cell zone of the adrenal gland was distinct from cells of the inner zone both histologically and histochemically. Against earlier findings of SAUER and LATIMER (1931) in our hands, neither the subcapsular nor the inner zone of the avian adrenal gland bore any structural resemblance to either zona granulosa or zona reticularis of the mammalian adrenal gland. If it is accepted that the subcapsular loop formation of the cortical cell cords indicates zoning (CHESTER et al., 1962), the subcapsular "zone" can be hypothetically regarded as a species-specific structure performing a specific function. KONDICS (1971) demonstrated that a considerable amount of corticosterone is produced in both zones, the inner one being controlled by ACTH, the outer one above all by the elec-

trolyte balance. In the light of these findings, the zoning of the adrenal gland might have been partly explained.

Histochemical examinations showed depletion of adrenal cholesterol and ascorbic acid in the sexually active period, taking place probably in response to the increased release of ACTH during this phase. Histologically the capsule and the ramified interstitial septa of the gland were found to be thicker in ducks than in geese, and ducks also had a relatively larger amount of chromaffin tissue.

The present histological and histochemical studies are, naturally, not sufficient to offer satisfactory explanation for zoning and its exact functions. They may facilitate the better understanding of these phenomena if additional evidence is sought by physiological and biochemical approach.

Summary

Studies on the adrenal glands of geese and ducks showed that the subcapsular zone of the cortical tissue was distinct from the inner zone both histologically and histochemically. Depletion of adrenal cholesterol and ascorbic acid was found during the sexually active period in both species. The adrenal gland of ducks had a thicker connective tissue capsule and interstitium and a larger proportion of chromaffin tissue, compared to geese.

*

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References

BELL, D. J. and FREEMAN, B. M.: Physiology and Biochemistry of Domestic Fowl. London—New York, 1971. BENOIT, J.: Traité de Zoologie. Paris, 1950. CHESTER JONES, I., PHILLIPS, J. G. and BELLAMY, D.: Br. Med. Bull. **18** (1961), 110. HARTMAN, F. A., MCNUTT, A. W. and CARVER, J. E.: Anat. Rec. **97** (1947), 211. KAR, A. B.: Anat. Rec. **97** (1947), 551. KONDICS, L.: Biológiai Közlöny **XIX** (1971), 17. MARSHALL, A. J.: Biology and Comparative Physiology of Birds. New York and London, 1954. MILLER, R. A. and RIDDLE, O.: Am. J. Anat. **71** (1942), 311. SAUER and LATIMER: Anat Rec. **50** (1931), 289. SIVARAM, F.: Anat. Rec. **48** (1964), 336. SIVARAM, F.: Can. J. Zool. **43** (1965), 1021. STURKIE, P. D.: Avian Physiology. New York, 1965. TURNER, C. D.: General Endocrinology. Philadelphia and London, 1966. UOTILA, U. U.: Anat. Rec. **75** (1939), 439.

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STUDIES ON ANTIGENIC PATTERNS OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM NEWBORN PIGLETS

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(Received March 22, 1974)

Escherichia coli-diarrhoea of newborn piglets is known to cause great economic losses. Usually haemolytic strains of *E. coli* have been isolated from small intestinal mucosa, mesenteric lymph nodes and occasionally from other organs of piglets died of diarrhoea between one to 10 days of age. The isolates, along with predisposing factors, have been shown to be responsible for the causation of the disease.

The *E. coli* strains involved in the aetiology represent as a rule only a few serogroups. The serogroups most frequently isolated from carcases of suckling pigs were given by PESTI (1960) as O28, O9 and O21, by SZABÓ (1964) as O8, O138 and O141, by SEMJÉN and PESTI (1973) as O147 : K89, K88ac and O149 : K91, K88ac. As to the most frequent antigenic patterns of *E. coli* isolates from suckling pigs, the following were reported by various authors: O147 : K89, K88ac; O8 : K87, K88ab, O141 : K88ab, K88ab; O138 : K81, K88ac (SOJKA, 1965), O141 : K85ab; O139 : K82, O138 : K81, O8 : K87, K88, O147 : K89, K88 (WITTIG, 1965), O8, O141, O101 (SALAJKA, 1966), O8 : K85, O138 : K81 (GOSSLING and RHOADES, 1966), O9, O8, O101 (MOON et al., 1966), O139 : K82, O8 : K87, O141 : K85ab (TRUSZCZYNSKI et al., 1965), O8, O141, O147 (SÖDERLIND, 1971), O8 : K87, K88ac, O116, O147 : K89, K88ac (GYLES et al., 1971).

In the present study, *E. coli* strains recently isolated from affected newborn piglets were examined for biochemical properties and O and K antigens.

Materials and methods

A total of 222 coliform strains were isolated from 88 piglets that died between 1 to 12 days of age in 30 swine farms. Most strains were isolated from the mucous membrane of the small intestine and mesenteric lymph nodes, a few from the large-intestinal mucosa. Biochemical tests, identification of O and K antigens, preparation of O and OK antisera, slide and tube agglutination tests and serum absorptions were performed according to the methods of KAUFFMANN (1966). The O and K antigens were examined with a total of 57 O and OK antisera to international *Escherichia* type strains, as described earlier

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(VARGA and FARID, 1974) and with all known (1—94) K antisera except three. Antigens K88ab and K88ac were examined with absorbed sera.

In the slide agglutination test, O and OK antisera were employed in pools, as proposed by EWING et al. (1956).

The antigens were prepared from cultures grown on the 0.1% glucoses-containing medium ($D_{1,5}$) described by SCHLECHT and WESTPHAL (1966).

Results

On the basis of biochemical tests, 218 of the 222 isolates were classified as *E. coli*, one strain was identified as *Klebsiella* and three strains could not be classified. All *E. coli* strains showed the biochemical properties characteristics of the genus, but apart from this 15 strains were urease-positive, one strain liquefied gelatine and six strains produced H_2S. The urease activity of the urease-

Table I

O and K antigens of *E. coli* strains isolated from newborn piglets

O Antigen	No. of strains	K Antigen	No. of strains	No. of strains with unidentifiable K antigen
O149	53	K91(B), K88ac(L)	49	—
		K—	4	
O147	30	K89(B), K88ac(L)	30	—
O41	21	—		21 K(A)
O75	18	K—	18	—
O9	10	K(A), K88ac(L)	3	2 K(A)
				5
O139	8	K82(B)	8	—
O2	8	K—	8	—
O8	4	K85(B), K88ac(L)	2	2
O20	4	—		4
O13	3	K11(L)	3	—
O23	3	K18(L)	3	—
O56	3	—		3
O154	3	—		3
O7	2	—		2
O86	2	K—	2	—
O138	2	K81(B), K88ab(L)	2	—
O141	2	K85(B)	2	—
Totals:	176 (80.7%)			

K—, strains not containing K antigen.

positive strains was similar in degree to the activity of *Klebsiella*, despite that the other characteristics were in every respect typical of *E. coli*. Seventy per cent of the *E. coli* strains caused beta haemolysis. All strains possessing K88ab or K88ac antigen were haemolytic, but at the same time certain strains having an identical antigenic pattern variously did or did not show haemolytic activity. Strains of S colony morphology, belonging to different O groups and not containing K antigen, were also found to cause haemolysis. Part of the strains lost haemolytic activity after several transfers to agar plates.

The results of antigenic studies are shown in Table I. Out of the 218 *E. coli* strains 176 (80.2%) could be typed with the available sera.

More than three quarters of these strains were found to belong to seven serogroups. The most frequent groups were O149, O147, O41, O75, O9, O139 and O2, the rest, including certain O groups which had formerly been frequently isolated from carcases of suckling pigs, were only sporadically encountered. The strain of O75, O2 and O86 did not contain K antigen as — in living state — all were agglutinable with the homologous O serum. *K* minus variants also occurred within serogroup O149. Part of the strains had in addition to K(B) antigen, a K88(L) antigen, usually in the form of K88ac, less often as K88ab. Some of the O9 strains and all O41 strains contained a thermostable, probably K(A), antigen which, although showing cross agglutination with several K(A) sera, could not be identified with the available range of K sera.

Summary

A total of 222 coliform strains, isolated from the carcases of 88 piglets that died between one and 12 days of age in 88 swine farms, were examined for biochemical properties, and the isolates identified as *E. coli* were typed serologically. O antigens were examined with a total of 57 O and OK sera, K antigens with all (1—94) except three K sera.

Two hundred and eighteen strains were identified as *E. coli*. Seventy per cent of the strains proved to be beta-haemolytic and 15 strains, although showing all biochemical characteristics of the genus *Escherichia*, were urease-positive.

On a total 176 strains (80.2%) could be typed with the available sera. More than two thirds of these strains were found to belong to seven serogroups.

The serogroups most frequenly isolated were O149 : K91, K88ac, O147 : K89, K88ac, O41 : K(A), O75 : K–, O9 : K(A), K88ac, O139 : K82, and O2 : K–. Nearly half of the strains contained one of the K(B) and K88(L) antigens, but some strains had a thermostable, probably K(A) surface antigen and there were also strains containing no K antigen.

References

Ewing, W. H., Tatum, H. W., Davis, B. R. and Reavis, R. W.: Studies on the Serology of the *Escherichia coli* Group. Public Health Service, Communicable Diseases Center, Atalanta, 1956. Gossling, J. and Rhoades, H. E.: Cornell Vet. **56** (1966), 344. Gyles, C. L., Stevens, J. B. and Craven, J. A.: Canad. J. comp. Med. **35** (1971), 258. Kaufmann, F.: The Bacteriology of *Enterobacteriaceae*. Copenhagen, 1966. Moon, H. W., Sorensen, J. H., Sautter, J. H. and Highbee, J. M.: Am. J. Vet. Res. **27** (1966), 1007. Pesti, L.: Acta vet. hung. **X**,

(1960), 365, SALAJKA, E.: Vet. Med. (Praha) **11** (1966), 283. SCHLECHT, S. and WESTPHAL, O.: Zbl. Bakt. I. Orig. **200** (1966), 241. SEMJÉN, G. and PESTI, L.: Acta vet. hung. **XXIII** (1973), 219. SOJKA, M. J.: *E. coli* in Domestic Animals and Poultry. C. A. B. England, 1965. SÖDERLIND, O.: Zbl. Vet. Med. B. **18** (1971), 569. SZABÓ, I.: Magyar Állatorvosok Lapja (MÁL), 1964, 368. TRUSZCZYNSKI, M., CIOSEK, D. abd TERESZCZUK, S.: Bull. Vet. Inst. Pulawy **9** (1965), 52. VARGA, J. and FARID, A. F.: Acta vet. hung. **XXV** (1975), 65. WITTIG, W.: Zbl. Bakt. I. Orig. **197** (1965), 487.

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MUCORMYCOSIS (*MUCOR PUSILLUS*) WITH ASTEROIDS IN A YOUNG BULL

By

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Visceral mucormycosis, either local or generalized occurs less frequently in cattle than any comparable disease in humans. In most hitherto published veterinary cases, however, the diagnosis was based on morphological criteria only or the isolated cultures were classified as *Mucoraceae* without closer identification (Cordes et al., 1964; Donnelly, 1967; Hogben, 1967). Less frequently, the fungal species was identified precisely. *Mucor pusillus* was isolated from mycotic lesions of mediastinal lymph nodes of a cow (Bühlmann and Werffeli, 1968) and from the brain of a calf (Ainsworth et al., 1955, cited by Ainsworth and Austwick, 1959) while *Absidia corymbifera* was identified in mycotic lesions of mesenteric lymph nodes of a young cattle (Davis et al., 1955; König et al., 1967).

Pulmonary mucormycosis in cattle was described by Davis et al. (1955). Some of these cases appeared as a complication of mycotic abortions (Cordes et al., 1964). Gastrointestinal mucormycosis in calves was described by Gitter et al. (1957). Cases of completely generalized mucormycosis occurred predominantly in newborn calves (Hogben, 1967; Cordes et al., 1967). Two such cases in older animals were observed by Donnelly (1967). A quite unusual location of granulomatous mycosis, presumably mucormycosis, in intervertebral discs was observed by Fankhauser et al. (1966).

Case report

Two enlarged mediastinal lymph nodes from a 6-month-old bull were submitted for examination. The animal was emergency-sloughtered because of retarded growth and impaired digestion. The inspecting veterinary suspected tuberculous lymphadenitis. Reportedly, there were no other macroscopic lesions either in lungs or elsewhere.

Macroscopically (Fig. 1), the specimen consisted of two lymph nodes measuring $17 \times 14 \times 14$ cm and $4 \times 3 \times 3$ cm, respectively. On the cut surface, there were patches of yellowish necrosis separated by strands either of fascicular or glossy and transparent grey tissue. There were densely disseminated small hemorrhages, especially in central parts of the lymph nodes. There were also small foci of dystrophic calcification.

Microscopically (hist. No. 1835/73) the necrosis was of the caseous type, in some places calcified. There was definite lining of necrotic foci with specific granulation tissue consisting of fibroblasts, collagen, epitheloid cells and multinucleated giant cells (Fig. 2). There was moderate cellular infiltration consist-

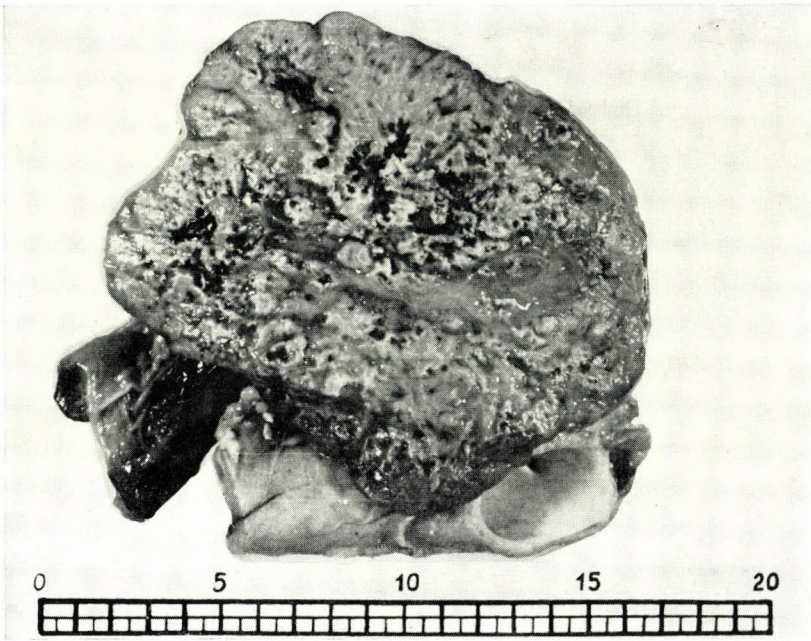

Fig. 1. Cut surface of the enlarged mediastinal lymph node with caseous granulomatous mucormycosis

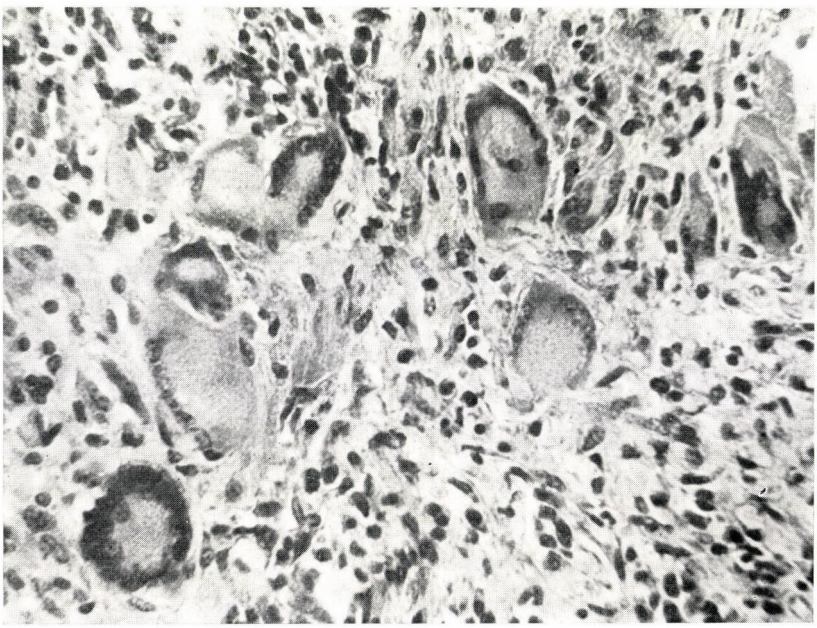

Fig. 2. Multinucleated giant cells in the specific granulation tissue. Hematoxylin-eosin. ×420

ing of lymphocytes and both neutrophilic and eosinophilic granulocytes. In some places, recent necrosis was separated from the granulation tissue by a very narrow demarcation zone of neutrophilic and eosinophilic leucocytes. In the necrotic tissue and in the plasma both of the fibroblasts and giant cells of the inner layer of granulation tissue, there were fragments of branching, non-septed, irregularly indented fungal hyphae measuring 4.3—6.5 μm in diameter. Around some of the fungal elements a definite lining of eosinophilic amorphous material was seen. The outer margins of this substance were either linear or irregularly outcropped or even of hairy or "cog-wheel" appearance (Fig. 3). In sections stained with FITC-labelled rabbit antibovine gamma-globulin (Fig. 4) there was positive specific fluorescence only in these linings. For this purpose standard products of the firm "Bioveta, n.p. Ivanovice" were used. The concentrated conjugate was absorbed to standard organ powder. Staining was performed after centrifugation and pH control in a wet chamber at 37°C for 30 min. Subsequently, the preparations were rinsed for 10 minutes in buffered saline and mounted in glycerol. Neither fungal elements nor other structures showed specific positive fluorescence in the examined sections from various tissue blocks. The fungal elements were stained positive by the Grocott and Mc Manus methods.

Cultures on Sabouraud's agar yielded *Mucor pusillus* Lindt (1886). The identification was performed by one of us (P.F.) using standard mycological procedures. A detailed description of these will be published elsewhere.

Discussion

Isolated mucormycosis of bronchial, mediastinal or mesenteric lymph nodes in cattle is not extremely rare (Gleiser, 1953; Davis et al., 1955; Bühlmann and Werffeli, 1968). Histopathologically the mycotic lesions in our case closely resembled those previously described. Davis et al. (1955) observed eosinophilic "asteroid" linings with mucormycotic hyphae in two of their 11 cases of glandular mucormycosis in cattle. These structures are interesting from the general point of view. They represent a "Splendore—Hoeppli" phenomenon in mycotic infections (Williams et al., 1969). There is a general agreement as to their basic nature as protein precipitate caused by antigen–antibody reaction. "Asteroid" linings are not uncommon with various fungal species in animals while rather rare in man. This fact, and also the higher frequency of trivial mycotic infections in animals, might be due to different immunity pattern of the host organism. In man, the resistance against fungi is controlled predominantly by the cell-mediated immunity, and generalized infections tend to occur mainly in persons where this is considerably impaired, as in Hodgkin's disease, leukaemia and other malignancies. The animals, on the other

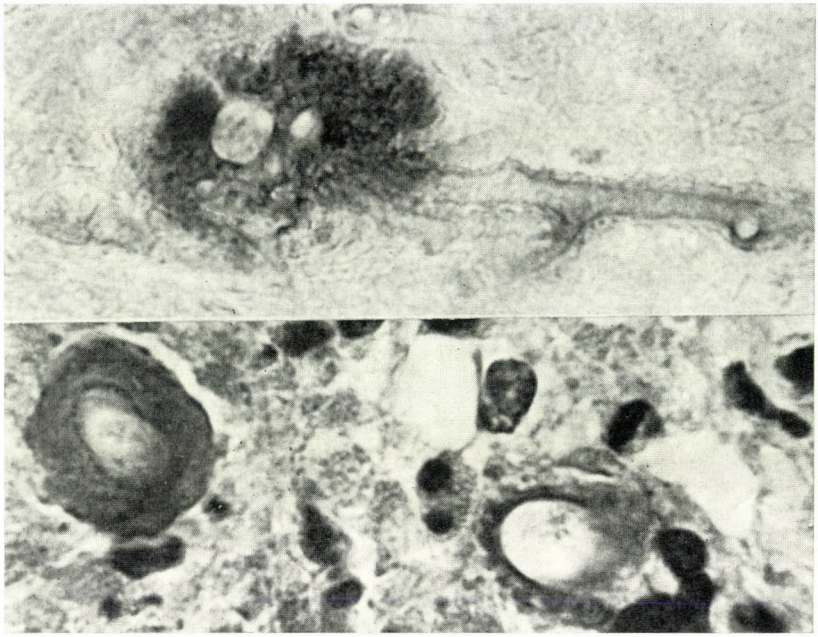

Fig. 3. Top figure. Branching nonsepted hypha with marked eosinophilic lining and with an "asteroid" in the necrosis. Hematoxylin-eosin. ×970. On the bottom, transverse sections of the hyphae with eosinophilic linings. "Cog-wheel" appearance at the right side. Hematoxylin eosin. ×1450

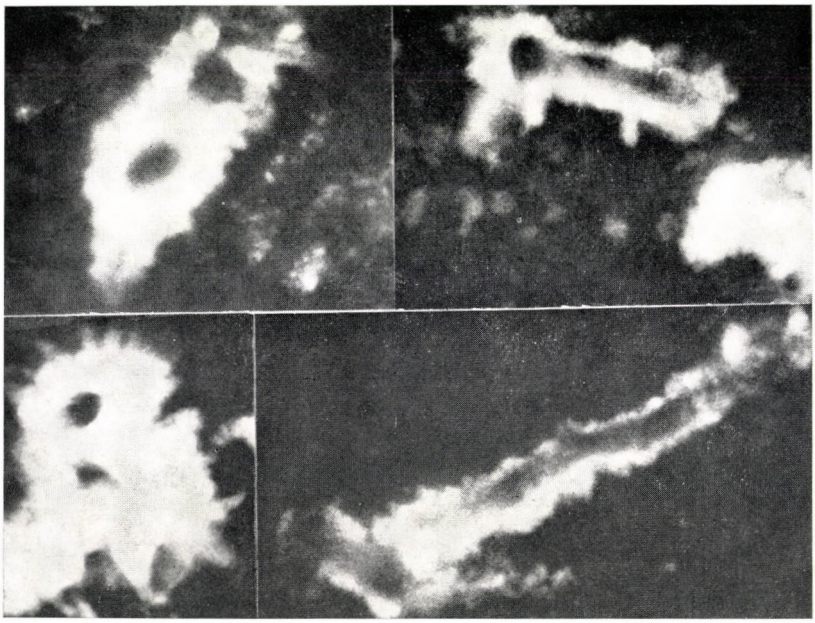

Fig. 4. "Splendore–Hoeppli" around mycotic hyphae. Immunofluorescence (FITC-labelled rabbit anti-bovine gamma globulin). ×1500

hand, might be more dependent on immunoglobulin systems, the cell-mediated immunity being generally less effective than in man. In our opinion asteroid linings around fungal elements might eventually prove to be a morphological evidence of an immunoglobulin response different from cases where no such structures are seen.

The results of immunofluorescent staining of these structures with host-specific anti-gamma globulin were quite impressive. The same method was used for demonstration of asteroid material with *Aspergillus fumigatus* Fresenius in pulmonary mycosis of hares (Prokš et al., 1972). We think that this relatively simple method will prove useful for identification of these structures in paraffin sections, even with the omission of strict qualitative and quantitative control criteria.

Summary

In two enlarged mediastinal lymph nodes of a 6-month-old bull there was granulomatous mycosis with morphological appearance of fungal elements suggesting *Mucor*. Around some of these, there were asteroid formations which displayed specific immunofluorescence with FITC-labelled host-specific anti-gamma globulin. The cultures yielded *Mucor pusillus* Lindt (1886).

References

Ainsworth, G. C. and Austwick, P. K. C.: Fungal Diseases of Animals. Commonwealth Agricultural Bureaux, Farnham Royal Books, England, 1959. Bühlmann, X. and Werffeli, F.: Mykosen **11** (1968), 41. Cordes, D. O., Dodd, D. C. and O'Hara, P. J.: N. Z. vet. J. **12** (1964), 101, Cordes, D. O., Royal, W. A. and Shortridge, E. H.: N. Z. vet. J. **15** (1967), 143. Davis, C. L., Anderson, W. A. and Mc Crory, B. R.: JAVMA **126** (1955), 261. Donnelly, W. J. C.: Irish vet. J. **21** (1967), 82. Fankhauser, R., Keller, H., Lanz, E. and Teuscher, E.: Schweizer Arch. Tierheilk. **108** (1966), 699. Gleiser, Ch. A.: JAVMA **122** (1953), 441. Hogben, B. R.: N. Z. vet. J. **15** (1967), 30. König, H., Nicolet, J., Lindt, S. and Raaflaub, W.: Schweizer Arch. Tierheilk. **109** (1967), 260. Prokš, C., Vítovec, J. and Vladík, P.: Mykosen **15** (1972), 427. Williams, A. O., Lichtenberg, F., Smith, J. H. and Martinson, F. D.: Arch. Path. **87** (1969), 459.

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VACCINATION EXPERIMENTS AGAINST TRANSMISSIBLE GASTROENTERITIS (TGE) OF SWINE

III. THE VIRUS-NEUTRALIZING TITRE OF VACCINATED SOWS' MILK AND ITS PERSISTENCE DURING THE LACTATION PERIOD

By

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(Received April 25, 1974)

In a previous experiment made during a natural outbreak of transmissible gastroenteritis (TGE), we investigated the antibody level of the sows' milk required for the protection of piglets born in an infected environment and the specific virus-neutralizing antibody levels necessary for stopping the epidemic in the suckling piglets (Benyeda et al., 1974). It was shown that the passive protection of the piglets born in an infected environment can be reckoned with from the 21st day after the TGE outbreak in sows when the average antibody titre of the sows' colostrum and milk is 1 : 8 or higher. However, we have no data concerning the time when the specific virus-neutralizing antibodies in the milk of immunized sows attain the level required for an effective protection of the newborn piglets. The same applies to the possible changes of the milk antibody level during the lactation period. The present work was undertaken to clear up these questions. The live TGE virus vaccine developed by Csontos et al. (1973) was used. This vaccine contains the CKp strain (Csontos and Szent-Iványi, 1971).

Materials and methods

The experiment was carried out in a sow herd of a large-scale farm, susceptible to the TGE virus. Twenty-seven sows were vaccinated intranasally once with a dose of 5 ml 9—27 days before farrowing. The titre of the vaccine was $10^{6.5}$ $TCID_{50}$/ml.

Blood and milk samples. Blood samples were collected from all the sows before vaccination, on the day of farrowing and 7 days later. Milk samples were collected on the day of farrowing and on the 3rd, 5th and 7th day afterwards. Further milk samples were collected from 9 sows which had farrowed between the 19th and 27th day after vaccination, till the end of the 35-day long lactation period. To obtain milk samples 15 IU oxytocin were injected into the vena cava superior.

Virus strain. In the virus-neutralization tests the CKp virus strain (Csontos and Szent-Iványi, 1971) was applied.

Virus-neutralization test. The milk whey was separated by clotting. Serum and whey samples were centrifuged, inactivated for 30 minutes at 56°C and stored at —20°C until tested.

The test was performed with a standard dilution of virus containing 100 $TCID_{50}$/0.1 ml and serial twofold dilutions of serum or whey, starting at 1 : 2.

Equal volumes of these were incubated at 37 °C for an hour. Finally, two or three tubes of secondary swine thyroid cell cultures were inoculated with 0.2 ml of each mixture. The test was evaluated two days post inoculation. As antibody titre, the highest serum or milk whey dilution was considered which entirely neutralized the viral cytopathic effect.

Results

The sows showed no symptoms after vaccination and their pregnancy took a normal course.

Specific virus-neutralizing antibodies in the serum and milk whey of sows appeared 10 days after vaccination. The antibody titres of the serum and milk samples were averaged (Fig. 1). The averages were compared with the corresponding average titres found during a natural TGE outbreak at the time when the outbreak reached an end in the suckling piglets (24 days after the outbreak of TGE in sows).

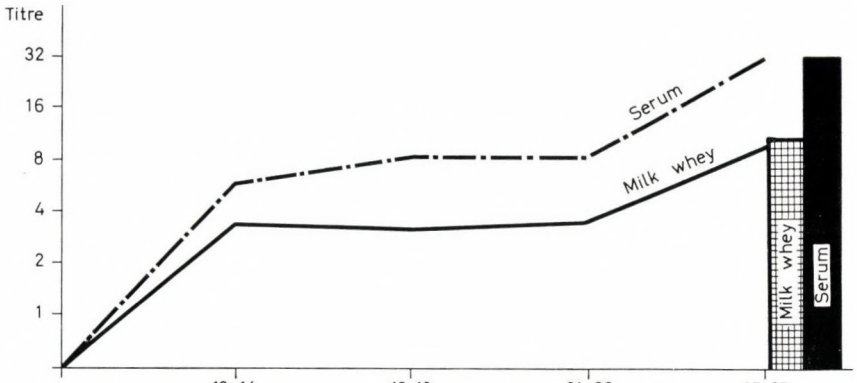
The persistence of the average antibody titre in the milk whey of nine sows farrowed between the 19th and 27th day after vaccination is shown in Fig. 2.

A significant difference was found among the individual sows' whey and serum titres. The serum titres of the sows farrowed 19—21 days after vaccination were between 1 : 4 and 1 : 8 and those of the sows farrowed 25—27 days after vaccination were 1 : 32. The whey titres of the sows farrowed 19—21 days after vaccination were 1 : 8 or 1 : 16 on the day of farrowing and 1 : 1 at the end of the lactation period, those of the sows farrowed 25—27 days after vaccination were 1 : 64 or 1 : 128 and 1 : 8, respectively.

Discussion

Antibodies could be demonstrated up to the 10th postvaccination day neither in the serum nor in the milk whey. In the sera and milk wheys of sows farrowed between the 10th and 14th day after vaccination, the specific virus-neutralizing antibodies appeared in different titres and the titre tended to increase. The average serum antibody titre of sows farrowed on the 27th day after vaccination was 1 : 30 and that of the milk whey was 1 : 9. The antibody titres obtained corresponded practically to those found in the sows' sera and milk wheys after natural TGE infection at the time when the epidemic ceased in the suckling piglets (24 days after the outbreak of TGE in sows).

Investigating the persistence of the milk whey antibody levels, the results obtained showed that on the day of farrowing the milk antibody level was



Serum	$\log_2$	2,5	3,0	3,0	4,8
Milk whey	$\log_2$	1,7	1,6	1,7	3,1

Fig. 1. The antibody level of sera and milk wheys of sows vaccinated once 10—27 days before farrowing

Note: The columns represent the average antibody titre of sows' sera and milk wheys during a natural TGE infection at the time when the epidemic reached an end in the suckling piglets

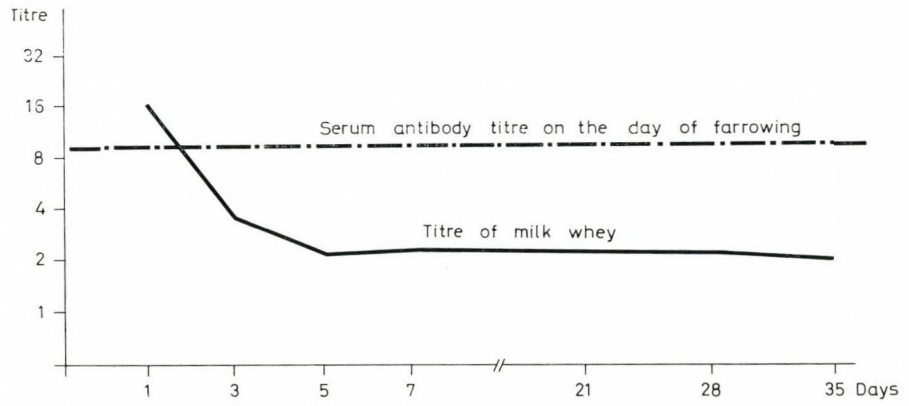


Fig. 2. The persistence of milk antibody titres of vaccinated sows during the lactation period

higher by one $\log_2$ unit than that of the serum. Then — between the 3rd and 5th day after farrowing — the milk antibody level fell by one or two $\log_2$ units compared to that of the serum and remained at that level to the end of the 35-day-long lactation period.

The results have shown that the specific virus-neutralizing antibodies in the colostrum and milk of sows properly immunized and thus having a high antibody level remain up to the end of the lactation period at a level which provides a firm protection for their piglets born during a TGE outbreak.

Summary

Twenty-seven sows from a sow herd of a large-scale farm susceptible to the TGE virus were vaccinated intranasally once with a dose of five ml 9—27 days before farrowing. The virus-neutralizing antibody titres of blood and milk samples collected were determined on the day of farrowing and at definite points of time thereafter.

Specific virus-neutralizing antibodies appeared in the sera and milk wheys of sows 10 days after vaccination and their titres increased gradually. On the 27th day after vaccination, the average serum and whey antibody titre was 1 : 30 and 1 : 9, respectively. These values corresponded practically to those found in the sows' sera and milk wheys after natural TGE infection at the time when the epidemic ceased in the suckling piglets (24 days after the outbreak of TGE in sows).

The persistence of the milk whey antibodies was investigated up to the end of the lactation period in the case of nine sows farrowed between the 19th and 27th postvaccination day. On the day of farrowing, the milk antibody level was higher by one $\log_2$ unit than the serum level, then the milk antibody level fell by one or two $\log_2$ units between the 3rd and 5th day after farrowing compared to that of the serum and remained at that level up to the end of the lactation.

The results showed that the specific virus-neutralizing antibodies in the colostrum and milk of sows properly immunized and thus having a high antibody level remained throughout the lactation period at a level which provides a firm protection for their piglets born during a TGE outbreak.

*

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References

BENYEDA, J., MOCSÁRI, E. and GAJDÁCS, GY.: Magyar Állatorvosok Lapja (MÁL), 1974, 145. IDEM: Acta vet. hung. **XXIV** (1970), 159. CSONTOS, L., SENT-IVÁNYI, T.: MÁL, 1971, 5. CSONTOS, L., SZENT-IVÁNYI, T. and BENYEDA, J.: MÁL, 1973, 22. IDEM: Acta vet. hung. **XXIV** (1974), 111.

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VACCINATION EXPERIMENTS AGAINST TRANSMISSIBLE GASTROENTERITIS (TGE) OF SWINE

IV. THE TITRE AND PERSISTENCE OF VIRUS-NEUTRALIZING ANTIBODIES IN THE SERA OF SOWS VACCINATED ONCE OR TWICE AT DIFFERENT INTERVALS

By

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(Received April 25, 1974)

The results of experimental vaccinations carried out in susceptible swine herds of large-scale farms as well as the persistence of postvaccination antibody titres and the antibody response to revaccination were described in previous papers (Csontos et al., 1973, 1974; Benyeda and Mocsári, 1974; Benyeda et al., 1974). The persistence of the postvaccination titres was examined in a single blood sample taken half a year after vaccination. The results obtained showed that the average titre of sows vaccinated on a single occasion was substantially higher six months after vaccination compared to the early postvaccination mean titre. The average antibody level of sows vaccinated twice at a two-week interval persisted at a constant level, while that of the sows vaccinated twice at a six-week interval markedly declined. Evaluating the results, we pointed to the necessity of (a) performing further investigation to reveal the cause of the titre increase observed in sows vaccinated once and (b) determining more exactly the time when the high antibody levels obtained by the double-dose vaccination schedule at a six-week interval decline.

In order to clarify these questions, in the present study the persistence of the specific virus-neutralizing antibodies was followed up in sows vaccinated once or twice at different intervals.

Materials and methods

The 132 sows of a herd susceptible to the TGE virus were divided into three groups. Sows of group I (58 sows) were vaccinated with a single dose, those of group II (41 sows) twice at a two-week interval while those of group III (33 sows) were vaccinated twice at a six-week interval. From the sows marked individually (group I, 20; group II, 20; group III, 16 sows) blood samples were collected every second to fourth week for half a year. The titre of the sera was determined by the virus-neutralization test.

The materials and methods applied were the same as described in the previous paper of this series (Mocsári et al., 1975) and in other papers related to the topic (Benyeda et al., 1974; Csontos et al., 1973, 1974). The titre of the vaccine was $10^{5.75}$—$10^{6.75}$ $TCID_{50}$/ml, the dose was 5 ml applied intranasally.

Results

The sows remained without symptoms after vaccination and their pregnancy took a normal course.

The titre and persistence of the specific virus-neutralizing antibodies which appeared in the serum of sows vaccinated once, or twice at an interval of two or six weeks, are demonstrated in Fig. 1.

In Figures 2a, 2b and 2c, the results are compared with those of the previous experiment carried out in a similar manner (BENYEDA et al., 1974).

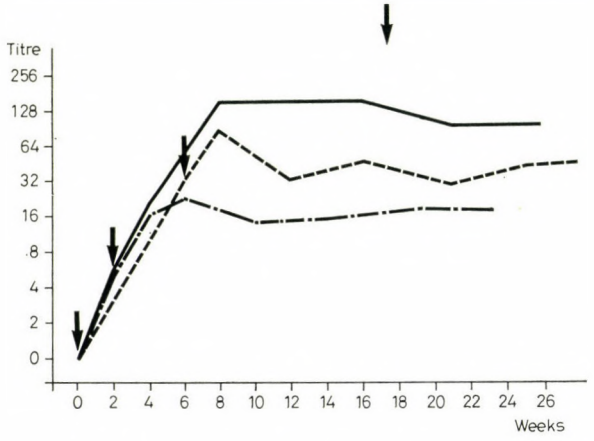

Fig. 1. Dynamics of antibody response to TGE virus in the sera of sows vaccinated once or twice at different intervals. Explanation to Figs 1, 2a, 2b and 2c: —·—·— sows vaccinated on a single occasion; ———— sows vaccinated twice at a two-week interval; - - - - - - - sows vaccinated twice at a six-week interval

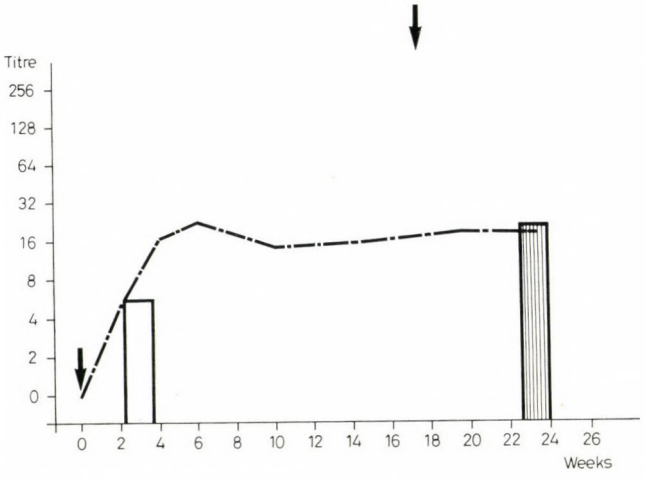

Fig. 2a. Persistence of antibody titres of sows vaccinated on a single occasion. Note to Figs 2a, 2b and 2c: The columns represent the results of the previous experiments

Discussion

The serum antibody level of the sows vaccinated on a single occasion was the highest on the 42nd day after vaccination (average 1 : 24). Then the antibody level fell by 0.5 $\log_2$ unit during the subsequent four weeks and remained unchanged for six months (average 1 : 16).

The specific virus-neutralizing antibody level of the sows vaccinated twice at a six-week interval was the highest in the 8th week after the first vaccination (average 1 : 94). Then it fell by 1.5 $\log_2$ unit during the subsequent four weeks and remained constant for six months average (1 : 32).

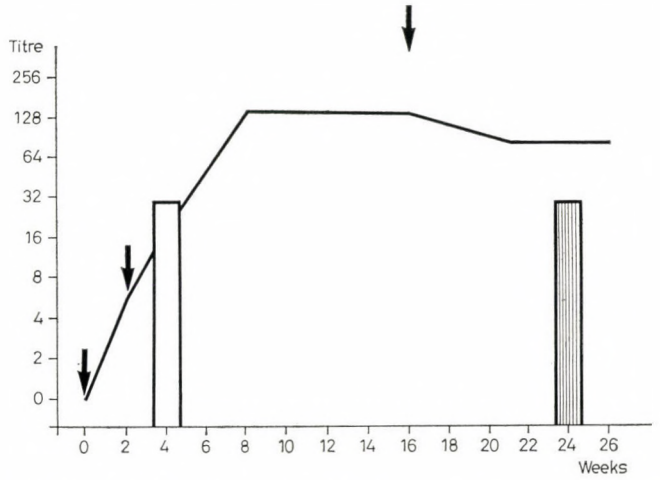

Fig. 2b. Persistence of antibody titres of sows vaccinated twice at a two-week interval

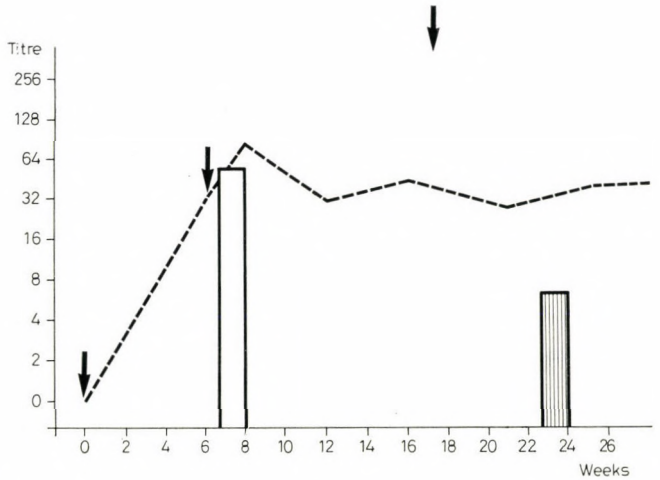

Fig. 2c. Persistence of antibody titres of sows vaccinated twice at a six-week interval

The level of the specific virus-neutralizing antibodies of the sows vaccinated twice at a two-week interval was the highest likewise in the 8th week after the first vaccination (average 1 : 120) and the titres did not fall substantially for six months (average 1 : 100).

In addition to these main results the present investigations have clarified all the questions that remained unanswered after the previous experiments. During the previous experiments the persistence of the postvaccination antibody titres of sows vaccinated once, or twice at a two-week or six-week interval, was determined by a single antibody titration carried out six months after vaccination. It was shown that the average antibody titre for sows vaccinated on a single occasion was substantially higher after half a year than immediately after immunization; the mean antibody titre of the sows vaccinated twice at a two-week interval persisted at a constant level, whereas that of the sows vaccinated twice at a six-week interval fell markedly (BENYEDA et al., 1974). The cause of this phenomenon has been revealed by following up the appearance and persistence of the specific virus-neutralizing antibodies. It has been shown that both the titre increase in sows vaccinated on a single occasion and the persistence of the titres in sows vaccinated twice at a two-week interval observed in the course of the previous experiment six months after vaccination derived from the untimely serological control (see columns of Figs 2a, 2b and 2c). The antibody titres of sows vaccinated according to these schedules increased for three to four weeks following the time of the serological control and then decreased slightly during the subsequent 4—8 weeks. Then the antibody titres remained at a constant level for six months.

The titres found in sows vaccinated twice at an interval of two or six weeks were lower during the previous experiment than in the present one (see the difference between the striped column and the curve of Figs 2b and 2c). This difference was probably caused by immunobiological differences between herds.

The results of the present experiment, in accordance with those of the previous one, have verified that the double-dose vaccination schedule at an interval of two weeks proved to be the best concerning the level and persistence of the specific virus-neutralizing antibodies in sows' sera. The antibody titres of sows vaccinated by this schedule corresponded to those found in the sows' sera after natural TGE outbreaks and the titres remained constant during the subsequent six months.

Summary

A hundred and thirty-two sows of a herd susceptible to the TGE virus were vaccinated on a single occasion or twice at an interval of two or six weeks with the live virus TGE vaccine prepared from the "CKp" strain. The appearance and the persistence of the specific virus-neutralizing antibodies were followed up for six months by serological tests carried out every second or fourth week.

The serum antibody level of the sows vaccinated on a single occasion was the highest on the 42nd day after vaccination (average, 1 : 24). The antibody level fell by 0.5 $\log_2$ unit during the subsequent four weeks and remained constant for six months (average, 1 : 16).

The specific virus-neutralizing antibody level of the sows vaccinated twice at a six-week interval was the highest in the 8th week after the first vaccination (average, 1 : 94), then fell by 1.5 $\log_2$ unit during the subsequent four weeks and remained constant for six months (average, 1 : 32).

The antibody level of the sows vaccinated twice at a two-week interval was the highest likewise in the 8th week after the first vaccination (average 1 : 120) and this did not fall substantially for six months (average 1 : 100).

It is concluded that the double-dose vaccination schedule at an interval of two weeks is the best, concerning the level and persistence of the specific virus-neutralizing antibodies in sows' sera.

*

Acknowledgements. The authors express their gratitude to Dr. E. KUDRON of Veterinary Institute of Szombathely, Dr. GY. BAZSIKA and Dr. A. HÉJJ of Veterinary Centre of Vas County for valuable services.

References

BENYEDA, J. and MOCSÁRI, E.: Developments in Biological Standardization **26** (1974). 13th Congress, Budapest 1973. Selected Veterinary Vaccines, 33. BENYEDA, J., MOCSÁRI, E. and SÁGHY, E.: Magyar Állatorvosok Lapja (MÁL), 1974, 26. IIDEM: Acta vet. hung. **XXIV** (1974), 121. CSONTOS, L. and SZENT-IVÁNYI, T.: MÁL, 1971, 5. CSONTOS, I., SZENT-IVÁNYI, T. and BENYEDA, J.: MÁL, 1973, 22. IIDEM: Acta vet. hung. **XXIV** (1974), 111. MOCSÁRI, E., BENYEDA, J. and SÁGHY, E.: MÁL, 1974, 479. IIDEM: Acta vet. hung. **XXV** (1975), XX.

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THE ULTRASTRUCTURE OF THE SPERMATOZOON OF THE DRAKE

I. HEAD

By

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(Received May 3, 1974)

The ultrastructure of the spermatozoon of vertebrates has been studied by a number of workers. Most of them examined mammalian spermatozoa.

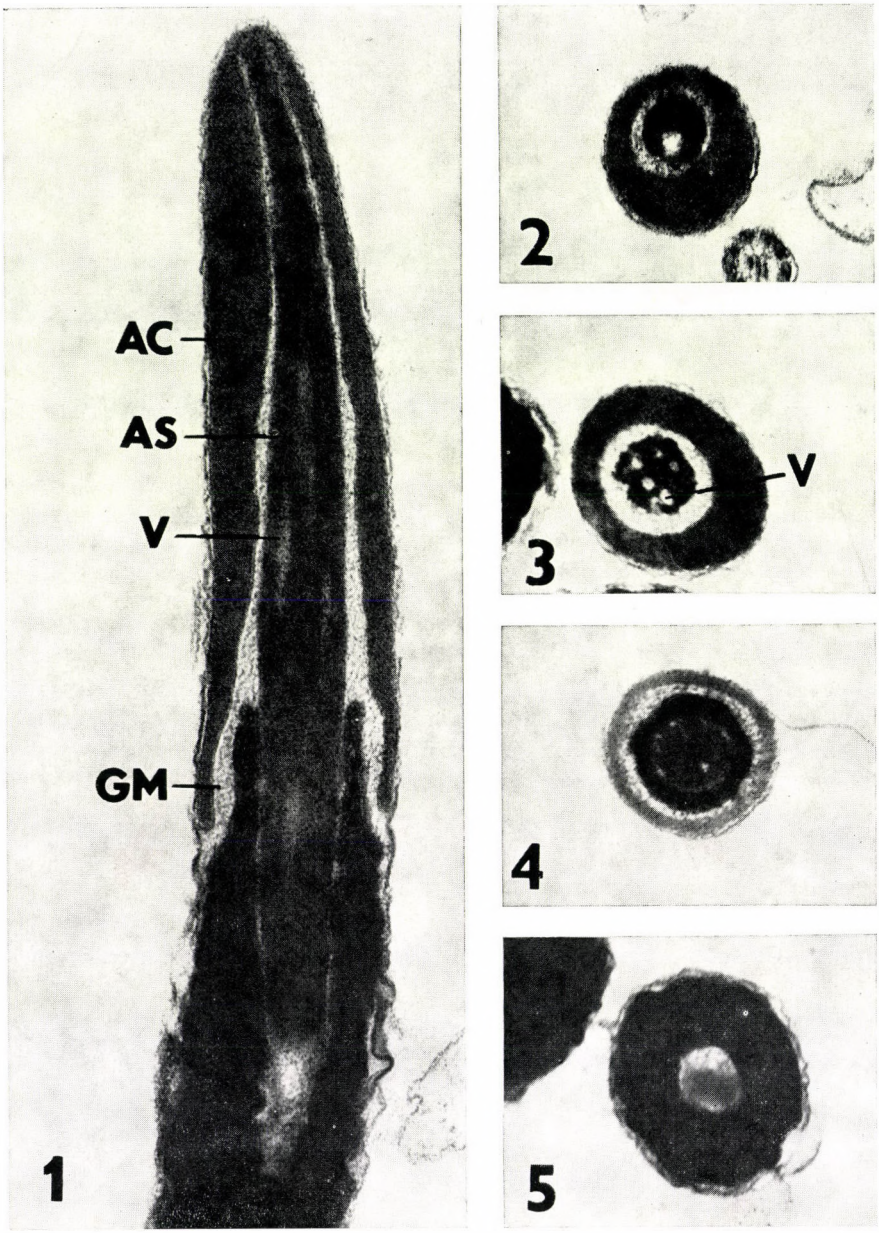
GRIGG and HODGE (1949) and BONADONNA (1954) were the first to report on the ultrastructure of the spermatozoon of the fowl, after having studied whole mounts with the electron microscope. More detailed knowledge of the ultrastructure of the fowl spermatozoon has been obtained only since the introduction of ultramicrotomy. (NAGANO, 1962; NICANDER, 1968 and LAKE et al., 1968).

Materials and methods

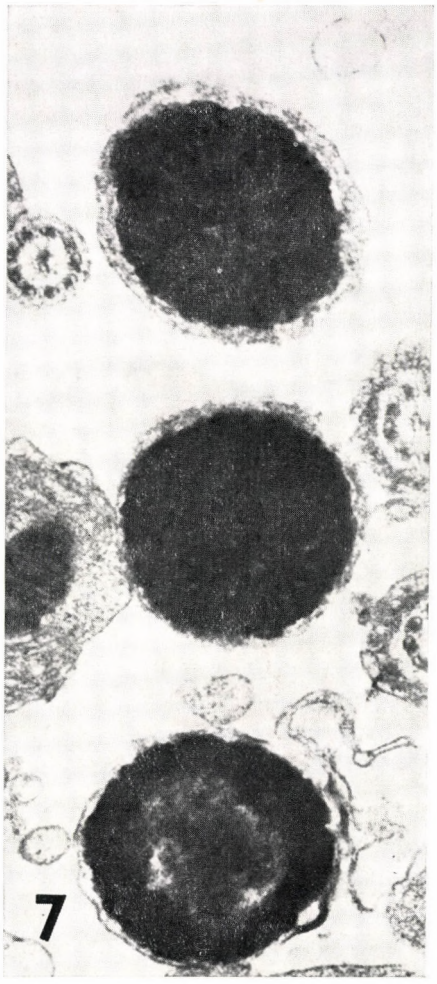
Semen was obtained by massage (BABUSHKIN, 1968) and from killed birds, from the ductus deferens. After microscopical examination the semen was fixed according to BLOM and BIRCH-ANDERSEN (1963) or transferred by means of a pipette into a phosphate-buffered 3% glutaraldehyde fixative containing 0.2 M sucrose (FAHIMI and DROCHMANS, 1965) and postfixed in 1% OsO_4 in phosphate buffer. After rinsing in the phosphate buffer the fixed material was dehydrated in a graded series of acetone or alcohol and embedded in Durcupan ACM and Epon 812 (LUFT, 1961). Ultra-thin sections were obtained by the ultramicrotome Tesla BS 490, and stained according to WATSON (1958) and REYNOLDS (1963). Sections stained were examined with the electron microscope Tesla BS 242 E and Tesla BS 613.

Results

Similar to other fowl species, the drake spermatozoon appears as a long, narrow cylinder, apically terminated by the acrosome. In its posterior region it freely passes into the neck and the middle piece of the tail. Its length and diameter range within 10—11 μm, and 0.6—0.7μm, respectively. Except in the proximal end where it is slightly tapering, the head appears to have the same diameter along its entire length. The tapering is more marked only in the place where the acrosome cap is attached from outside to a portion of 0.3 μm length.



Figs 1—5. 1, Longitudinal section of an acrosome. Acrosome cap (AC) covers the acrosome spine (AS). Along the inner part of the spine vacuoles (V) are seen. The space between the spine and the cap are filled with granular material (GM). ×90,000. *2—5*, Transversal sections of an acrosome at different levels. The vacuoles (V) inside the acrosome spine are well-visible. *2*, ×49,200; *3* and *4*, ×51,500; 5, ×63,200



Figs 6—7. 6, Longitudinal section of the caudal region of the head. The chromatin displays a coarse granular structure with a small vacuole present in the middle. Cytoplasmic membrane closely applied to the nuclear surface (N). ×51,000. *7*, Transversal section of the head of a spermatozoon. On the bottom of the picture, a section at the invagination, with remnants of the proximal centriole

The acrosome is composed of two parts: the acrosome cap and the acrosome spine, which differ both in structure and nature. The acrosome cap consists of homogeneous material of moderate density surrounded by a single membrane; it is reversed "V"-shaped and reaches 2.2 μm in length. The thickness of its wall is 0.1—0.2 μm, being thinnest at the site where the nucleus is attached. The thickness of the wall, however, is thinner at some sites than at others. The base of the acrosome cap is approximately as wide as the latter at the site beneath the head. The acrosome spine, composed of electron-dense material

is needle-shaped, reaching an average length and diameter of 2.6 μm and 0.2 μm respectively. With 1 μm of its length it lies in an invagination of the proximal portion of the head, whereas its remaining part projects and is covered with the acrosome cap. Inside the spine, along its entire length, longitudinally running vacuoles are seen (Fig. 1). Their number moves within 2 to 5 and sometimes even more. Neither the size nor the number of vacuoles are constant. The vacuoles are able to communicate with the outer space between the acrosome cap and the acrosome spine. The vacuoles appear to be either empty or filled with a small amount of a moderately dense substance.

The acrosome cap and the acrosome spine are separated from each other by a 10—40 nm wide space. The widest point of this space is above the processes of the nucleus. This space becomes narrower proximally. It is filled with a low electron-dense granular substance. In the anterior portion the acrosome spine touches the acrosome cap, whereas distally it may, but need not, reach as far as the base of the invagination of the nucleus (Fig. 1).

The chromatin of the head is composed of large granules of about 60 nm which are closely attached together. It is therefore difficult to differentiate its granular structure in the sections. Only a small number of the spermatozoa displayed a marked granular structure. The density of chromatin is similar along the entire length of the head. The nucleus is surrounded by a double nuclear membrane, which in adult spermatozoa is so closely applied to the chromatin that it hardly can be differentiated from the nucleus. Sometimes the nuclear membrane becomes obvious only in the anterior region of the head, at the site where the acrosome cap is attached, in the place of the invagination and in the posterior part of the head at the place where the tail is articulated with the head. Even in this portion the nuclear membrane follows the chromatinic content and does not form any processes or folds (Fig. 6).

The cell membrane covering the acrosome cap and the nucleus is either closely applied to this structure or bulges slightly. These bulgings are more frequent in the region of the nucleus than in that of the acrosome cap.

Discussion

The first descriptions of the shape and structure of the fowl spermatozoon were made on the occasion of their observation in a distilled water medium. Exerting a hypotonic effect the above medium revealed the inner structure of the head in the fowl spermatozoon (GRIGG and HODGE, 1949; BONADONNA, 1954). These observations considered mainly the description of the shape and size of the single parts of the spermatozoon and only partly their inner structure. The latter was observed in adult spermatozoa as late as 1968 by LAKE et al. in sections of fowl spermatozoa. The sizes established in the spermato-

zoon of the male duck are in general agreement with those reported by BONADONNA (1954) in the fowl spermatozoon and differ only little from the data reported by LAKE et al. (1968).

The acrosome of the male duck as in the fowl is composed of the acrosome cap and the acrosome spine, called "apical cap" and "apical spine" by GRIGG and HODGE (1949). Both elements differ not only in shape but also in their inner structure. Whereas the acrosome cap appears homogeneous in structure consisting of moderately dense substance, the acrosome spine is composed of an electron-dense substance displaying several longitudinally-arranged vacuoles inside the spine. In the latter formation no lamellar structures, similar to those described by LAKE et al. (1968), could be seen. It is possible that the longitudinally running vacuoles had caused the lamellar appearance of the acrosome spine. At its anterior end this formation slightly touches the acrosome cap and its termination is not well visible whereas it caudally reaches as far as the base of the invagination. The granular material filling the space between the acrosome cap and the acrosome spine is not present at the site of the nuclear invagination. LAKE et al. (1968) admit that it may be of cytoplasmic origin, having perhaps been derived from the Golgi zone during acrosome development.

Throughout its entire length the nucleus reveals a similar thickness, only in the proximal portion it is slightly tapering, thus forming a smooth junction of the nucleus with the acrosome cap. In contrast to the homogeneous appearance in most mammalian species (FAWCETT, 1958; NICANDER and BANE, 1962a, b; SAACKE and ALMQUIST, 1964; BLOM and BIRCH-ANDERSEN, 1965), the nucleus of the drake spermatozoon contains large granules of chromatin. A granular structure of the chromatin had been observed only in the human spermatozoon (ANBERG, 1957; SCHULTZ-LARSEN, 1958; BEDFORD, 1967; PEDERSEN, 1969). Chromatin granulation is generally ascribed to the presence of immature spermatozoa as a result of incomplete condensation (ANBERG, 1957; HORSTMANN, 1961; NAGANO, 1968). A marked granular structure of chromatin could be well observed in the spermatozoon of the male duck after exposure to an isotonic medium (MARETTA, 1971). Transverse segmentation of the nuclear content of the head as shown by LAKE et al. (1968) in the fowl spermatozoon was not seen in our material. The nucleus is surrounded by a double nuclear membrane following the nuclear surface and even at the place where the head articulates with the tail. No caudal processes of the nuclear membrane similar to those described by LAKE et al. (1968) and wich are a common feature in mammalian spermatozoa (BEDFORD, 1965; FAWCETT, 1965; FAWCETT and ITO, 1965) were seen.

Both the acrosome and the nucleus are surrounded by a cytoplasmic membrane being as a rule applied to the above formations. LAKE et al. (1968) stated periodical bulging of the cytoplasmic membrane in the region of the nucleus. According to the above authors these bulgings can form gaps filled

with a granular substance, considered by the authors as remmants of the spermatid cytoplasm. Although slight bulging of the cytoplasmic membrane in the region of the nucleus could be observed in some spermatozoa of the male duck, gaps containing granular material as described by LAKE et al. (1968) were not seen. It is suggested that the high requirements of the fowl spermatozoon to the medium may be reflected in the relation to the fixatives. In detailed evaluation of the structures it is therefore necessary to consider the above fact. Even so some ultrastructural differences between the acrosome and the nucleus are present in the spermatozoa of the drake and those of fowls.

Summary

A study was undertaken on the ultrastructure of the head of the drake spermatozoon. The latter consists of an acrosome cap, an acrosome spine and the nucleus. The acrosome cap presents the anterior apex-like termination of the head and appears homogeneous in structure. The acrosome spine is needle-shaped displaying a number of vacuoles within. The space between the spine and the acrosome cap is filled with a granular substance. The nucleus is composed of chromatin arranged into large granules. The nuclear membrane is applied to the surface of the nucleus. The cell membrane is closely applied to the acrosome cap and the nucleus.

References

ANBERG, A.: Acta obstetr. gynec. scand. **36** (1957), Suppl2. BABUSHKIN, V.: Drůbežnictví, **10** (1966), 151. BEDFORD, J. M.: Amer. J. Anat. **121** (1967), 443. BIRCH-ANDERSEN, A. and BLOM, E.: Nature **199** (1963), 201. BLOM, E. and BIRCH-ANDERSEN, A.: Nord. Vet. Med. **17** (1965), 193. BONADONNA, T.: Poultry Sci. **33** (1954), 1151. FAHIMI, H. D. and DROCHMANS, P.: Microscopie **4** (1965), 737. FAWCETT, D. W.: Int. Rev. Cytol. **7** (1958), 195. IDEM: Z. Zellforsch. **67** (1965), 279. FAWCETT, D. W. and ITO, S.: Amer. J. Anat. **116** (1965), 567. GRIGG, G. W. and HODGE, A. J.: Aust. J. Sci. Res. **2** (1949), 271. HORSTMANN, E.: Z. Zellforsch. **54** (1961), 68. LAKE, P. E., SMITH, W. and YOUNG, D.: Q. J. exp. Physiol. **53** (1968), 356. LUFT, J. H.: J. biophys. biochem. Cytol. **9** (1961), 409. MARETTA, M.: Folia vet. **XVIII** (1974), XXX. NAGANO, T.: J. Cell biol. **14** (1962), 193. IDEM: Z. Zellforsch. **89** (1968), 39. NICANDER, L.: VI. Congr. Intern. Reprod. Anim. Insem. Artif. Paris I (1968), 89. NICANDER, L. and BANE, A.: Z. Zellforsch. **57** (1962a), 390. IIDEM: Int. J. Fertil. **7** (1962b), 339. PEDERSEN, H.: Z. Zellforsch. **94** (1969), 542. REYNOLDS, E. S.: J. Cell. Biol. **17** (1963), 208. SAACKE, R. G., ALMQUIST, J. O.: Amer. J. Anat. **115** (1964), 163. SCHULTZ-LARSEN, J.: Acta Pathol. Microbiol. Scand. Suppl. P. **128** (1958). WATSON, M. L.: J. biophys. biochem. Cytol. **4** (1958), 475.

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THE ULTRASTRUCTURE OF THE SPERMATOZOON OF THE DRAKE

II. TAIL

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(Received May 3, 1974)

Most of the knowledge of the ultrastructure of the fowl spermatozoon has been supplied by the authors cited in our accompanying paper dealing with the ultrastructure of the head of the drake spermatozoon. The fine structure of the tail of the fowl spermatozoon has been partially described by NAGANO (1962), NICANDER (1968), NICANDER and HELLSTRÖM (1968) and TINGARI (1973).

Materials and methods

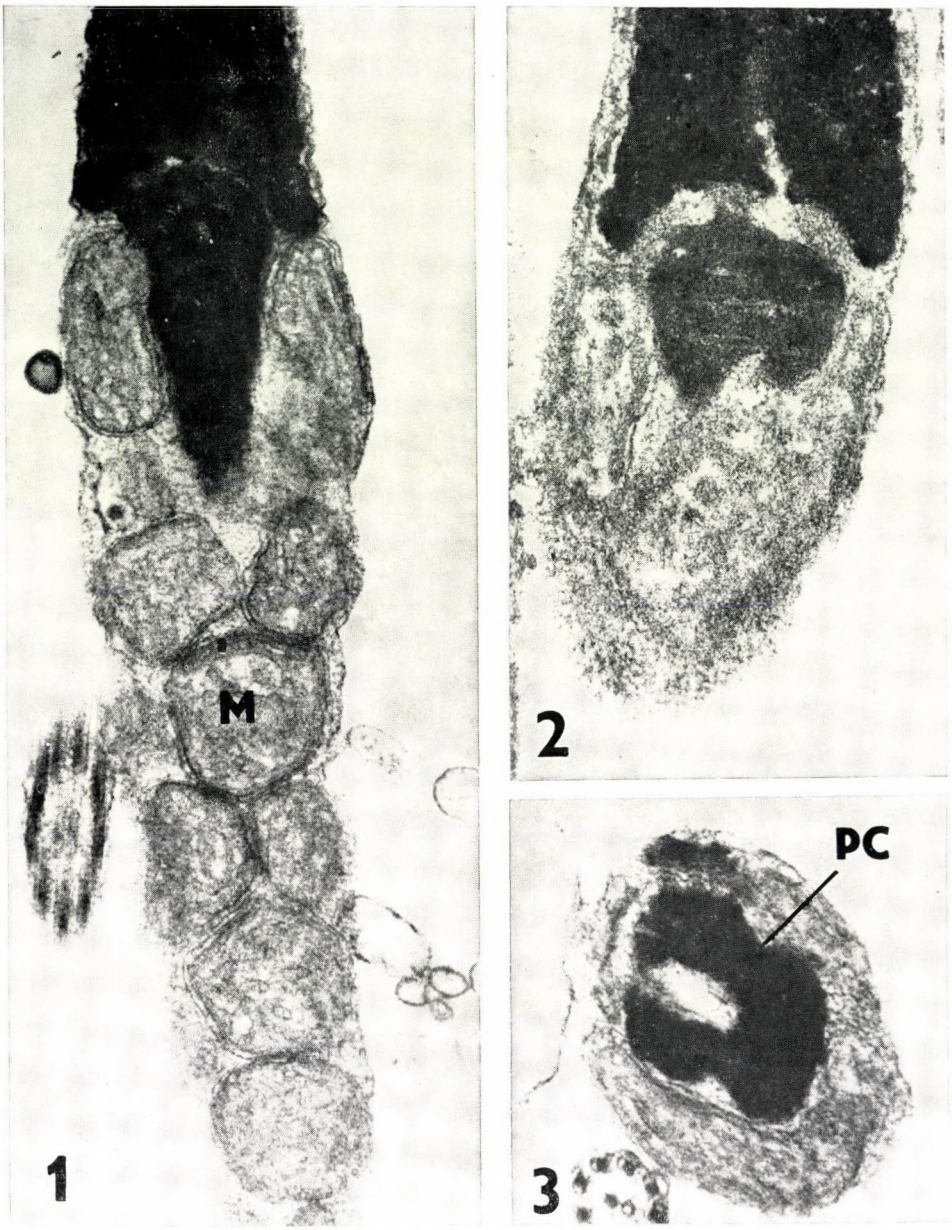
In the present studies the same material was used as that described in the accompanying paper. The methods were completed by fixation according to STEFANINI et al. (1967).

Results

The tail of the drake spermatozoon is composed of four parts: a neck, a middle piece, a principal piece and an end piece.

The neck is the place of articulation between the head ant the tail, which may comprise the proximal centriole and an adjacent electron-dense material filling the area around the proximal centriole. In contrast to the distal centriole, the proximal centriole maintains its original size. At one end of its lumen an electron-dense material can be seen. Its wall consists of nine triplet tubule structures and forms a right angle to the distal centriole. In cross sections it appears as a thick-walled ring in which some tubules of the triplets can be partly identified. (Figs 1, 2, 3).

The middle piece is defined by the length of the mitochondrial sheath measuring in the drake sperm 3—4 μm, and having a diameter of 0.6—0.7 μm. The main portion of the middle piece consists of the centrally placed distal centriole and part of the axial filament complex. In adult spermatozoa the distal centriole reaches a length of up to 2 μm and a diameter of 0.2 μm. The lumen of the proximal portion of the centriole is usually filled with electron-dense material (Fig. 5). The distal centriole is connected with the proximal centriole by a dense material, which also fills the space under the projecting

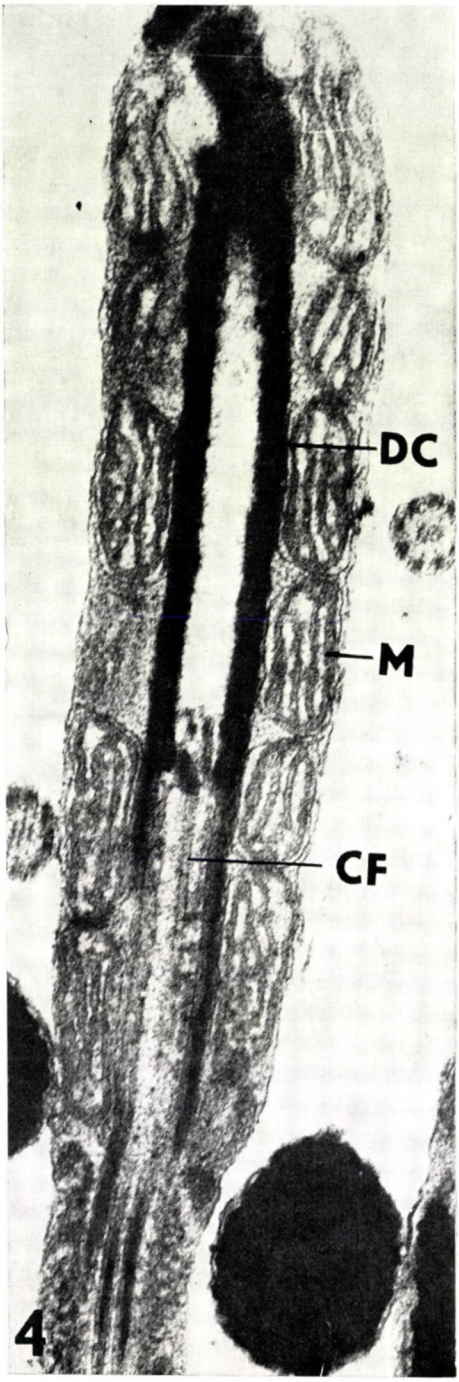


Figs 1—3. 1, Longitudinal section of the neck and part of the mitochondrial sheath. Mitochondria (M) are of irregular spheric shape. ×51,000. *2*, Transverse section of the caudal part of the head and anterior region of the middle piece. The proximal centriole is surrounded by electron-dense material. ×68,800. *3*, Transverse section of the spermatozoon's neck in the region of the proximal centriole (PC). ×71,000

parts of the proximal centriole. This dense material partly replaces the segmented coarse fibres occurring in the mammalian spermatozoa. Within its caudal end a small amount of electron-lucent material is placed wherefrom the central fibrils arise. The general structure of the axial complex is well known from spermatozoa of other species, cilia and flagella, having 9 + 2 pattern. The two central fibrils are circular in cross section, about 25 nm in diameter. An open lumen in one of them was not always demonstrable. The nine outer doublets are composed of two subfibrils which differ in density. The denser member of the pair (subfibril A) is smaller and lies nearer to the axis of the tail than does the larger, less dense subfibril B. Subfibril A carries two "arms" directed towards the adjacent doublet and one "spoke" which runs to the pair of central fibrils. The outer dense fibres were only partly preserved reaching a diameter of about 40 – 50 nm and are closely attached to the doublets. They are visible only in the posterior region of the middle piece reaching as far as the beginning of the principal piece. From outside mitochondria are attached to the distal centriole and part of the axial fibres. In the anterior part of the middle piece mitochondria closely reach the head and are caudally defined by the annulus. They are of irregular spherical shape (Fig. 1) and their total number ranges between 24 – 30. They are flattened at the sides and about 0.2 μm thick. Mitochondrial cristae run parallel to the flattened mitochondrial wall (Figs 3, 4, 5).

The annulus (Jensen's ring) presents a ring-shaped formation located between the mitochondrial sheath of the middle piece and the amorphous sheath of the principle piece (Fig. 7). It is well-defined by these structures, its cross section showing an almost triangular shape with a wall length of 100—150 nm. The base is turned towards the cytoplasmic membrane, the termination aiming at the centre of the tail. It is composed of homogeneous material suggesting sometimes a fibrous structure (Fig. 8).

The principal piece of the tail is defined by the length of the amorphous sheath. Cranially, it starts at the annulus and passes caudally into the end piece. The centrally placed axial filament complex is running throughout the principal piece. Only in the anterior region, remnants of the outermost dense fibres are visible. The amorphous sheath consists of a moderately electron-dense material and its wall has a diameter of 0.1 μm in its proximal portion. The amorphous sheath is not closely applied to the axial filament but appears to be separated from it by a space 20 nm in width. In cross, and partly also longitudinal, sections the sheath can be seen to be composed of two layers, namely, the inner layer revealing greater density and the outermost less dense layer. Caudally, the sheath gradually becomes narrower, whereby its outermost layer disappears first; gradually the innermost layer disappears, too. The site where the amorphous sheath disappears presents the place where the principal piece passes into the end piece of the tail (Fig. 12).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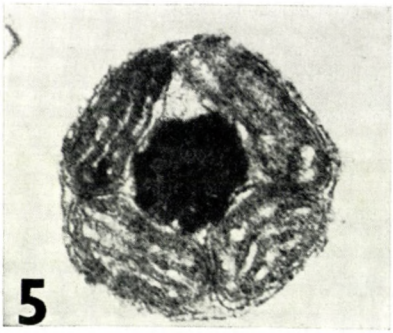
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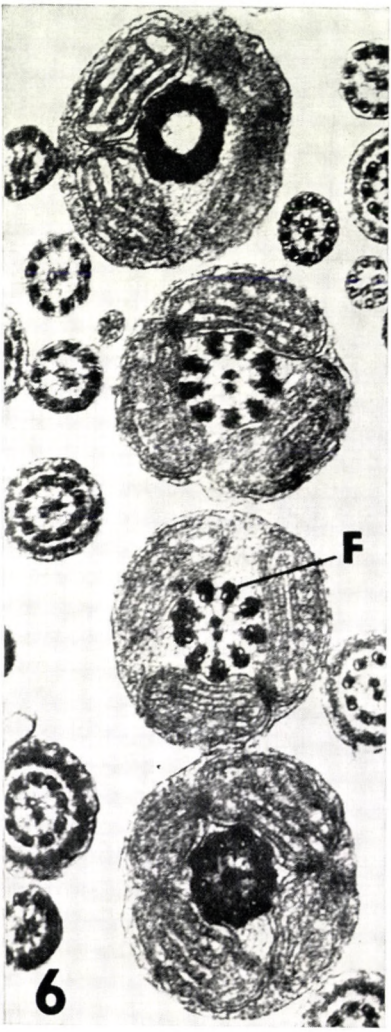
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The end piece is composed of an axial filament complex. The fibrous arrangement as we observed in this region is similar to that described above. Near the tip of the end piece the arms of the doublets disappear and subfibril A takes on a hollow appearance. The doublets are reduced to single fibrils. The peripheral fibrils gradually decrease in number.

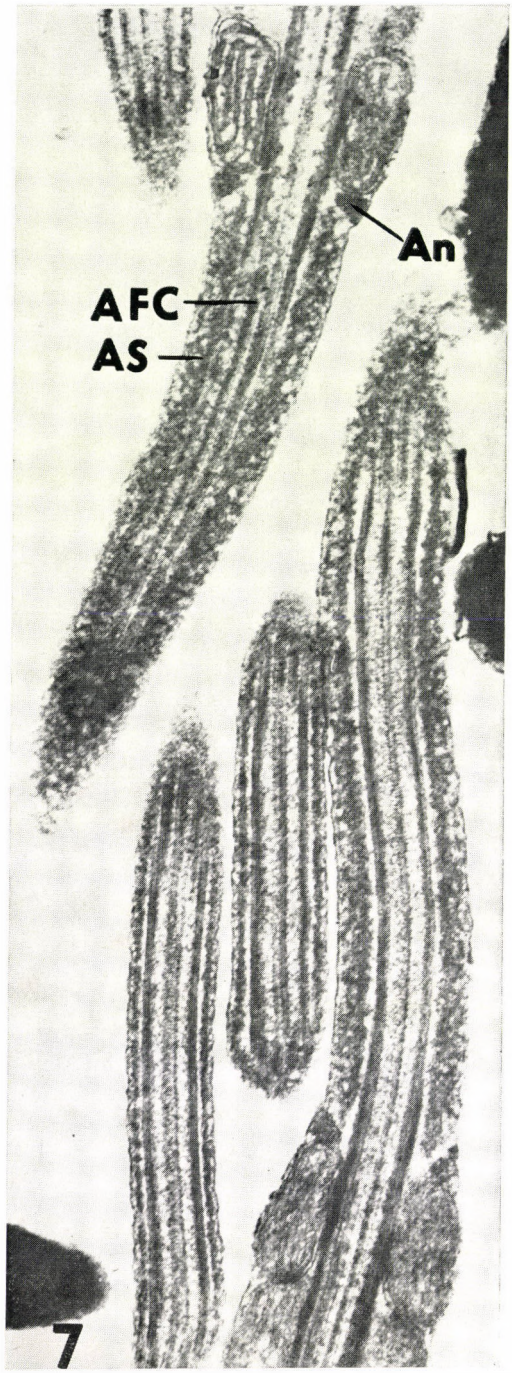
The cytoplasmic membrane covering the entire length of the tail is closely applied to the underlying structures. It is fixed only to the annulus. In the posterior portion of the principal piece it appears to be separated from the amorphous sheath by a narrow lighter space.

Discussion

The region defined by the posterior border of the head and the beginning of the mitochondrial sheath or the middle piece is in general considered as the neck in the mammalian spermatozoon. According to FAWCETT (1958) it is 0.5 μm in length. In the spermatozoa of drakes some mitochondria reach closely to the head; they agree in arrangement and structure with the mitochondria of the remaining part of the sheath. This region may therefore be considered the site of articulation between the head and the tail without any exact definement of space.

The nuclear membrane lining the invagination of the nucleus closely follows its surface and no projections into the neck region, similar to those seen in mammalian (BEDFORD, 1967; FAWCETT, 1965; FAWCETT and ITO, 1965; NICANDER and BANE, 1962; 1966; PEDERSEN, 1969, 1972) and fowl spermatozoa (LAKE et al., 1968) are visible. No segmented dense fibres characteristic of the normal mammalian spermatozoa have been observed in the fowl or drake spermatozoa. Instead, a small amount of electron-dense material is present. This is placed closely to the proximal centriole and filling up the empty space around it, simultaneously presenting the fusion of the two centrioles. The distal centriole which is transformed in the spermatozoa of mammals (FAWCETT, 1958, 1965; SOTELO and TRUJILLO-CENOZ, 1958; FAWCETT and ITO, 1965) reveals however a well-preserved structure in the spermatozoon of the male duck. It appears to be 3—4 times longer than the proximal centriole. Maintenance of the distal centriole two or three times longer than the proximal centriole has also been reported by AUSTIN (1965) in the spermatozoa of some

Figs 4—6. 4, Longitudinal section through the middle piece of the tail. Central fibrils (CF) arise from the caudal end of the distal centriole (DC). The mitochondrial cristae are arranged parallel to the flattened mitochondrial wall (M). ×53,300. *5*, Cross section through the tail in the proximal portion of the distal centriole. Inside the centriole is filled with electron-dense material. ×49,200. *6*, Cross section through the middle piece at different levels. Outer dense fibres are present (F). ×43,000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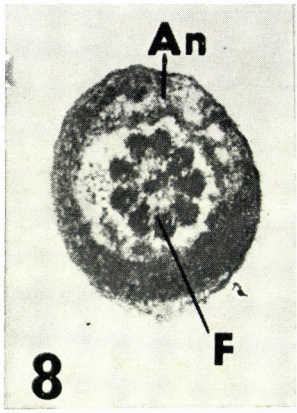
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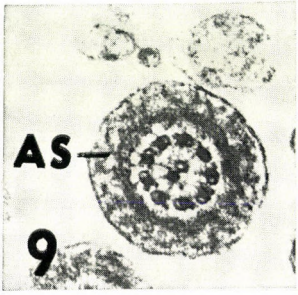
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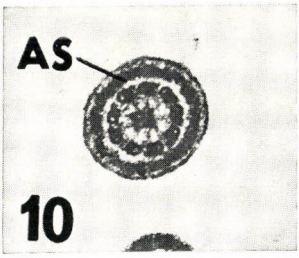
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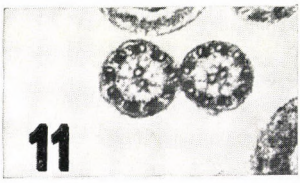
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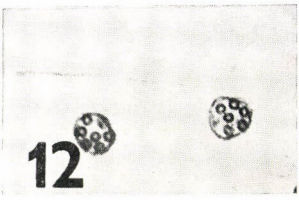
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snake species. Two tubules of the distal centriole run caudally as doublets of the axial filament complex (MARETTA, 1971). On the other hand, central fibrils proceed from the site of caudal termination of the distal centriole. LAKE et al. (1968) reported the presence of central fibrils in the lumen of the distal centriole of the fowl spermatozoa. The findings of the present study are in agreement with those of NAGANO (1962) and NICANDER (1968) in the fowl spermatozoon. The above authors failed to observe any central fibrils in the distal centriole. The outer dense fibres, so marked in the spermatozoa of mammals, appeared to be considerably reduced in size in the drake spermatozoon and are visible only in the posterior region of the middle piece, or at the onset of the principle piece. They are somewhat larger than stated by LAKE et al. (1968) in the fowl spermatozoon. From outside, mitochondria are attached to the distal centriole and the axial filament in the region of the middle piece, thus forming the mitochondrial sheath. They are predominantly spherical in shape and arranged in a way that they seem to fuse. LAKE et al. (1968) described them in the fowl spermatozoon as rectangular platelets being arranged helically. No such arrangement, so marked in the spermatozoa of mammals (FAWCETT, 1965; HANCOCK, 1967; BLOM and BIRCH-ANDERSEN, 1960; SAACKE and ALMQUIST, 1964), has been found in the male duck. The mitochondrial cristae in the spermatozoa of drakes are arranged parallel to the flattened mitochondrial wall. The annulus which is placed between the mitochondrial and the amorphous sheath is similar in shape and structure to that described in the spermatozoa of a number of mammals. No similar findings as to different density of the amorphous material of the sheath could be found in the works of authors having investigated the fine structure of the fowl spermatozoon. This structure seems to occur only in the spermatozoa of the male duck. Like in mammalian spermatozoa the structure of the axial fibrils was similar along their entire course, except of the termination.

Figs 7—12. 7, Longitudinal section through the principal piece of the tail in the anterior and in the posterior region. The middle piece and the principal piece are separated by an annulus (An). The sheath consisting of an amorphous material (AS) is attached to the axial filament complex (AFC). ×41,200. *8*, Cross section through the tail in the region of the annulus (An). The outer dense fibres (F) are still present. ×51,600. *9*, Cross section of the tail in the proximal portion of the principal piece. The amorphous sheath (AS) is composed of an outer less dense and an inner denser layer. ×51,600. *10*, Cross section of the principal piece of the tail in the posterior part. The amorphous sheath (AS) consists only of an inner denser layer. ×51,600. *11*, Cross section of the end piece of the tail. The structure of the axial filament complex (AFC) is similar to that in the spermatozoa of mammals. ×49,200. *12*, Transversal section through the tail in termination of the end piece. The full filament is missing on the doublets and their arrangement is disturbed. ×49,200

Summary

A study has been conducted on the ultrastructure of the tail of the drake spermatozoon. The tail consist of a neck, a middle piece, a principal piece and an end piece. The neck includes the proximal centriole and an adjacent electron-dense material. The middle piece is defined by a mitochondrial sheath consisting of 24—30 mitochondria of irregular spheric shape flattened at the sides. The axis of the middle piece is presented by a distal centriole, the proximal part of the axial filament complex and the outer dense fibres which are considerably reduced in size. The principal piece consists of an axial filament complex and an amorphous sheath enveloping it. The end piece contains only an axial filament complex revealing an arrangement of 9 + 2 pattern. The cell membrane is closely applied to the surface structures of the tail.

References

AUSTIN, C. R.: J. Ultrastruct. Res. **12** (1965), 452. BEDFORD, J. M.: Amer. J. Anat. **121** (1967), 443. BLOM, E. and BIRCH-ANDERSEN, A.: Nord. Vet. Med. **12** (1960), 261. FAWCETT, D. W.: Int. Rev. Cytol. **7** (1958), 195. IDEM: Z. Zellforsch. **67** (1965), 279. FAWCETT, D. W. and ITO, S.: Amer. J. Anat. **116** (1965), 567. HANCOCK, J. L.: In: Advances in Reproductive Physiology. N. Y., 1966. LAKE, P. E., SMITH, W. and YOUNG, D.: Q. J. exp. Physiol. **53** (1968), 356. MARETTA, M.: Vývoj a ultraštruktúra spermie hydiny. Thesis. Košice, 1971. NAGANO, T.: J. Cell biol. **14** (1962), 193. NICANDER, L.: VI. Cong. Intern. Reprod. Anim. Insem. Artif. Paris **I** (1968), 89. NICANDER, L. and BANE, A.: Z. Zellforsch. **57** (1962a), 390. JIDEM: Ibidem **72** (1966), 496. NICANDER, L. and HELLSTRÖM, B.: Exptl. Cell. Res. **48** (1967), 622. PEDERSEN, H.: Z. Zellforsch. **94** (1969), 542. IDEM: Ibidem **123** (1972), 305. STEFANINI, M. C., DEMARTINO and ZAMBONY, I.: Nature **216** (1967), 173. SAACKE, R. G. and ALMQUIST, J. O.: Amer. J. Anat. **115** (1964), 163. SOTELO, J. R. and TRUJILLO-CENÓZ, O.: Z. Zellforsch. **48** (1958), 565. TINGARI, M. D.: J. Reprod. Fert. **34** (1973), 255.

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OCCURRENCE OF K80(B) ANTIGEN IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM SUCKLING CALVES

By

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Strains showing the antigenic pattern O78 : K80(B) have been frequently isolated from suckling calves died of *E. coli* diarrhoea, as reported by ÁLDÁSY (1955, 1959) from Hungary and by WRAMBY (1946), ULBRICH (1954), FEY (1957), SOJKA (1963) and others from abroad. As such strains often produce the septicaemic form of the disease, they have been isolated not only from the small intestine and mesenteric lymph nodes, but also from other organs. In the course of serological examinations performed in this laboratory recently, mainly with the aim to study the K antigens of *E. coli* strains causing disease in calves, several *E. coli* strains have been isolated with different O antigen, which are inagglutinable in the living state with the homologous O antiserum but agglutinable with the OK serum O78 : K80(B) up to the end titre of the serum. Details of the serological examinations have been reported elsewhere (VARGA and FARID, 1974).

In this paper the occurrence of antigen K80(B) in various *E. coli* strains and the serological examination of the K antigen of the international type strain E38 (antigenic pattern: O78 : K80(B) : NM) are described.

Materials and methods

A total of 288 coliform strains, isolated from the small intestine and, occasionally, from other organs of 117 suckling calves died of diarrhoea when less than two weeks old, were examined for biochemical properties, and O and K antigens. Primary isolations as well as culturing for antigen production were both made on the $D_{1,5}$ medium proposed by SCHLECHT and WESTPHAL (1966).

The biochemical examinations, production of O and OK antisera, antigen preparation, absorption and slide and tube agglutination tests were carried out as described by KAUFFMANN (1966). The O and K antigens of the strains classified into the genus *Escherichia* on the basis of biochemical properties were regarded as indentified when the strain was agglutinable by one of the O or K sera up to the end titre of the serum.

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Results

Two hundred and sixty-three isolates were classified into the genus *Escherichia* on the basis of biochemical behaviour. Out of them 33 strains belonged to seven different O groups, a single strain to group O17, and four to six strains to each of the remaining six serogroups, but all strains were agglutinable with the OK antiserum to the international type strain E38 (O78 : K80(B)) up to the end titre of the serum. The O group distribution of the strains as well as agglutination titres obtained with O78 : K80(B) O and OK sera are shown in Table I. Strains belonging to the various O groups were in a living state not agglutinable either by the homologous O serum or the O78 serum, whereas the O78 : K80(B) OK serum agglutinated them up to its end titre (1 : 1280). K agglutination was lost after absorption of the O78 : K80(B) OK serum with the live homologous strain, but it did not change if the homologous antigen was heat-treated for 2.5 hours at 100 °C before absorption. The O78 : K80(B) OK serum, after absorption with the live strain No. 231, O153 : K80? lost agglutinating activity for all strains except the isolate No. 252, O78 : K80(B) and the international type strain E38. The latter two strains were, in living state, agglutinable with the absorbed serum up to 1 : 80. The O78 : K80(B) OK serum however, lost activity for all examined strains including E38, if it had been absorbed with the live strain No. 252. The live strains listed in the Table I were tested with all (1—94) K sera except K65, K90 and K92 but only the serum O78:K80(B) agglutinated them.

Table I

Agglutination reactions of living *Escherichia coli* strains belonging to different O groups with whole and absorbed E38 — OK antisera

Serial no. of strains	O group	E38 antisera		E38 OK antisera absorbed with			
		O	OK	E38 live	E38 killed (2.5 hr at 100°C) antigens	231 live	252 live
45	O17	—	+	—	+	—	—
123	O8	—	+	—	+	—	—
128	O20	—	+	—	+	—	—
231	O153	—	+	—	+	—	—
261	O21	—	+	—	+	—	—
252	O78	—	+	—	+	+a	—
494	O9	—	+	—	+	—	—
E38 type strain	O78	—	+	—	+	+a	—

Symbols used: — negative slide agglutination; + 1 : 1280 tube agglutination titre; a, 1 : 80 tube agglutination titre

Since the various group-O isolates were, in the living state, agglutinable by the OK antiserum to type strain E38, which was found to maintain an unchanged agglutinating activity even after absorption with the heat-killed (2.5 hours at 100 °C) homologous strain, it seemed to be of interest to examine the agglutinability as regards the K antigen of the type strain E38 with differently prepared antigens, and absorbed homologous O and K sera. The results are shown in Table II. The living type strain was not agglutinable by

Table II

Agglutination reactions of E38 *Escherichia* type strain with whole and absorbed OK antisera

E38 antigens	E38 antisera		E38 OK antisera absorbed with homologous antigen			
	O	OK	Live	Heat-treated 1 hr, 60 °C	Heat-treated 2.5 hr, 100 °C	Ethanol-treated 50%, 20 hr, 37°C
Live	—	1280a	—	—	1280	—
Heat-treated, 1 hr, 60 °C	2560	2560	—	—	—	—
Heat-treated, 2.5 hr, 100 °C	2560	2560	—	—	—	—
Ethanol-treated, 50%, 20 hr, 37 °C	—	1280	—	—	1280	—

— negative slide-agglutination; a, tube-agglutination titre

the O78 serum, but the OK serum agglutinated it up to 1 : 1280. This K agglutinating capacity was lost after absorption with the homologous strain eithe- untreated or preincubated for 1 hour at 60 °C, or pretreated with ethanol, but maintained it if the absorbing antigen had been treated for 2.5 hours at 100 °C. The heat-treated antigens were agglutinated up to same titre by sera O and OK, but no agglutination took place in the absorbed sera, indicating that the agglutinability of the K antigen of the type strain E38 was lost upon heat treatment. The ethanol-treated antigen reacted similarly to the live strain. The antigen of the type strain was also examined after treatment with 1 *N* hydrochlorid acid for 20 hours at 37 °C, but acid treatment resulted in auto-agglutination.

Discussion

The strains of different O groups shown in Table I were, in the living state, inagglutinable by both the homologous O serum and the O78 serum. The same strains were inagglutinable by all examined K sera except the OK serum to type strain E38, O78 : K80(B), with which they showed an agglutination up to its titration endpoint. This suggested that the strains were in pos-

session of a K80(B) antigen, but absorption tests showed that exclusively those strains having the antigenic pattern O78 : K80(B) are capable of complete absorption, whereas the various group-O strains reacting positively with the O78 : K80(B) OK serum are not. It follows that strains having the antigenic pattern O78 : K80(B) possess the same K antigen as type strain E38, O78 : K80(B), whereas strains of different O groups have a K antigen not identical with, only closely related to, K80(B). The agglutination produced by the strains shown in Table I with the E38, O78 : K80(B) OK serum should be regarded as a K agglutination, because the reaction is shown only by the living strains, the presence of H agglutinins in the antiserum can be excluded, for type strain E38 O78 : K80(B) is a variant having no flagella and treatment of the type strain with 50% ethanol did not alter the K agglutination titre. The presence of natural alpha or beta agglutinins in the serum can also be excluded because the preimmunization rabbit serum did not agglutinate the type strain O78 : K80(B) and because the OK antiserum could be completely absorbed with the living homologous strain.

The closer study of the K antigen of type strain E38, O78 : K80(B) had a surprising result. According to KAUFFMANN (1966), K(B) antigens maintain agglutinability antibody-binding capacity and immunogenicity after heat treatment for 1 hour at 60 °C, but lose all properties except antibody-binding capacity when treated for 2.5 hours at 100 °C. In our hands, type strain E38, O78 : K80(B) lost K agglutinability, but maintained antibody-binding capacity after heat treatment for 1 hour at 60 °C, but lost all three properties after heating for 2.5 hours at 100 °C, so that it failed to remove K antibodies from the OK serum by absorption, the result being a pure K serum. In view of this, there is reason to suppose that the type strain E38, O78 : K80(B) has a thermosensitive, probably K(L) surface antigen. At the same time, the agglutination results do not accord with those obtained by ØRSKOV et al. (1971, 1972) in agar gel diffusion and immunoprecipitation studies, in which the B antigen of many K(B) type strains, including E38, O78 : K80(B), did not form an independent precipitation band in agar gel, which would correspond to their K specificity and therefore these B antigens might be only parts of the respective O antigens.

Summary

O and K antigens of a total of 263 *Escherichia coli* strains, isolated from 117 suckling calves, were identified. Thirty-three isolates were, in the living state, inagglutinable by all (1—94) K sera except the OK serum to type strain E38, O78 : K80(B). The 33 strains belonged to seven different O groups (O8, O9, O17, O20, O78 and O153) and K agglutination showed them to possess the K80(B) antigen. Tests with absorbed E38, O78 : K80(B) antiserum however showed that only the isolates of O78 : K80(B) antigenic pattern had a K antigen identical with K80(B) of the type strain, whereas the common surface antigen of the isolates belonging to the remaining six O groups was related, but not identical, with K80(B) of the type strain.

Closer studies on the K antigen of type strain E38, O78 : K80(B) showed that, since the antigen pretreated for 2.5 hours at 100°C could not absorb K antibodies from the homologous OK serum the strain might well contain a thermolabile, probably K(L) surface antigen to which antiserum could be produced in rabbits and absorption of this OK serum results in a pure K antiserum.

References

ÁLDÁSY, P.: Thesis. Budapest, 1955. IDEM: Magyar Állatorvosok Lapja (MÁL), 1959, 195. FEY, H.: Zbl. Vet. Med. **4** (1957), 447. KAUFFMANN, F.: The Bacteriology of Enterobacteriaceae. Copenhagen, 1966. ØRSKOV, I., JANN, B. and JANN, K.: Acta path. microbiol. scand. Section B **79** (1971), 142. ØRSKOV, F. and ØRSKOV, I.: Ibidem **80** (1972), 905. SCHLECHT, S. and WESTPHAL, O.: Zbl. Bakt. I. Orig. **200** (1966), 241. SOJKA, W. J.: *Escherichia coli* in Domestic Animals and Poultry. C. A. B. England, 1965. ULBRICH, F.: Zbl. Vet. Med. **1** (1954), 603. VARGA, J. and FARID, A. F.: Acta vet. hung. **XXIV** (1974), 421. WRAMBY, G.: Skand. Vet. Tidskr. **36** (1946), 370.

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SWINE LYMPHADENITIS DUE TO *MYCOBACTERIUM AVIUM* AND ATYPICAL *MYCOBACTERIA*

I. PATHOLOGICAL STUDIES

By

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During the recent years, the frequency of tuberculous involvement of mesenteric and submaxillary lymph nodes has been increasing among slaughtered pigs originating from certain farms. The abattoir statistics and investigations conducted on the respective herds (tuberculin test and subsequent pathological and other studies) both showed that the condition also occurred in some modern large-scale pig units where neither other animals, nor poultry were kept and the pigs had no access to contact with other species. No dairy products were included in the pig diets either. The problem of tuberculous infection in swine is stressed by the fact that the national bovine tuberculosis eradication program has already made a great progress.

These practical observations contradict the earlier view that swine tuberculosis is caused either by feeding of milk or dairy products originating from an infected cow herd or by ingestion of manures or organs of tuberculous poultry.

In 1972, investigations started along several lines to obtain more informations on the problem. This paper is a report of the pathological, histopathological and bacteriological findings, with special regard to their epizootological importance.

English, Danish, German and American authors pointed out in the 'thirties that *Mycobacterium avium* infection tended to increase among pigs, although bovine tuberculosis infections were declining (cit. LUKE, 1958; LESSLIE et al., 1968; KARLSON and THOEN, 1971; RAY et al., 1972). In Great Britain, *M. tuberculosis* was not encountered among 318 *Mycobacterium* strains isolated from 563 pigs over the period 1952—1966. Among 45, 134 and 139 strains isolated in the periods 1952—56, 1957—61 and 1962—66, 44, 81 and 92%, respectively, were identified as *M. avium*, the rest as *M. bovis*. While 48% of the *M. bovis* infections identified over 15 years was generalized, only 12% of the *M. avium* infections involved, also the liver, spleen and lungs apart from lymph nodes of the head, neck and mesentery (LESSLIE et al., 1968). The low rate of occurrence of the generalized form in pigs has, among others, been attributed to the shorter lifetime of the pigs compared other species (LUKE, 1958).

BANG established already in 1913 (cit. LUKE, 1958) that swine tuberculosis due to *M. avium* generally involves only lymph nodes and takes a milder course than *M. bovis* infection. According to LUKE (1958), *M. avium* infection of pigs is characteristically an alimentary process which rarely becomes generalized and does not involve massive shedding of the bacteria. RAY et al. (1972) reported that experimental intracutaneous and oral infection of pigs with *M. avium* and other *Mycobacterium* strains belonging to the Runyon III group never spread to contacts.

In the GFR, SCHLIESSER (1964) isolated 69 *M. avium*, 14 *M. fortuitum*, 4 *M. phlei* and 10 not nearer identified scotochromogenic strains from lymph nodes of slaughtered pigs, and

pointed out that the control of avian tuberculosis cannot resolve the problem of the tuberculous involvement of lymph nodes in pigs, because possibilities of infection by various saprophytic *Mycobacterium* species continue to exist.

PIENING et al. (1972) identified 71% of 134 *M. avium* isolates from pigs as belonging to serotype II and classified the rest as serotype Davis, Watson, III and IV. ONET (1971) found that 20 *M. avium* isolates from slaughtered pigs were all pathogenic for poultry and in another series (ONET, 1972), identified 5, 61 and 3 of 69 Runyon group III strains as belonging to serotype I, II and Davis, respectively. In Japan, MIYASHITA et al. (1972) isolated Runyon group III strains from 173 mesenteric and 16 submaxillary lymph nodes of 6129 clinically healthy pigs examined in the slaughter house. YUGI et al. (1972) reported the occurrence of six serotypes, including *M. avium* serotype II and Davis.

In Australia, usually serotype VI *M. intracellulare* has been isolated from the frequent mesenteric lymph node involvement of slaughtered pigs, and in several herds lesions occurring exclusively in the submaxillary lymph nodes of 2—3% of the animals have been the source of isolations of several atypical *Mycobacteria*, some of which occur in nature as saprophyte. *Corynebacterium equi* has been found to occur in the altered submaxillary lymph nodes either alone or together with *Mycobacteria* (REZNIKOV et al., 1971, 1973).

Isolations of *Corynebacteria*, above all *C. equi*, as well as of streptococci and staphylococci, from tubercle-like or purulent-caseous lesions mainly in the submaxillary lymph nodes have also been reported from other countries at a varying frequency (NAGY, 1944; LESSLIE et al., 1968; LUKE, 1958; ROBERTS and HAMILTON, 1968).

On histological examination of 100 lymph nodes condemned as tuberculosis at meat inspection, RETZLAFF (1966) found tuberculous lesions in 91, of which only 8 showed productive processes regarded as characteristic of avian tuberculosis, although *M. avium* had been isolated from most of them, and only in some cases could be isolated atypical *Mycobacteria*.

Materials and methods

The examinations were limited to clinically healthy, slaughtered pigs rom large-scale farms and did not include similar animals originating from small herds, or pig showing clinical signs of tuberculosis. Those lymph nodes and parenchymatous organs were examined which, at meat inspection, were grossly diagnosed as tuberculosis or suspected of TB. In a total 142 submaxillary lymph nodes, 227 mesenteric lymph nodes, 49 peribronchial lymph nodes, 4 hepatic lymph nodes, 19 lungs and 12 livers of pigs from 41 farms were examined.

Histological technique

Lymph nodes and other organ specimens were fixed in 10% formalin solution immediately after slaughter and were embedded in paraffin. The sections were stained with haematoxylin and eosin for general orientation and the following selective techniques were applied: Farkas—Mallory's staining, Gömöri's silver impregnation and the Ziehl-Neelsen technique.

Isolation and culturing of bacteria

To one volume homogenized lymph node or organ specimen five volumes of 6% w/v sulphuric acid were added and the mixture was allowed to stand for 25 minutes after which pH was brought up to 7.0 by adding 1.0 *N* NaOH.

The fluid decanted from the coarse sediment was centrifuged for 10 minutes at 3000 r.p.m., the supernatant was decanted and the sediment was used for isolation experiments. Aliquots of each sample were transferred to Petragnani's glycerol agar, Petragnani's glycerol-free agar, as well as Dorset and Sula media. Cultures were incubated at 37 °C.

Positive isolates were subcultured on Loewenstein—Jensen medium, to test the morphological properties, temperature requirement, growth rate and pigment production of the strains.

The biochemical properties were assayed with the nitrate reductase, catalase, peroxidase and aryl sulphatase tests, and Bönicke's carbonamide decomposition test (acetamide, benzamide, urea, isonicotinic amide, nicotin amide, pyrazinamide, salicylamide, allantoin, succinic amide, malonic amide). If required, additional biochemical test were used for typing. The biochemical tests were performed according to the standard methods (TUBOLY, 1969).

Results

Gross and microscopic lesions

Tuberculous foci of various sizes were found in one or more lymph nodes of the same animal. The presence of small, pin-point size foci altered neither the size, nor the shape of the involved lymph node. Larger foci of the size of a bean or groups of several small foci, however, caused a slight enlargement of the lymph node and made its surface appear deformed or knobby. At this stage, parts of the foci were grossly visible through the capsule of the lymph node or — those in the mesenteric lymph nodes — even through the peritoneum. The cavity of the focal lesions was filled by a yellowish or greyish-yellow, caseous or, occasionally, friable or calcified substance which was as a rule easily detachable from the thin, membrane-like capsule. Adjacent foci often became confluent and the capsule became lobular.

Microscopically, single and confluent foci were found in the lymph nodes. The necrotic, or already calcified, central part of the foci was surrounded by angiofibroblast tissue and by a fibrous capsule of varying thickness (Fig. 1). In other foci, a specific layer of tissue, composed of epitheloid cells and containing a few giant cells, was sandwiched between the necrotic or calcified core and the angiofibroblast outer layer (Fig. 2).

Less often the foci showed the specific structure of a tubercle, consisting of epitheloid cells and Langhans-type giant cells (Figs 3a, b). In some of the tubercles, a central necrotic process was already in progress. The presence of an abundant reticulum fibre network around the tubercles and, occasionally, of many reticulum fibres inside them, appears to be a characteristic feature.

Occasionally, tubercles consisting exclusively of epitheloid cells, with no giant cells among them, were found (Fig. 4) and still fewer lymph nodes had

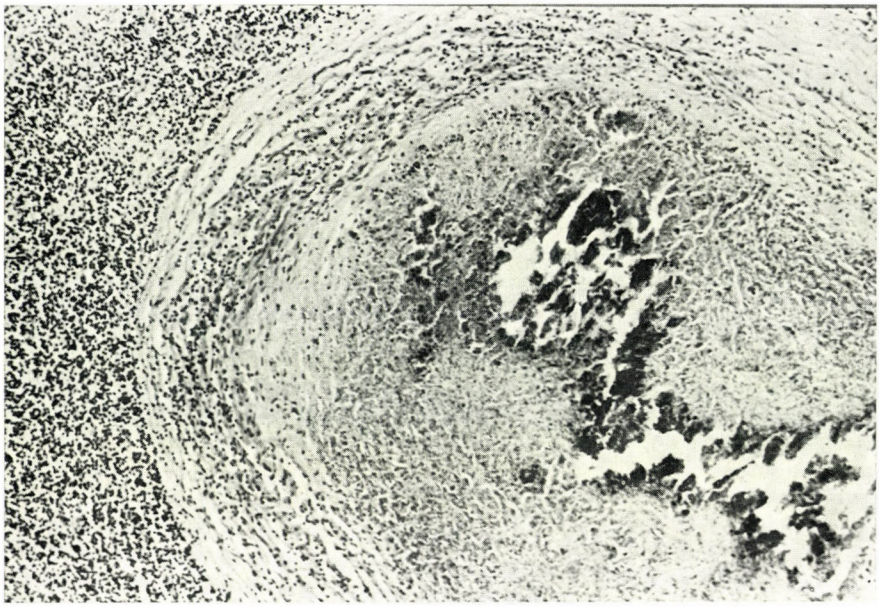

Fig. 1. Focus with a necrotic-sclerotic centre, surrounded by angiofibroblastic tissue and a connective tissue capsule. H–E staining. × 95

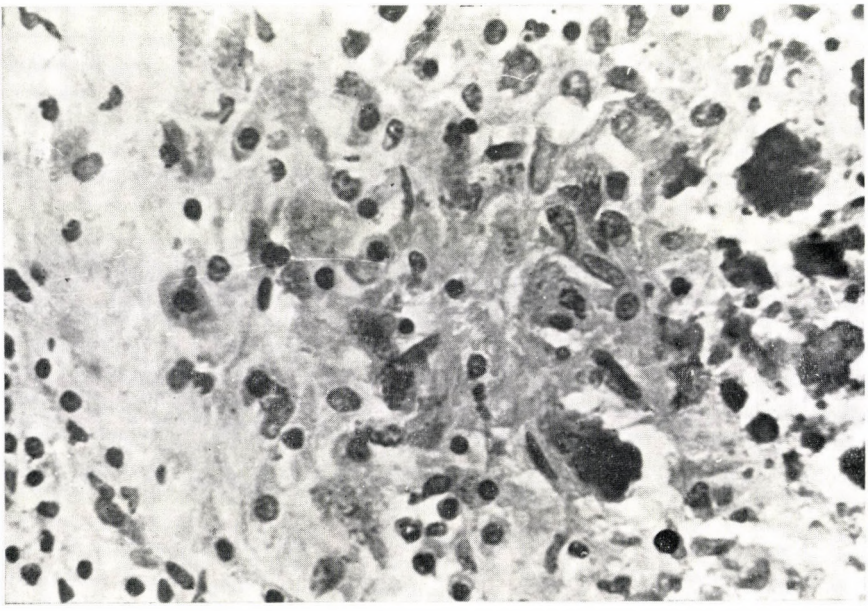

Fig. 2. Necrotic-sclerotic focal lesion. Note specific structure of surrounding tissue. Haemalaun and eosin staining. × 600

in the lymphoid tissue only giant cells, without epitheloid cells (Figs 5a, b). In the parenchyma of the latter type, argyrophilic reticulum fibres were present in abundance, particularly around the giant cells (Fig. 6).

Most of the foci, above all the first two types were found to contain acid- and alcohol-fast rod-shaped bacteria, occurring either singly or in groups. The liver and lung foci, which grossly seemed suspected of tuberculosis, showed neither the above changes, nor contained acid-fast bacteria, these were identified on microscopic examination as parasitic or fibrotic lesions.

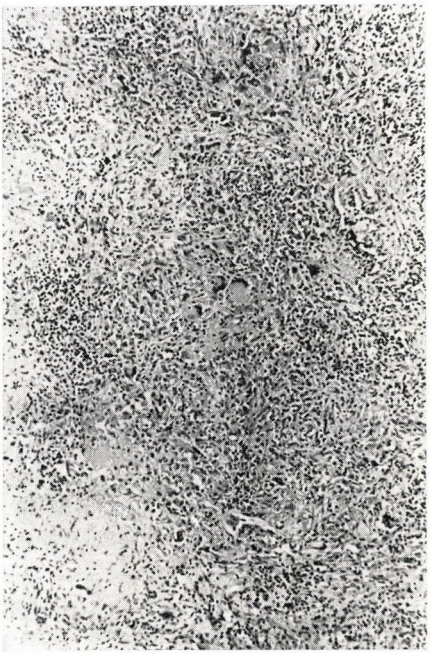

Fig. 3a. Tubercle composed of epitheloid cells and giant cells. Haemalaun and eosin staining. ×95

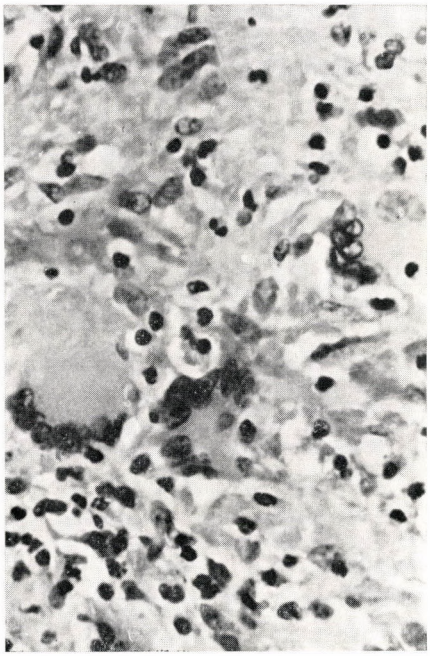

Fig. 3b. Same as in Fig. 3a at a higher magnification. Haemalaun and eosin staining. ×600

Bacteriological findings

Neither *M. bovis* nor *M. tuberculosis* could be isolated from the lymph nodes (142 submaxillary, 225 mesenteric, 49 peribronchial, 4 hepatic) obtained from 71 samplings from slaughtered pigs of 41 farms. The 115 isolates were identified as follows:

Mycobacterium avium	65
Runyon II	1
Runyon IV	49

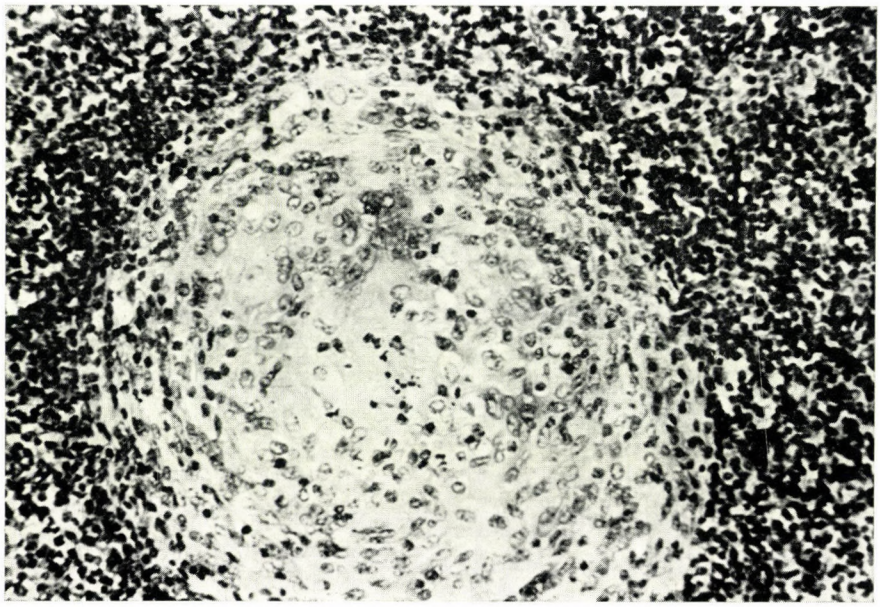

Fig. 4. Focal lesion composed exclusively of epitheloid cells. Haemalaun and eosin staining. × 240

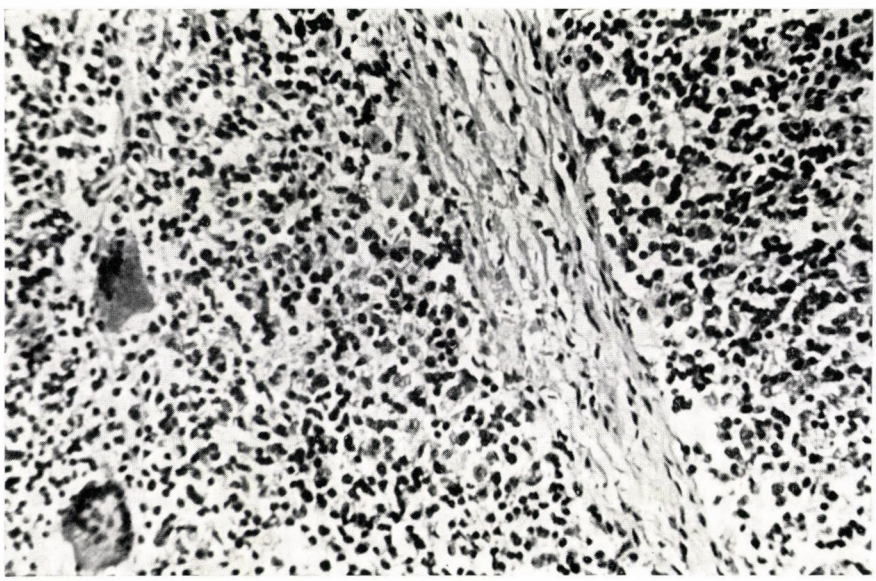

Fig. 5a. Exclusively Langhans-type giant cells are seen in the lymphoid tissue of the changed lymph node. Haemalaun and eosin staining. × 240

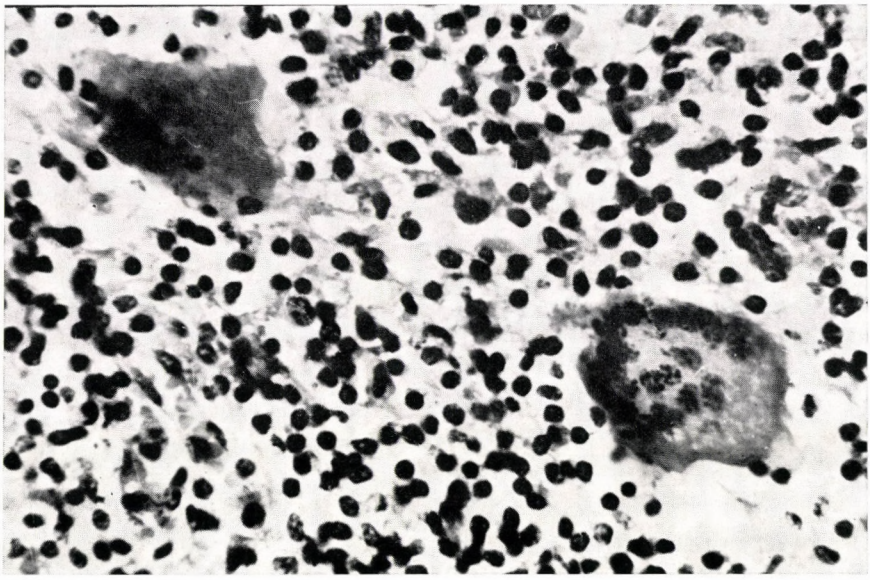

Fig. 5b. Same as in Fig. 5a at a higher magnification. Haemalaun and eosin staining. ×600

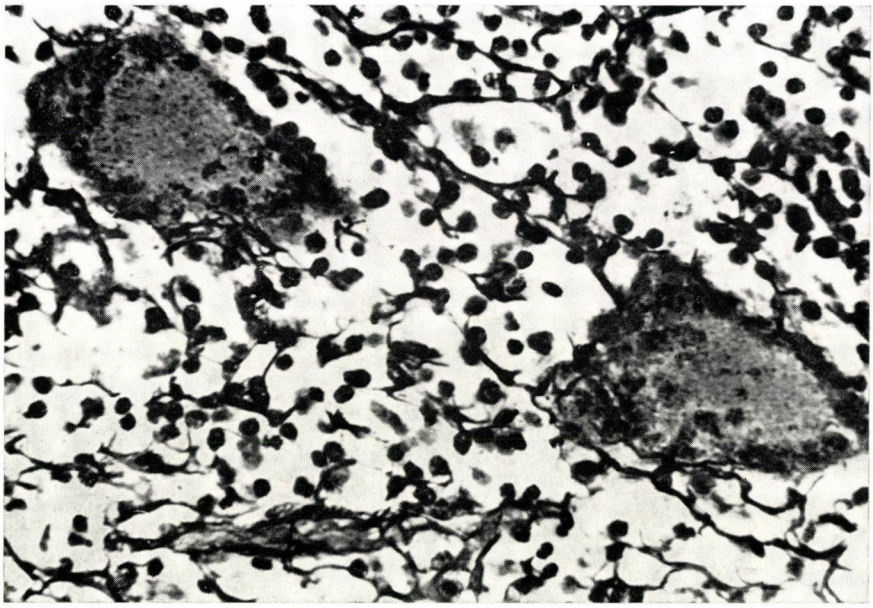

Fig. 6. The giant cells are surrounded by a network of argyrophilic fibres. Gömöri's silver impregnation technique. ×600

Up to now 30 *M. avium* isolates have been typed serologically by Shaefer's method with the following result:

Schaefer's serotype	I	5 strains
	type II	15 strains
	type III	—
	type IV	1 strain
	type V	4 strains
	type VI	—
	type VII	1 strain
	type Davis	3 strains
	type Watson	1 strain

Two, three or even four types of *Mycobacteria* were found to occur simultaneously in certain swine herds, especially in those from which a higher number of samples were examined on several occasions.

No *Mycobacteria* were demonstrable in livers and lungs.

Corynebacteria and *streptococci* were isolated from a few small abscesses found in lymph nodes of the head.

Discussion

Gross lesion identical with tuberculosis have frequently been found at meat inspection in lymph nodes of slaughtered pigs originating from large pig farms. Organisms belonging to the *Mycobacterium avium* group and various atypical *Mycobacterium* strains have been isolated from such lesion. It was found that, although several avian or atypical *Mycobacterium* strains may occur simultaneously in the same swine herd, neither *M. bovis*, nor *M. tuberculosis* have ever been isolated.

Unlike the data in the literature on swine tuberculosis, neither *M. tuberculosis*, nor *M. bovis* infections have been encountered among pigs in the present study. This might be attributed firstly, to the fact that the test materials originated from closed large pig units in which skim milk and/or dairy products are not included in the diet, secondly, to the advanced stage of the national bovine tuberculosis eradication program at the time of the examinations. The lymph nodes and organs supplied by the abattoirs had also been taken from pigs from large herds, for the slaughterhouses collaborating in the study do not slaughter commercialy collected or back-yard pigs.

The structure of the lymph node lesions was identical in most cases with that of a tubercle. Although the strains isolated from the typical tubercle-like lesions were usually identified as *M. avium*, the correlation was not regular enough and borderline cases were too numerous to draw conclusions by histo-

logical changes only on the *Mycobacterium* species involved. Consequently histological examinations of the preparations, even the use of special staining method at meat inspection are not sufficient for identifying which species of *Mycobacteria* are involved.

Epizootological data can be used conclusively rather than the histological findings on the causative role of *M. avium* or atypical *Mycobacteria* in tuberculosis of pigs. *E.g.* the origin of the pig from a large-scale unit *per se* informs on lack of contact with mammals, and on absence of milk and dairy products from the diet. Furthermore, the exclusive occurrence of lesions in lymph nodes of the head and mesentery strongly suggests the involvement of *Mycobacteria* other than *M. bovis* and *M. tuberculosis*.

Data in the literature and our own experience show that under closed systems of management, the *Mycobacterium* infections of pigs may be attributed to environmental factors. Out of the latter, soil can be excluded when the animals are kept in closed houses with solid floor, the main sources being the litter, feed and drinking water. However indirectly the soil and birds, including domestic fowl, can be taken into consideration. The main preventive measure is, therefore, the elimination of the sources of infection, with special regard to identification and elimination of the main source.

According to RAY et al. (1972), transmission of the infection by tuberculin-positive pigs with the above lymph node lesions are practically negligible, but this remains to be confirmed by further study.

Summary

Change in the epizootological conditions of swine tuberculosis under large-scale management systems is evaluated on the basis of literature and the authors' own experience. The histological and bacteriological study of specimens (142 submaxillary, 225 mesenteric, 49 peribronchial and 4 hepatic lymph nodes, 19 lungs and 12 livers) collected on 71 occasions in 41 large swine herds is described. Tuberculous lesions were not found in livers and lungs, and no *Mycobacteria* could be isolated from them. Isolates from lymph nodes included 65 *M. avium* strains and 1 and 49 strains belonging to the Runyon II and IV groups, respectively. Out of the 30 *M. avium* isolates typed serologically up to now, 5, 15, 1, 4 and 1 were identified as Schaefer's serotype I, II, IV, V and VII, respectively, 3 as type Davis and 1 as type Watson. Two, three or even four different strains were found to be present simultaneously in several herds. Under closed management systems the litter, feed and probably, the drinking water, seem to be the main sources of infection, domestic poultry, wild birds and soil being only indirect transmitter. In not fully closed swine herds, however, nearby flocks of domestic poultry as well as wild birds and soil may serve as the primary source of transmission.

References

KARLSON, A. G. and THOEN, Ch. O: Am. J. Vet. Res. **32** (1971), 1257. LESSLIE, I. W., BIRN, K. J., STUART, P., O'NEILL, P. A. F. and SMITH, J.: Vet. Rec. **83** (1968), 647. LUKE, D.: Vet. Rec. **70** (1958), 529. MIYASHITA, T., YACHIDA, S., NARA, H., SHIMIZU, K. and ONO, T.: J. Jap. Vet. Med. Ass. **25** (1972), 76. NAGY, D. E.: A Corynebaktérium előfordulása sertések nyirokcsomóiban. Thesis. Budapest, 1944. ONET, E.: Rec. Med. Vet. **147** (1971), 1349. IDEM: Ibidem **148** (1972), 959. PIENING, C., ANZ, W. and MEISSNER, G.: Dtsch. Tierärztl. Wschr., 1972, 316. RAY, J. A., MALLMAN, V. H., MALLMAN, W. L. and MORILL, C. C.: Am. J. Vet. Res. **33** (1972), 1333. RETZLAFF, N.: Arch. Lebensmittelhyg. **17** (1966), 56. REZNIKOV, M., LEGGO, J. H. and TUFFLEY, R. E.: Aust. Vet. J. **49** (1973), 264. ROBERTS, F. J. and HAMILTON, J. M.: Vet. Res. **83** (1968), 215. SCHAEFER, W. B.: Amer. Rev. Resp. Dis. **92** (1965), 85. SCHLIESSER, TH.: Arch. Lebensmittelhyg. **15** (1964), 250. TUBOLY, S.: Különféle Mycobacterium fajok antigénszerkezetének vizsgálata és összehasonlítása. Thesis. Budapest, 1969. YUGI, H., NEMOTO, H. and WATANABE, K.: Nat. Inst. Anim. Hlth. Qu. **12** (1972), 168.

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SWINE LYMPHADENITIS DUE TO *MYCOBACTERIUM AVIUM* AND ATYPICAL *MYCOBACTERIA*

II. STUDIES ON THE ROLE OF LITTERING IN MYCOBACTERIAL LYMPHADENITIS INCIDENCE IN LARGE-SCALE PIG UNITS

By

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Isolation of *Mycobacterium avium* and various atypical mycobacteria from more or less tuberculosis-like changes of lymph nodes of breeding and fattening pigs originating from large-scale units was reported earlier. The present studies were focused on the transmission of the infection through the environment, above all by the litter, because the rising incidence of lymphadenitis was observed in those units in which sawdust littering had been employed after the failure of keeping without litter.

Swine tuberculosis incidence began to increase simultaneously with the conclusion of the bovine tuberculosis eradication programmes and the aetiological role of *M. avium* was soon recognized, but then already it was suspected that the source of infection should also be sought in factors other than poultry (Schliesser, 1964).

Kleeberg and Nel (1969) found that the bulk of the mycobacteria are taken up by the pigs from the environment. Loveday (1969) observed that the infection usually occurs after weaning and the process is tending for spontaneous recovery.

On examining the role of sawdust litter and feed, Kauker and Rheinwald (1972) isolated serotype II *M. avium* from 11.8% of 59 sawdust samples, and serotype II, and in a single case serotype III, *M. avium* from 19 out of 58 various feed samples (fish meal, 6; concentrate, 7; animal protein, 3; plant protein 3). The mycobacterial contamination of sawdust litter and its role in the spread of mycobacteriosis has chiefly been studied by German (Beerwerth and Popp, 1971; Popp, 1971; Kauker and Rheinwald, 1972) and Australian authors (Tammemagi and Simmons, 1968; Resnikov, 1970; Brooks, 1971). The latter demonstrated apart from other mycobacteria, the serological type VI (Schaefer's type) in deep litter, drinking water and in pathologically altered mesenteric lymph nodes of the animals as well.

Beerwerth and Popp (1971) isolated type II, III and IV Runyon-group mycobacteria from 57.4, 52.5 and 61.8% of 108 tree bark samples, 440 sawdust samples and 285 faecal samples from pigs, respectively.

The epizootological role of freshwater (large rivers) (Viallier and Viallier, 1971) and surface waters (muddy swamps) (Kazda, 1973) in the spread of facultative pathogenic mycobacteria has also been investigated.

Materials and methods

Samples submitted for bacteriological examination were decontaminated by various methods depending on their nature.

Samples of sawdust, straw litter and soil were treated with five volumes of 3% NaOH, allowed to stand for 20 minutes and neutralized with 2% HCl.

The coarse sediment was discarded and the fluid phase was centrifuged for 10 minutes at 3000 r. p. m.

Samples of feed and fish meal were decontaminated with trisodium phosphate (Na_3PO_4 = TNP). One volume of test material was homogenized in five volumes of TNP and kept at room temperature for 16 hours. Subsequently, neutralization was made with 10% HCl, the rough sediment was discarded and the supernatant centrifuged for 10 minutes at 3000 r.p.m. The sediment was used for isolation attempt. The primary and subcultures were prepared as described previously (SZABÓ et al., 1974).

Statistical analysis of mycobacterial lymphadenitis incidence was made in farms in which

(A) sawdust litter was abundantly employed to absorb humidity,
(B) the pigs were either kept without litter or only straw was used for littering and
(C) sawdust litter was only temporarily used or sawdust was occasionally strewn over wet floor areas.

Data on the postmortem findings were kindly supplied by the abattoirs and of them only those were considered in this study which reflected the conditions of environment and management checked by ourselves directly in the herd of origin. In various infected pig farms, where sawdust litter was used, the offspring or part of the offspring of tuberculin-tested sows were themselves tested with tuberculin before weaning, *viz.*, before grouping and, if required, also later, and as far as possible these animals were followed up until slaughter, after which we examined them for gross and microscopic lesions, and bacteriologically.

The tuberculin tests were performed by intraderma administration of mammalian tuberculin on the left side and avian tuberculin on the right side, or of avian tuberculin alone, into the zone of transition between head and ear skin. The reaction was read 48 hours after administration. Swelling, erythema, necrosis and oedema were diagnosed by visual and manual examination and thickening of the skin fold or the diameter of the cutaneous reaction were measured in most cases with a slide gauge.

Results

The frequency of lymph node mycobacteriosis incidence in different littering conditions can be seen in Table I.

In the course of the reading of tuberculin tests it was found that the evaluation by slide gauge thickening is not without difficulty because, owing to the great variations in skin thickness even in nearby areas, the basic data cannot be always precisely taken. The diameter of the cutaneous reaction

Table I

Incidence of lymph-node mycobacteriosis in pig under different conditions of littering

Litter	No. of units	No. of slaughtered pigs	Lymph- node mycobacteriosis		
			No. of pigs involved	Mean incidence %	Extreme values %
A	17	14, 419	4054	28.1	(6.7—53.2)
B	21	36, 550	808	2.2	(0—7.3)
C	10	31, 476	629	2.0	(1.0—6.0)

can be more precisely measured, but a basis for correlation is lacking. Accordingly, these measurements were omitted after the evaluation of several hundred reactions and reading was further on based on visual and manual examination. When avian and mammalian tuberculin were applied simultaneously, positive reactions to mammalian tuberculin were less and less pronounced.

In one of the farms studied (Farm N), two herds of the same origin and identical in number, were kept in two independen t units (K and T) (Table II)

Table II

Results of tuberculin test in two pig herds kept on different litter

Unit	Litter	Positive reactions to tuberculin/animals in the group	
		Sows	Piglets
K	sawdust in farrowing house	28/40 = 70.0%	58/110 = 48.7%
T*	straw	15/52 = 28.8%	0/105 = 0.0%

* The sows in unit T were of the same origin as those in unit K and the greater part of them had been transported from K to T

In unit K the animals were kept under a closed system of management and abundant sawdust litter was used with regard to dysentery among the piglets and to the prevention of foot injuries caused by hard floor. In unit T, the management system was still traditional, including yards attached to the houses, the hygienic conditions were acceptable and straw was used as litter. In neither unit were kept poultry by the attendants serving the area. Mycobacterium infection was significantly lower in the latter unit.

In farm R (Table III), two large groups of swine were formed in a closed unit for littering experiments with straw and sawdust. No poultry were kept in the unit. Among group I pigs which had been kept on straw litter in the farrowing house and without litter during the fattening period, *Mycobacterium* infection was less than 10% throughout, whereas among group II swine,

Table III

Results of tuberculin test of sows and progeny under conditions of keeping on straw and sawdust littering

R

Group	Bedding	Response of sows to tuberculin	Response of piglets to tuberculin at the age of	
			75-90 days	6-8 months
I/1	straw	27/29 = 93.0%	4/236 = 1.7%	6/109 = 5.5%
2	straw	N.t.	0/38 = 0	—
3	straw	N.t.	0/251 = 0	4/47 = 8.5%
4	straw	N.t.	0/251 = 0	—
II/1	sawdust	26/30 = 86.7%	28/232 = 12.1%	10/53 = 19.0%
2	sawdust	N.t.	13/54 = 24.1%	—
3	sawdust	N.t.	N.t.	107/330 = 32.4%
4	sawdust	N.t.	N.t.	32/135 = 23.7%

N.t., not tested. See footnote Table II

which had been kept on sawdust litter, it figured 20—30% by the age of 6—8 months.

In three farrowing houses of farm K (Table IV), piglets were kept (a) on sawdust, (b) on straw and (c) without litter. Fatteners were kept without litter throughout. The floor of the pig houses was heavily worn, the hygienic conditions were poor and consequently the floor was covered with urine and in some places also with dung, especially under the heavier animals. The manage-system was semi-closed and no poultry were kept on the farm.

Table IV

Response to tuberculin of sows and progeny kept under different conditions of littering

K

Farrowing house No.	Litter	Sows positive/No.	Piglets positive/No. at the age of	
			60—70 days	6—8 months
8	sawdust	24/33	96/269 = 36.4%	191/228 = 84.2%
6	straw	28/32	7/304 = 2.3%	41/171 = 23.9%
1	without bedding	22/30	19/245 = 7.7%	111/183 = 56.9%

In farm St (Table V), the swine herd was under a closed system of management, straw litter was always used in the farrowing houses and in the fattening house abundant sawdust litter was spread on a single occasion

for each newly formed group. Sawdust was regularly strown over the dividing corridor between the two rows of farrowing pens. It is clear from the data shown in Table V that the repeated use of sawdust promoted the spread of the infection.

Table V

Mycobacteriosis followed up from weaning into the fattening period under different conditions of littering

Farrowing house No.	Litter	Weanlings (72—80 days)	Fattener house No.	Litter	Fatteners (160—200 days) incidence in %
I	straw	0/297	VIII	No litter	23.1
VI	sawdust + straw	7/311	VIII	sawdust for one week	47.9
IX	straw	3/298	III	sawdust for one week	25.6
X	straw	3/295	VI	No litter	23.6

The results of isolation experiments from samples of the litter, feed and soil were the following:

Sawdust samples (26)	6 strains	2 *M. avium*, 4 Runyon-IV
Straw samples (3)	1 strain	*M. avium*
Fish meal samples (3)	1 strain	Runyon-IV
Fodder samples (23)	2 strains	Runyon-IV
Soil samples (4)	1 strain	Runyon-IV

Discussion

The experiments conducted in infected large pig herds located in different geographical areas of Hungary have unequivocally shown that littering, especially sawdust litter, plays an important role in the incidence of mycobacterial lymphadenitis of pigs (Tables II—V). Incidence of mycobacteriosis was much higher than elsewhere in units in which sawdust litter was used. In certain farms, omission of sawdust littering was followed by a singificant decrease in mycobacteriosis among the progeny of the infected sows, whereas in others the reduction of its incidence did not exceed about one half of the original level. In the latter herds a source other than sawdust may also have played a role. We agree with BEERWERTH and POPP (1971) that the origin of the sawdust (forestry byproduct, containing also the soil-contaminated bark or industrial byproduction) as well as its storage and the frequency of manure removal are further decisive factors. SCHLIESSER and WEBER (1973) found

that in experimentally infected sawdust, two *M. avium* strains virulent to poultry (serological type II) survived for 153—160 days at 18—22 °C whereas two other strains, avirulent to poultry (Davis-serological type strains of the intermediary group), survived for 169—214 days under identical conditions. The respective survival times were 35—42 and 49—63 days in the incubator at 37 °C. No multiplication of *Mycobacteria* in the sawdust was observed under the given conditions of experiment; the higher (37 °C) temperature even seemed to promote the gradual decrease of *Mycobacterium* counts. STOLL (1973) however holds the view that certain atypical *Mycobacteria* do not only occur ubiquitously outside animal reservoirs but may even multiply there if the conditions are favourable. In view of this it cannot be excluded that in wet sawdust or in sawdust litter long retained in the pig house, atypical *Mycobacteria* may multiply at the same rate as in surface water (KAZDA, 1974) or in freshwater (VIALLIER and VIALLIER, 1971). Ingestion of sawdust by the pigs has often been observed. This weighs in favour of the view (STOLL, 1973) that mechanical injury of the mucous membranes by sawdust favours the establishment of *Mycobacteria* in them, to the analogy of silico-tuberculosis in man. Also, while with straw littering the manure is removed at least once daily, there is no such technological requirement with sawdust littering.

The atypical *Mycobacterium* strains isolated from samples of sawdust straw, *etc.*, were similar to those demonstrated in the affected lymph nodes of pigs. However, in those units in which attendants living in the farm keep their own poultry flocks, and *M. avium* infection occurs among the birds, the main source of swine mycobacteriosis should of course be sought in this. Wild birds may also spread the infection either directly or indirectly, by contaminating the fodder or the sawdust or straw used as litter.

In swine groups kept without litter, mycobacteriosis incidence was higher than among those kept on straw litter, from which it is concluded that the moisture of the litter, the frequency of manure removal and general hygiene also play a role in the frequency of infection.

Naturally, transmission by contact within the herd should also be taken into consideration. The question arises whether the animals shed the micro-organisms only as long as they may take them from the environment or food. We have performed several experiments to obtain more information, but no unequivocal evidence was emerging to the role of sows in transmitting the infection. Experiments based on a different approach are now in progress and it also remains to be clarified whether *M. avium* and the other atypical *Mycobacteria* play an equally important role in swine tuberculosis.

Summary

The role of litter as an environmental factor in the incidence of mycobacterial swine lymphadenitis was studied chiefly under field conditions. Atypical *Mycobacteria* were demonstrated in samples of litter and in different food rations as well. Experiments conducted in several pig units clearly showed that under large-scale management systems, litter plays an important role in the spread and incidence of lymph-node mycobacteriosis in swine. The highest incidence was found in those units in which sawdust was used as litter, it was lower among pigs kept without litter, but still higher compared to those kept on straw. It appears that the origin and storage conditions of sawdust as well as the frequency of manure removal also have a significant influence on the incidence of swine mycobacteriosis. Since, however, the infection — although at a lower rate — also occurred in those units in which no sawdust litter had been used, other sources of infection should also be taken into consideration. One main source is the soil which may contaminate the food and drinking water. *M. avium* infection may be transmitted by infected poultry kept nearby the pig unit. Also other domestic birds or wild birds may spread the infection indirectly, by contaminating the litter or the fodder ration of the pigs with their droppings. Such indirect contamination can, of course, take place not only in the pig unit itself, but also in the food-mixing plant or food store. It appears that spread of *M. avium* or atypical *Mycobacterium* infection by pig-to-pig contact plays little role in herd infections, but its importance cannot be disregarded.

References

Beerwerth, W. and Popp, K.: Zbl. Vet. Med. Reihe B **18** (1971), 634. Brooks, O. H.: Aust. Vet. J. **47** (1971), 424. Kauker, E. and Rheinwald, W.: Berl. Münch. Tierärztl. Wschr., 1972, 384. Kazda, J.: Zbl. Bakt. Hyg. I. Abt. Orig. B **158** (1973), 151. Kleeberg, H. H. and Nel, E. E.: J. South. Afr. vet. med. Ass. **40** (1969), 233. Loveday, R. K.: J. South. Afr. vet. med. Ass. **40** (1969), 253. Popp, K. D. I Inaug. Diss., 1971. Resnikov, M.: Aust. Vet. J. **46** (1970), 239. Schliesser, Th.: Arch. Lebensmittelhyg. **15** (1964), 250. Schliesser, Th. and Weber, A.: Zbl. Vet. Med. Reihe B **20** (1973), 710. Stoll, L.: Dtsch. Tierärztl. Wschr., 1973, 548. Szabó, I., Széky, A. and Tuboly, S.: Magyar Állatorvosok Lapja (MÁL), 1974, 511. Tammemagi, L. and Simmons, G. C.: Aust. Vet. J. **44** (1968), 21. Viallier, J. and Viallier, G.: Rev. Inst. Pasteur **4** (1971), 27.

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SERO-GROUPING OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM CASES OF WHITE SCOURS IN EGYPT

By

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The economic losses due to neonatal diseases in young calves were recognized many years ago. Diarrhoea during the first two weeks of life is considered the commonest symptom of these diseases. By nature of its habitats, *Escherichia coli* can be isolated from the faeces in different diseased conditions and routine cultural methods tend to select for it. For this reason, it is difficult to assess the significance of an isolation of *E. coli* from a specimen, especially with diseases manifested primarily by diarrhoea. Yet, the major aspect of this organism in the pathogenesis of diarrhoea of newborn calves has been clearly delineated.

Escherichia coli has been incriminated by many authors to be most important bacterial agent causing scours of calves. Different O groups have been isolated from diseased or dead calves. WRAMBY (1948) found O groups 15, 8, 9, 30W, 78, 45 and 27W in calves.

BOKHARI and ØRSKOV (1952) reported that the O groups most frequently encoutered were 78, 15, 27W, 30W, 8, 9, 45 and 26. Since then the 27W and 30W strains have been accepted as international type strains O115 and O117, respectively (ØRSKOV, 1952). FEY (1957) reported that O groups 78, 115, 86, 15 and 117 were dominant in coli septicaemia in calves, ÁLDÁSY (1959) found the O groups 41, 8, 9 and 101 most frequently, whereas according to DAM (1960) the O groups 78, 115 and 15 were responsible for 57% of colisepticaemic cases. GOSSLING et al. (1964) found 9, 101, 15 and 17 as the commonest groups with the absence of O78. GLANTZ et al. (1968) reported that three O groups OX 28, and 117 were associated with calf mortality in a tested herd. Recently, the most commonly occurring O groups have been 78, 8 and 20 (MANZ, 1971) and 101, 20, 41, 8 and 7 (VARGA and FARID, 1974). In Egypt, the first trial was done by ABD EL GHAFFAR et al. (1972) and eleven strains (resemble 11 dead calves) could by typed as O groups 8, 26 (two from each), 10, 20, 45, 73, 78, 114 and 135 (one from each), but no futher investigations have been done so far.

The present work deals with other strains isolated from dead young calves with enteritis.

Materials and methods

One hundred strains were sent from the Veterinary and Research Laboratories at Cairo to be identified. These strains were isolated from 100 dead calves with enteritis at different farms in Egypt.

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Biochemical studies and serogrouping were done according to KAUFFMANN (1966). The nitrate reduction, lactose decomposition, citrate utilization, indole and urease production, hydrogen sulphide production and gelatine liquefaction as well as the Voges-Proskauer and methyl red tests were performed.

For serological identification, strains were cultured on $D_{1.5}$ media suggested by SCHLECHT and WESTPHAL (1966) and used in living, boiled and autoclaved forms. International O type strains, namely, 2—5, 7—11, 13, 15, 17, 20, 21, 23, 25, 28, 41, 44, 56, 69, 75, 78, 86, 93, 101, 111—113, 115, 117, 119, 124—126, 128, 136—139 and 141—157 and all international K type strains (1-94) except K65, K90 and K92 were used to produce O and/or OK serum for serological typing. Antigens were tested with pooled antisera on slide. Each pool contained about five different antisera. The strains giving positive reaction with the pools were then tested with the sera within the pool separately. The slide agglutination was confirmed in every case by the tube agglutination test and strains were only approved to be identified if they gave the same titre as the international standard type strain with the homologous serum; otherwise the agglutination was considered a cross- reaction.

Results

Out of the 100 coliform strains, 91 could be identified as follows: 85 *Escherichia coli*, 3 *Klebsiella*, 2 *Citobacter* and one *Proteus*.

The results of the biochemical tests are shown in Table I. The remaining 9 strains were discarded as they differed in some of the biochemical reaction and could not be attributed to any genus.

Table I

The biochemical behaviour of 91 isolated strains

	No. of strains	nitrate	lactose	indole	M.R.	V.P.	urea	citrate	H_2S	gelatine
Escherichia	85	+	+	+	+	—	—	— (8+)	—	— (3+
Citrobacter	2	+	+	—	+	—	—	+	+	—
Klebsiella	3	+	+	—	—	+	+	+	—	—
Proteus	1	+	—	—	+	—	+	+	+	+

Using 57 O and/or OK sera thirty strains could be typed within 16 O groups. Table II shows the OK grouping of the strains. It is evident that 7 strains were K minus and 23 contained capsular antigen either a thermostable A (in 4 strains of O41) or a thermolabile B and/or L antigen (in 19 strains).

Moreover, 14 strains gave a slide and tube agglutination reaction with 6 different O sera. These results were omitted and considered "cross- reaction" as none of the strains were agglutinated up to the end titre of the corresponding serum.

Table II

shows the O and K typing of the identified *E. coli* strains

O group	No. of strains	K antigen	No. of strains	No. of strains with unidentifiable K antigen
08	4	K—	2	2
041	4			4K(A)
0145	4	K—	2	2
0128	3	K67(B)	3	
015	2	K14(L)	2	
020	2			2
0115	2			2
02	1	K—	1	
017	1	K16(L)	1	
023	1			1
028	1	K—	1	
0125	1	K70(B)	1	
0142	1	K—	1	
0147	1	K89(B)	1	
0152	1			1
0154	1	K94(B)	1	
Total:	30	K minus	7	
		With K antigen	23	

Discussion

E.coli diarrhoea of calves during the first two weeks of life may be associated with a number of factors (BARNUM et al., 1967; MEBUS et al., 1969). The role of *E.coli* in neonatal diseases of calves has been reviewed by several authors (GAY, 1965; SOJKA, 1965; BARNUM et al., 1967).

Classification of coliform isolates by biochemical reactions has always been useful. The majority (91) of our 100 strains could be classified into four genera *viz.*, *Escherichia*, *Citrobacter*, *Klebsiella* and *Proteus*.

It has been reported that certain serogroups are frequently associated with calf diseases (WRAMBY, 1948; FEY, 1957; DAM, 1960), while recent

reports have shown that there are geographical differences as to the important groups, in some countries being one of the serogroups dominant in others not (GOSSLING et al., 1964; VARGA and FARID, 1974). In the present study groups O8 and O20 seem to play an important part in calf diseases in Egypt as shown by their repeated isolation during the first trial (ABD EL GHAFFAR et al., 1972), while all other groups have been isolated for the first time. Strains belonging to O8, O41, O145 and O128 contribute 50% of the identified strains. The serogroup O41 was recorded to be associated to calves (FEY, 1957; ÁLDÁSY, 1959; VARGA and FARID, 1974). In addition, strains belonging to O15 and O115 showed some importance as they amount to 13.3% of the identified strains and their importance has been reported by several authors (WRAMBY, 1948; ØRSKOV, 1951; DAM, 1960; SÖDERLIND, 1965).

Two different serogroups O128 : K67 (B12) and O125 : K70 (B15) could be found in 3 and one case, respectively. This finding is in agreement with other reports (ØRSKOV, 1951; GUINÉE, 1963) which recorded that certain groups which are known to be related to human disease could be isolated from cases of white scours.

The group O78 could not be found in these strains, though it was recorded before (ABD EL GHAFFAR et al., 1972). It is also worth to denote that O9, O101 and O117 which are known to be important in Europe could not be isolated up to now in Egypt while O147 : K89(B), which is always related to pigs, was encountered in one case.

The importance of the capsular antigen in masking O agglutination is well-known (SOJKA, 1965; KAUFFMANN, 1966; GLANTZ, 1968) while its suspected importance in pathogenesis has not been proved yet (SMITH and LINGGOOD, 1972). Here 76.7% of our identified strains contained K antigens. The A form could be found only associated with O41 while other groups contained B or L forms. During our investigations strains of O8 and O145 signed as K minus seems that they may have lost their capsular antigens due to repeated subculturing.

It is worth to mention that in the present work serogroups never isolated in Egypt before have been isolated from calf scours and some light has been thrown on the presence of K antigens in these strains. Further efforts are needed for clarifying the important serotypes before introducing new prophylactic measures.

*

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Summary

One hundred coliform strains isolated from dead calves with enteritis in Egypt, were sent to be identified. Biochemical studies showed that 85 strains were *E. coli*. Serologically, using 57 O and/or OK sera thirty strains could be grouped. The most important O groups were 8, 41, 145, 128, 15, 20 and 115.

As the O groups 8 and 20 had already been isolated in Egypt, they were considered to be important, 77% of the O grouped strains contained K antigens, 4 of which as A form while the rest as B or L.

References

Abd El Ghaffar, M. M., Lotfi, Z. S., Abd El Malek, A. S. and Farid, A. F.: Proceeding of the 10th Arab Annual Veterinary Congress, Cairo, 1972. Áldásy, P.: Magy. Áo. Lapja (MÁL), 1959, 145. Barnum, D. A., Glantz, P. J. and Moon, K. W.: Colibacillosis. Ciba Veterinary Monograph Series, 1967 1. Bokhari, S. M. H. and Ørskov, F.: Acta Pathol. Microbiol. Scand. **30** (1952), 87. Dam, A.: Nord. Vet. Med. **12** (1960), 321. Fey, H.: Zbl. Vet. Med. **4** (1957), 309. Gay, C. C.: Bact. Rev. **29** (1965), 75. Glantz, P. J.: Appl. Microbiol. **16** (1968), 435. Glantz, P. J., Rothenbacker, H. and Hokanson, J. F.: Am. J. Vet. Res. **29** (1968), 1561. Gossling, J., McKay, K. A. and Barnum, D. A.: Can. Vet. J. **5** (1964), 151. Guinée, P. A. M.: Zbl. Bakt. Orig. **188** (1963), 201. Kauffmann, F.: The bacteriology of Enterobacteriaceae. Copenhagen, 1966. Manz, J.: Thesis. München, 1971. Mebus, C. A., Underdahl, N. R., Rhodes, M. B. and Twiehaus, M. J.: Research Bulletin, University of Nebraska **233** (1969). Ørskov, F.: Acta Path. **29** (1951), 373. Idem: Acta Path. Microbiol. Scand. **31** (1952), 51. Schlecht, S. and Westphal, O.: Zbl. Bakt. Orig. **200** (1966), 241. Smith, H. W. and Linggood, M. A.: J. Med. Microbiol. **5** (1972), 243. Sojka, W. J.: *E. coli* in animals. C. A. B. England, 1965. Söderlind, O.: Zbl. Vet. Med. Reihe B **12** (1965), 13. Varga, J. and Farid, A. F.: Acta vet. hung. **XXIV** (1974), 421. Wramby, G.: Acta Path. Microbiol. Scand. Suppl. **76** (1948).

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EXPERIMENTAL INFECTION OF LAMBS WITH AN ADENOVIRUS ISOLATED FROM SHEEP AND RELATED TO BOVINE ADENOVIRUS TYPE 2.

I. VIROLOGICAL STUDIES

By

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Natural respiratory diseases of mammalian domestic animals, caused by adenoviruses, have been reported only in calves (ÁLDÁSY et al., 1964), in horses (JOHNSTON and HUTCHINS, 1967) and in dogs (DITCHFIELD et al., 1962) so far.

Adenovirus isolations from sheep have been done only in Northern Ireland (MCFERRAN et al., 1969, 1971). The aetiological role of these strains is unknown.

In late October, 1973, an adenovirus, designated Het/3 was isolated from the nasal discharges of 6—8 weeks old merino lambs showing mild respiratory symptoms (BELÁK and PÁLFI, 1974). The flock of lambs had no connection with cattle. The Het/3 virus could readily be distinguished from any of the prototype strains of ovine adenoviruses, but proved to be closely related to, if not identical with type 2 bovine adenovirus. The isolate appeared to play an aetiological role in the disease, so it seemed to be justified to study its pathogenicity in lambs infected experimentally.

Materials and methods

Experimental animals. Thirteen colostrum-deprived merino lambs, aged one week, were used. The lambs had proved free of neutralizing antibodies against the Het/3 virus strain.

Virus. The Het/3 virus was propagated in secondary fetal lamb kidney cultures as described elsewhere (BELÁK and PÁLFI, 1974). As inoculum the third passage of the virus was used.

Experimental infection. Seven lambs were given 1 ml suspension of virus (10^3 $TCID_{50}$) intratracheally and 1.5 ml intranasally. These inoculations were repeated on the subsequent day. Two further lambs were kept together with the infected group from the second day after inoculation to serve as contact control. Four control lambs were inoculated with the supernatant of uninfected tissue cultures in the same way as the first group. Nasal and rectal swabs were collected daily and temperatures were taken twice a day. Three animals of the infected group died, the other lambs were killed between 4—13th days post infection (p.i.). One control animal was killed on each of 2, 4, 7 and 13 days p.i.

Eight infected lambs and the controls were involved into detailed pathological and histopathological studies. The lungs, the peribronchial and intestinal

lymph nodes, the intestinal tracts and the spleens were examined bacteriologically. Reisolation of the virus from the respiratory and intestinal tracts as well as from the nasal and rectal swabs was attempted in secondary fetal lamb kidney cultures.

The preinoculation sera and the sera collected from exsanguinated lambs were tested for virus-neutralizing antibodies, using lamb kidney cell cultures, and 100 $TCID_{50}$/tube.

Results

Clinical signs. One lamb (No. 1639) which died on the first day following infection was not involved into the detailed studies. A further lamb (No. 1640) showing no clinical signs died on the second day. From the second day all animals except one showed raised temperature, which reached the maximum generally 3—4 days p.i. (a rise of 0.7—1.5 °C). Detailed results are presented in Table I.

Table I

The result of experimental infection with Het/3 virus

	Lamb No.	Maximum temperature		Respiratory symptoms	Diarrhoea	Killed on day	Died p.i.	Virus reisolation from		
		day	C_s					organs	nose on days	faeces p.i.
I.n. and i.t. infect.**	1636	3rd	39.3	+	+	7		resp.tract	3—6	3—4
	1590	3rd	40.0	+	+	13		—	3—8	3—4
	1638	—	—	+	+		9	—	3—7	3—4
	1640	—	—	—	—		2	resp.tract	—	—
	1593	3rd	40.2	+	+	4		resp.tract	—	—
	1639	—	—	—	—		1	NT	NT	NT
	1591	3rd	39.9	+	+	7		—	—	—
Contact	1637	3rd*	39.8	+	+	7 (4*)		resp.tract	—	—
	1589	5th*	39.5	+	+	13 (10*)		—	5—7	5—6

NT, not tested; * After supposed uptake of virus; ** Intranasal and intratracheal infection

Serous nasal discharge, sneezing and dyspnoea were observed from the second day p.i. on five lambs infected intratracheally and intranasally. Respiratory signs were accompanied by diarrhoea from day 4 p.i.

Clinical signs showing the dysfunction of the intestinal tract were seen for 3—4 days. The respiratory signs were still present in the final phase of the experiment.

In the lambs exposed to contact infection (Nos 1589 and 1637) similar respiratory and enteric symptoms were observed from days 3—5 p.i. on.

The lambs inoculated with uninfected supernatant showed no clinical signs.

Pathological and histopathological studies. The result of these observations will be presented in detail in the second article of this series (TURY et al., in press).

Bacteriological findings. No bacteria could be cultivated from the lungs, peribronchial and intestinal lymph nodes and spleens. From the small intestines non-haemolyzing *E. coli* bacteria and enterococci were cultivated.

Virological studies. Shedding of virus with the nasal discharge was demonstrated in three lambs (Nos 1590, 1636 and 1589), one of which had been exposed to contact infection. On days 3—7 p.i. the virus was also reisolated from lamb No. 1638, which showed respiratory and intestinal symptoms, but no elevated temperature.

The virus was reisolated from the rectal swabs of four animals (Nos 1636, 1590, 1638 and 1589). The presence of the virus in the faeces was demonstrated between days 3 and 6 p.i.

Virus was detected in the homogenized lower respiratory tracts of four lambs (Nos 1636, 1640, 1593 and 1637), but it could not be isolated from the homogenized intestines and intestinal lymph nodes of the same animals.

The reisolates proved to be identical with the Het/3 virus in the virus-neutralization test.

Serological observations. Virus-neutralizing antibodies (a titre of 1 : 8) could be first time demonstrated in the blood of the lamb killed on day 7 p.i. In the sera collected later (from animals Nos 1638, 1589, 1590) antibody titres up to 1 : 32—1 : 128 were found. The same titres were observed against 100 $TCID_{50}$ of either Het/3 virus or bovine adenovirus type 2.

Discussion

No data are available on natural diseases or successful experimental infections of sheep as far as adenoviruses are concerned. The pathogenicity of ovine adenovirus isolates to other species is also unknown. The adenoviruses isolated from different species are regarded to be highly host-specific. The most detailed studies have been made with human strains of the adenovirus family. Except for the oncogenicity of some strains — generally animals could not be infected experimentally with human isolates (ANDREWES and PEREIRA, 1972). Among the limited number of successful experimental infections can be mentioned the work of BETTS et al. (1962), who were able to produce pneumonia with a human strain in colostrum-deprived piglets. Latent infection of rabbits (PEREIRA

and KELLY, 1957) and inapparent infection of dogs (SINHA et al., 1960) were also reported. GENOV et al. (1971) infected lambs with the bovine adenovirus strain Sofia 4/67 and observed an antibody response, but no pathological alterations.

The natural transmission of an adenovirus between different species has been very rarely found (CABASSO and WILNER, 1969; KAPP and LEHOCZKI, 1966).

The Het/3 virus, which was isolated from a naturally diseased sheep, was unrelated to the sheep adenovirus types known so far but closely related to type 2 of bovine adenoviruses. Type 2 bovine adenovirus was originally isolated from the faeces of a clinically normal calf (KLEIN et al., 1960). DARBYSHIRE (1968) observed serological response against this type after respiratory disease of cattle. Mild respiratory symptoms and diarrhoea were observed in calves infected experimentally with type 2 bovine adenovirus (DARBYSHIRE et al., 1969).

In the experiment presented here a serologically closely related virus was capable of causing moderate respiratory and intestinal symptoms in lambs. From the second day after infection the lambs showed similar respiratory disease as in the field. These symptoms were later accompanied by rise of temperature and diarrhoea. The virus spread to susceptible lambs by contact, causing similar symptoms in the latter.

From the third day p.i. on, the lambs shed the virus in their nasal secretions and faeces. It is worth to note, that in the nasal discharge the virus was present for a longer time than in the faeces, and the respiratory symptoms prevailed longer, too. The virus could be reisolated from the tissues of the lower respiratory tracts of the animals died or killed during the first week of the experiment. These findings in correlation with the clinical observations in the field suggest, that the Het/3 virus causes more severe processes in the respiratory tract than in the intestines.

Further experiments are in progress to study the relationship or identity of Het/3 virus to bovine adenovirus type 2 and to investigate the pathogenicity of the former in calves.

Summary

Colostrum-deprived lambs were experimentally infected with adenovirus strain Het/3. The strain, isolated from sheep with respiratory symptoms, is related to type 2 bovine adenovirus. The virus was found to be pathogenic for lambs. Respiratory and intestinal symptoms were observed with a mild rise of temperature. Susceptible lambs showed similar symptoms after contact infection. Three out of nine infected animals died on post- infection days 1, 2 and 9, the others were killed between days 4 and 13. The lambs shed the virus both in their nasal discharges and faeces. Virus-neutralizing antibodies up to a serum dilution of 1 : 8 — 1 : 128 appeared in the sera of the infected animals. On the basis of detailed observations evidence is presented that the Het/3 virus can cause a respiratory and intestinal disease in lambs.

References

ÁLDÁSY, P., CSONTOS, L. and BARTHA, A.: Magyar Állatorvosok Lapja (MÁL), 1964, 457. ANDREWES, C. and PEREIRA, H. G.: Viruses of Vertebrates. London, 1972. BELÁK, S. and PÁLFI, V.: Arch. ges. Virusforsch. **44** (1974), 177. IIDEM: Ibidem **46** (1974), 366. BETTS, A. O., JENNINGS, A. R., LAMONT, P. H. and PAGE, Z.: Nature (Lond.) **193** (1962), 45. CABASSO, V. J. and WILNER, B. I.: Adv. Vet. Sci. Comp. Med. **13** (1969), 158. DARBYSHIRE, J. H.: JAVMA **152** (1968), 786. DARBYSHIRE, J. H., KINCH, D. A. and JENNINGS, A. R.: Res. Vet. Sci. **10** (1969), 39. DITCHFIELD, J., MCPHERSON, L. W. and ZBITNEW, A.: Canad. Vet. J. **3** (1962), 238. GENOV, I., ENCHEV, S. and MERMERSKI, K.: Vet. Med. Nauki **9** (1972), 29. JOHNSTON, I. G. and HUTCHINS, D. R.: Austr. Vet. J. **43** (1967), 600. KAPP, P. and LEHOCZKI, Z.: Acta vet. hung. **XVI** (1966), 429. KLEIN, M., ZELLAT, J. and MICHAELSON, T. C.: Proc. Soc. exp. Biol. Med. (N. Y.) **105** (1960), 340. MCFERRAN, J. B., NELSON, R., MCCRACKEN, J. M. and ROSS, J. G.: Nature (Lond.) **221** (1969), 194. MCFERRAN, J. B., NELSON, R. and KNOX, E. R.: Arch. ges. Virusforsch. **35** (1971), 232. PEREIRA, H. G. and KELLY, B.: Nature (Lond.) **180** (1957), 615. SINHA, S. K., FLEMING, L. W. and SCHOLES, S.: JAVMA **136** (1960), 481. TURY, E., BELÁK, S. and PÁLFI, V.: Acta vet. hung. (in press)

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SOME FEATURES OF ANTIGEN DOSE-ANTIBODY RESPONSE RELATIONSHIP DURING THE PRIMARY ANTI-BSA RESPONSE IN THE CHICKEN

By

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A mathematical equation describing the quantitative relationship between antigen dose and antibody response was formulated early in this century (SMITH and St. JOHN-BROOKS, 1912). However, the equation is not universally applicable to any experimental system, owing to the great dissimilarity of the methods used for antibody detection on the one hand and the relatively narrow range of doses used in the experiments on the other (STEVENS, 1956). *In vivo* studies concerning the relationship between antigen dose, number of antibody-producing cells and quantity of antibodies are also scarce (LEVI et al., 1973, 1973a).

In this laboratory, the effect of optimal and large doses of antigen on the primary response of chickens was investigated (NAGY and FEHÉR, 1972, 1972a). It was shown that the cytological changes taking place in the spleen are dose-dependent, thus closely correlating with the quantity of antibodies produced. In the present experiments, the use of a wider dose range enabled us to clarify whether the dose response data obtained fit to the SMITH and St. JOHN-BROOKS's formula. The influence of different antigen doses on the quantities of antibodies belonging to the two main immunoglobulin classes (IgG and IgM) and the kinetics of their production were also studied.

Material and methods

Chickens. A total of 118 eight-week-old brown Leghorn SPF chickens, weighing 780 $\pm$ 115 g, were used.

Antigen. A 10% sterile solution of bovine serum albumin (BSA), fraction V, was used throughout.

Schedule of experiment. The chickens were divided into four groups. Those in experimental groups 1, 2 and 3 were given intraperitoneally 10, 50 and 400 mg BSA, respectively. The fourth, non-immunized group served as control. Three chickens from each experimental group and two from the control group were killed by bleeding 1, 2, 3, 4, 5, 6, 7, 9, 11, 16 and 19 days after antigen administration.

Serological methods. Quantitative precipitation was carried out as described by WILLIAMS and CHASE (1971), in the presence of 8% NaCl (GOODMAN et al., 1951). The biuret reaction was used for the determination of protein in the immune precipitate.

Passive haemagglutination (HA). Sheep red blood cells (SRBC) obtained throughout from one and the same animal were washed with buffered saline

and treated with a 1 : 150,000 dilution of tannin (Reanal Co., Budapest) for 10 minutes at 37 °C. As shown by ourselves as well as by others (NÉMETH, 1965), the tannin preparation used caused autoagglutination of SRBC at dilutions lower than 1 : 100, 000. BSA solutions 0.25 or 0.125% were used to sensitize the tanninized SRBC at 37 °C for 30 minutes. Passive HA test was done with Takátsy's microtitrator device, using two parallels of serial two-fold dilutions for each test serum with each sensitized lot of SRBC. Thus, four titrations were made for each serum, and the geometric mean was regarded as the antibody titre. The sera were inactivated and absorbed with normal SRBC before tested.

Sensitivity of antibodies to 2-mercaptoethanol (2-ME, Calbiochem) was determined by the method of HEGE and COLE (1966), using 0.1 *M* 2-ME.

Histological methods. Tissue specimens were taken from the plane of the largest transversal section of the spleen and were fixed in Carnoy's solution. Paraffin sections 4 μ thick were stained with methyl-green–pyronine.

Plasmoblast and plasma cell counts were assessed from cell numbers counted in 100 fields of vision in each section, at a 1260-fold magnification.

The calculations required for statistical evaluation were made by means of a Hewlett—Packard Model 10 calculator.

Results

Antibody-producing cells and antibodies. Changes in plasmoblast and plasma cell counts as well as the quantities of precipitating antibodies are shown in Fig. 1. It is evident that both increased with the rising antigen dose in the dose range studied.

The kinetics of plasmoblast transformation was also dose-dependent. Peak values were reached on the 5th day with a small or medium dose and on the 6th day with a large dose. The slope of the ascending limb of the curve was approximately the same for each of the three doses. On administration of small doses, transformation commenced one day later (from the 3rd day on) than in the other two cases (from the 2nd day); thus the peak on day 5 was lower, despite the similar rate of transformation. With a high dose level, the number of plasmoblasts and plasma cells was as large on day 5 as that found with medium doses. Transformation is, however, prolonged at the same rate, resulting in a higher peak of cell count on day 6.

Minor dose-dependent alterations in the kinetics of precipitin production were also found (see Fig. 1). Maximal antibody concentration was attained at day 7 with a medium dose, whereas with small and large doses uniformly two days later (at day 9). This phenomenon reflects, to a certain extent, the kinetic differences of plasmoblast transformation, but the different quantities of anti-

body bound by the antigen not yet eliminated from the blood may also have played a role. Moreover, the persistence of antibodies in the blood was slightly prolonged with the rise of the antigen dose (Fig. 1). With small and medium doses, antibodies were demonstrable in the blood of all animals during the detectability period of precipitating antibodies, with large doses, however, one nonresponder was found on each of days 7 and 11.

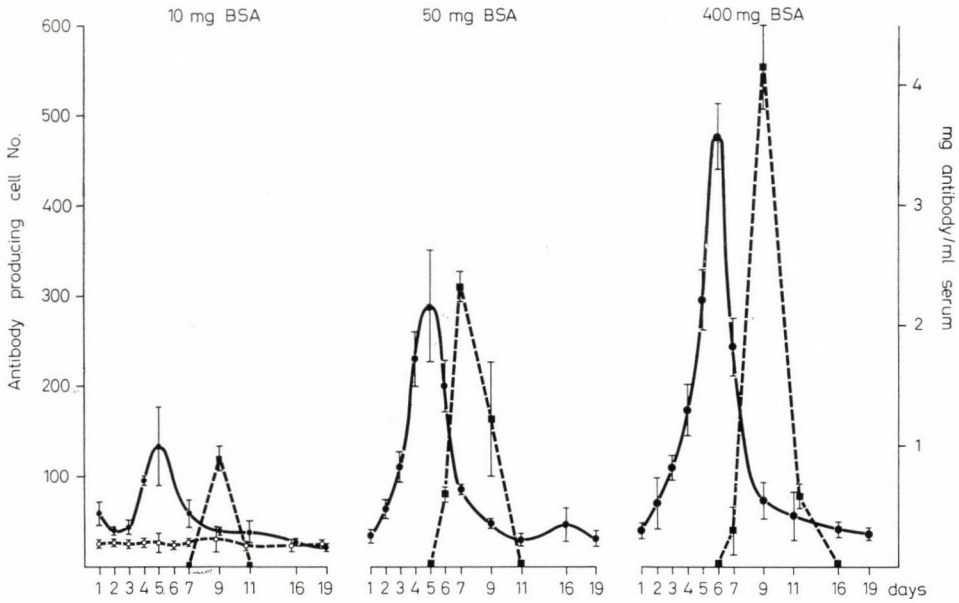

Fig. 1. Dynamics of plasmoblast + plasma cell counts (left ordinate, ●——●, cell No./0.1 mm² area of spleen section), and precipitating antibody titres (right ordinate, ■ — — ■, mg antibody protein/ml serum) after administration of 10, 50 or 400 mg BSA. Each dot represents the mean ± S.D. for three chickens, except the control values for plasmoblast + plasma cell counts (○ . . . ○), where the mean ± S.D. for two animals per dot is presented

Quantitative changes in antibodies demonstrable with passive HA are shown in Fig. 2. This again indicates an increase in maximal titres with the elevation of the antigen dose. Titre exaltation was approximately three-fold between small and medium doses, and eightfold between medium and large doses. The times of maxima corresponded to those obtained with precipitation (see Fig. 1). No further similarities were found between the two groups of curves. Antibody was detectable for longer period than with the precipitation test, but by the 19th day the HA titres were also minimal. Only a single nonresponder was found with HA; the serum haemagglutinin titre of the other animals were found to be responder while precipitation was relatively low. These differences point to a greater sensitivity of the passive HA method.

Quantitative changes in 2-ME-resistant (MER) antibodies, regarded as belonging to the IgG class, are also shown in Fig. 2. Unlike the findings outlined in the foregoing, MER antibodies exhibited no appreciable antigen dose dependence. Their maximal titre was the highest with small doses, and the lowest with medium doses. However, the larger the dose, the earlier antibodies could be detected in the blood. This phenomenon was particularly conspicuous with

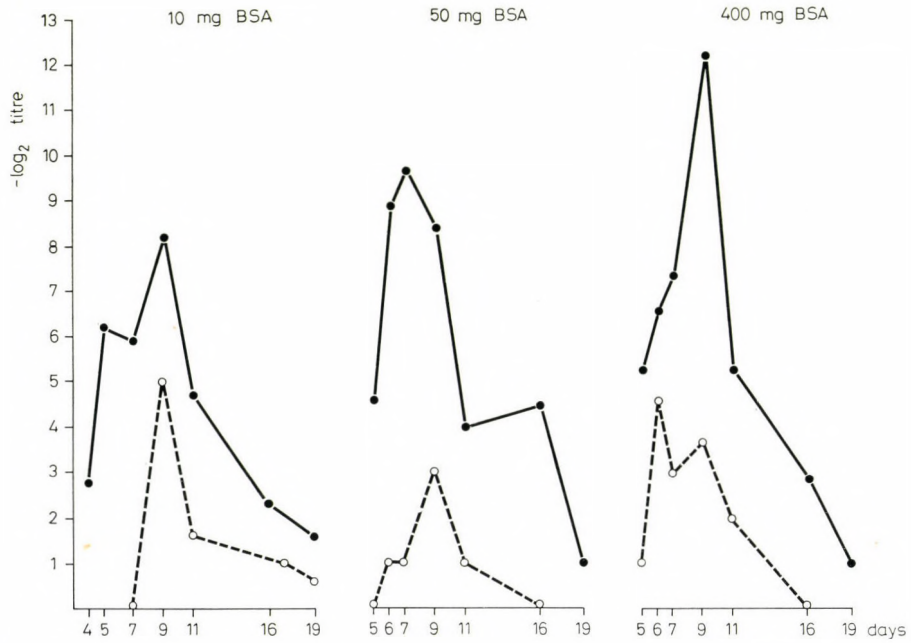

Fig. 2. Dynamics of the total antibody level demonstrable with passive haemagglutination (●———●), and the level of MER antibodies (○ — — ○) on administration of 10, 50 or 400 mg BSA. Each dot represents the $-\log_2$ mean titre for three chickens

the largest dose, after which MER antibodies reached maximal titre as early as on the 6th day.

It seems therefore that the antigen dose-dependence of the primary antibody response is chiefly due to IgM type (2-ME sensitive) antibodies, at least in the given experimental system. In contrast, the antigen dose had no notable influence on the quantity of IgG-type (MER) antibodies, but altered the kinetics of their production.

Statistical evaluation. According to the Smith and St. John-Brooks' formula, there is a linear relationship between the logarithms of maximal antibody concentrations and the logarithms of antigen doses. This log-log-inear function is valid only for a medium dose range. At low dose levels accord-

ing to STEVENS (1956), the dose response relationship is rectilinear, while the response to very large doses is constant, *i.e.*, no enhancement can be achieved by further elevation of the antigen dose. Out of the above dose intervals, the medium range seems to be the widest, thus a log-log-linear relationship should be valid for most dose response investigations. The data obtained in the present experiments also originate from the medium dose range. The largest dose used (400 mg BSA) represented about the upper limit of this interval as shown by the lack of response to this dose by two animals.

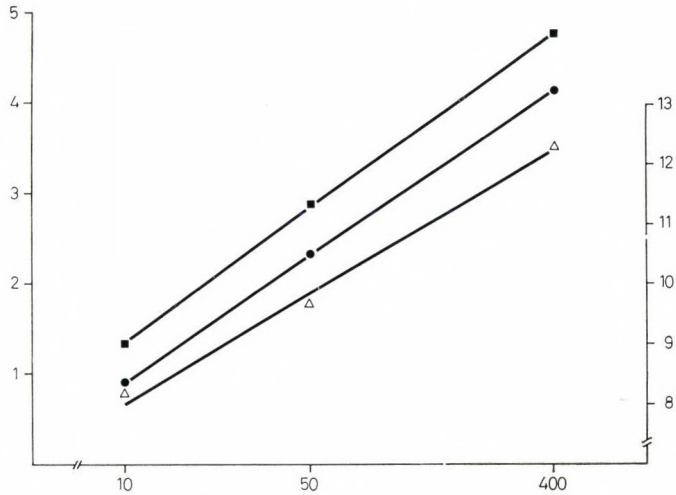

Fig. 3. Relations of various manifestations of immune response to the antigen dose. Left ordinate: maxima for plasmoblast + plasma cell counts (■, $\times 10^2$ cell/0.1 mm^2 area of spleen section) and precipitating antibody concentrations (●, mg antibody protein/ml serum), on the right ordinate, maximal $-\log_2$ titres of passive haemagglutination (△) are demonstrated, in function of the logarithm of antigen dose

On the basis of the foregoing considerations, attempt was made at fitting our experimental data to a log-log relationship. Maxima of the antibody-producing cells and those of precipitating and haemagglutinating antibodies were plotted against the logarithms of the antigen doses (Fig. 3). As can be seen in Fig. 3, the maximum counts of plasmoblasts and plasma cells as well as the maximum precipitin concentrations fitted well to a log-linear system, and only the maximal passive HA titres fitted more or less precisely to the log-log-linear relationship accepted in the literature.

This unexpected result was also checked by calculations. The three groups of data were fitted to both log-linear and log-log-linear systems by means of regression, and the "goodness" of fitting was checked by correlation analysis (Table I).

Table I

Parameters of regression and values of correlation obtained by fitting of the experimental data to both log-lin and log-log relationships

Experimental data	Regression				Correlation (r^2)	
	Slope		Ordinate intercept			
	log-linear	log-log	log-linear	log-log	log-linear	log-log
Max. antibody-producing cells	$9.29+10^1$	3.40×10^{-1}	-7.85×10^1	6.61×10^1	0.9997	0.9631
Max. precipitating antibodies	8.81×10^{-1}	4.06×10^{-1}	-1.12×10^0	3.97×10^{-1}	0.9999	0.9567
Max. passive HA titres	1.34×10^3	7.76×10^{-1}	-3.39×10^3	4.57×10^1	0.8810	0.9913

The values obtained proved the correctness of the relationships represented graphically in Fig. 3. Accordingly, the first two groups of data fit better with log-linear than with log-log-linear relationship, while the reverse is true for the third group of data.

It appears therefore that the log-log-linear relationship which has generally been accepted by the authors does not always precisely characterize the antigen dose–antibody response relationship, a log-linear relationship being in certain cases more conclusive.

Discussion

Many workers (Smith and St. John-Brooks, 1912; Markovich and Vorobyev, 1952; Edsall, 1955; Stevens, 1956; Backhausz, 1967) have agreed that the quantitative relationship between antigen dose and antibody response can be described with a reasonable exactitude as a log-log-linear function in the medium dose range. The present investigations, however, have shown that the dose-dependence of the number of antibody-producing cells and the quantity of precipitating antibodies are more precisely characterized by a log-linear function, which has a character of saturation curves, being fairly general for a variety of natural phenomena. However, a discrimination between the correctness of the two theoretical curves requires scores of experimental evidence, because certain parts of the log-linear function may be replaced with a satisfactory accuracy by a log-log-linear function having an exponent less than 1. In principle, however, it is not indifferent which of the two relationships is in fact valid. The correctness of the log-linear relationship did not appear unequivocally universal even in our studies, for one group of the data (passive HA titres) fits better to the log-log function. This discrepancy

might be explained by the finding of BENEDICT et al. (1962) that the two serological methods used (quantitative precipitation and passive HA) detect antibodies of different physicochemical properties.

We believe, however, that the crucial point of the present observations is not as much the fact that antibodies of different physicochemical properties had been detected, but rather that a possible discrepancy between the accuracy of the two methods has emerged. In the case of passive HA, supposing a twofold dilution series of ideal accuracy, the concentration difference of serum and accordingly also of specific antibodies between two adjacent wells is 100%. In principle, end titre determination means that the titre is within the concentration interval between the last positive and the first negative well. Thus even in the ideal case, the range of the relative error of measurement is $\pm 50\%$.

Accordingly, the higher the titre, the greater the absolute error. This considerable source of error is concealed, but not eliminated, by the usual logarithmic expression of titre. Consequently, it is postulated that selection of values corresponding to the log-linear relationship would also be possible even within these theoretical limits of accuracy. Quantitative precipitation is, however, free from a similar source of error, hence its relative error is as little as 1—2%.

Now if the log-linear relationship is correct, the present studies also permit the conclusion that a rectilinear relationship exists between the number of antibody-producing cells and the quantity of antibodies produced. This would mean that each antibody-producing cell secretes antibodies at an approximately constant rate, as suggested, although not proved, by UHR (1964).

Out of the effects of antigen dose on kinetics of antibody production, the early IgG response induced by large doses is most conspicuous. This phenomenon resembles, to a certain extent, the finding of IVÁNYI et al. (1966) and ČERNÝ and IVÁNYI (1966) that large doses of human serum albumin induced a strong IgG response, accompanied at times by the selective inhibition of IgM antibody production.

For both phenomena the following explanations may be offered:

(a) Antibodies belonging to the classes IgG and IgM are produced by different cells with dissimilar antigen sensitivity.

(b) IgM- and IgG-type antibodies are produced by the same cells, but large doses cause an earlier "switchover" (NOSSAL et al., 1964) from IgM to IgG production.

It seems possible that both (a) and (b) may occur in a cell population in response to one and the same antigen. However, the validity of these explanations demands further experimental support.

Summary

The quantitative and kinetic changes of plasma cell reaction in chicken spleen, and antibodies demonstrable with quantitative precipitation and passive haemagglutination tests were studied after administration of 10, 50 or 400 mg bovine serum albumin (BSA). It was shown that in case of maximum counts of plasmoblasts + plasma cells and maximum concentrations of precipitating antibodies, the dose response relationship is log-linear and only maximum passive haemagglutination titres fit better to the log-log-linear function generally accepted in literature.

It was also demonstrated that in the model investigated, the antigen dose-dependent increase of primary antibody response was chiefly due to IgM type antibodies. An elevation of the antigen dose did not notably influence the quantity of IgG type antibodies, but altered the kinetics of their production.

References

Backhausz, R.: Immunodiffusion und Immunoelektrophorese. Grundlagen, Methoden und Ergebnisse. Budapest, 1967. Benedict, A. A., Brown, R. J. and Ayenger, R.: J. Exp. Med. **115** (1962), 195. Černý, J. and Iványi, J.: Folia Biol. **12** (1966), 343. Edsall, G.: Ann. Rev. Microbiol. **9** (1955), 347. Goodman, M., Wolfe, H. R. and Norton, S.: J. Immunol. **66** **66** (1951), 225. Hege, J. S. and Cole, L. J.: J. Immunol. **95** (1966), 559. Iványi, J., Valentova, V. and Černý, J.: Folia Biol. **12** (1966), 158. Levi, M. I., Basova, N. N., Livshitz, M. M., Zotova, W. W. and Basova, H. N.: Immunology **24** (1973a), 1051. Levi, M. I., Durikhin, K. V., Basova, N. N., Fomenko, G. A. and Kravtsov, F. E.: Folia Microbiol. **18** (1973 b) 237. Markovich, A. V. and Vorobyev, A. A.: Doklady Akad. Nauk SSSR **87** (1952), 665. Nagy, Z. A. and Fehér, Gy.: Z. Immun.-Forsch. **143** (1972a), 223. Idem: Ibidem **143** (1972b), 323. Németh, I.: Z. Immun.-Forsch. **128** (1965), 468. Nossal, G. J. V., Szenberg, A., Ada, G. L. and Austin, C. M.: J. Exp. Med. **119** (1964), 485. Smith, J. H. and St. John-Brooks, R.: J. Hyg. **12** (1912), 77. Stevens, K. M.: J. Immunol. **76** (1956), 187. Uhr, J. W.: Science **145** (1964), 457. Williams, C. A. and Chase, M. W. (Eds): Methods in Immunology and Immunochemistry. New York and London, 1971.

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ANTHELMINTIC ACTIVITY OF MEBENDAZOLE AGAINST NATURALLY ACQUIRED GASTRO-INTESTINAL NEMATODES IN SHEEP

By

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Mebendazole (methyl-5-benzoyl-benzimidazole-2-carbamate) synthesized by the Janssen Pharmaceutica has been shown a potent anthelmintic against numerous species of worms in man, domestic as well as zoo animals. Biochemically its action in the helminths is to inhibit the uptake and/or transport of glycose (VAN DEN BOSSCHE, 1972).

Apart from the manufacturer's interim records, the efficacy of mebendazole against gastrointestinal nematodes in sheep was first reported by GUILHON et al. in 1972. Treating groups of 3 to 5 animals each with a single dose of 35 down to 8 mg/kg of body weight proved highly effective as judged by the reduction of faecal egg output. Next experiment on a total of 12 sheep of GUILHON and BARNABÉ (1973) attested to similar results. Using a minimum dosage rate of 10 mg/kg, TABBAA (1972) corroborated the remarkable anthelmintic activity of mebendazole in field trials carried out on four flocks of sheep. More recently, KUTZER et al. (1974) reported on favourable results obtained with several species of wild ruminants and also three sheep being treated at 15 mg/kg on three consecutive days.

The aim of our field trials was to convey some further assessment of this comparatively new anthelmintic produced by the G. Richter Pharmaceutical Company (Hungary) in cooperation with the Janssen Pharmaceutica (Belgium).

Material and methods

Hungarian merino ewes, aged between 2 and 5 years, with mild naturally acquired gastrointestinal nematode infection on different farms were used in these trials.

In a pilot experiment designed to determine the optimum dose rate and the tolerance of mebendazole, more or less heavily pregnant ewes were employed. The animals were randomized into subgroups of 10 animals each, ear-tagged, weighed at, and one week after, the treatment being carried out on individual body weight basis. The sheep were observed for abnormal behaviour for two hours after the treatment and then daily for a week.

In other experiments, only some representatives of the flock were weighed, and the body weight of the rest of animals was merely estimated and the dose accordingly adjusted. The sheep were at random allocated to appropriate groups, marked, and drenched by a single administration of mebendazole in 10% (occasionally 5%) suspension using a semi-automatic drenching gun or a syringe for administration of small volumes of the drugs. One group of animals were treated by feeding pulverous mebendazole mixed into a reduced amount of feed. As reference drug, 10% solution of tetramisole was subcutaneously injected into ewes at a dose rate of 10 mg/kg of tetramisole hydrochloride, and untreated animals served as controls.

Rectal samples of faeces were collected from possibly each experimental animal on the day of, and between 4 to 7 days after, the treatment. Strongyloid nematode egg counts in 3 g faecal samples were determined by slightly modified McMaster technique. The anthelmintic efficacy in different groups was calculated from the mean egg counts in 1 g of faeces (EPG) prior to, and after the treatment, and was expressed as per cent reduction.

Controlled anthelmintic test was also performed in one trial. To preclude a newly acquired infection, these sheep were maintained indoors on concrete pens following the medication with mebendazole or tetramisole, and 11 to 14 animals were slaughtered from different groups 4 to 7 days later. The abomasa and small intestines were separately washed out and the washings were sedimented in jars for 30 minutes. The supernatant was subsequently sucked off, and the sediments were made up to 0.5 or 1 litre. While thoroughly mixing the contents of abomasa and the small intestines 20% and 10% aliquots, respectively, were withdrawn and stained with iodine to facilitate the search for worms with the aid of a stereomicroscope. The worms were transferred to formol saline, identified on generic level and counted. The anthelmintic efficacy was assessed by comparing the mean worm counts recovered from the the treated animals to those of unmedicated controls.

Pregnant ewes, lambing and the offspring were carefully observed for symptoms of toxicity.

Body weight data were subjected to analysis of Student's *t*-test.

Results

In the pilot experiment on pregnant ewes treated with mebendazole orally or with tetramisole subcutaneously (Table I), no side effects were to be seen. Figures pertaining to body weight changes seem to indicate some reduced weight "gain" in groups dosed with mebendazole at 5, 10 and 320 mg/kg or tetramisole as compared to unmedicated controls. The anthelmintic efficacy revealed by faeces examination proved complete in all the groups.

Table I

Body weight and faecal egg counts (EPG) of gastrointestinal nematodes in groups of ten pregnant ewes on the day of (0), and 7 days after (A), the treatment with mebendazole (M) or tetramisole (T)

Dose mg/kg	Body weight (kg) Mean ± SD			No. of animals Positive/Total		Mean EPG		Efficiency %
	0	A	"Gain"	0	A	0	A	
M 5	58.8±3.6[b]	59.8±4.1[b]	1.0±1.0[b]	8/10	0/10	225	0	100
M 10	55.3±6.8[a]	55.4±7.4[a]	0.1±1.4[c]	7/10	0/10	120	0	100
M 20	50.4±6.4[a]	52.6±6.8[a]	2.1±1.9[a]	8/10	0/10	160	0	100
M 40	53.6±5.3[a]	56.5±5.9[a]	2.9±1.8[a]	6/10	0/10	75	0	100
M 80	54.1±7.5[a]	56.4±6.7[a]	2.3±1.5[a]	7/10	0/9	80	0	100
M 160	53.8±7.1[a]	55.9±6.1[a]	2.1±1.6[a]	7/10	0/10	160	0	100
M 320	54.2±4.4[a]	55.7±5.2[a]	1.5±1.2[b]	9/10	0/10	140	0	100
T 10	57.0±2.2[b]	58.7±2.7[a]	1.7±1.7[b]	10/10	0/10	140	0	100
Control	49.9±9.5	53.0±8.7	3.2±1.0	7/10	8/10	100	135	—

Superscript letters indicate the level of significance: a, $p > 0.05$; b, $p < 0.05$; c, $p < 0.01$

Thus, to check upon the efficacy of low dose rates of mebendazole, dosages of 5 and 10 mg/kg of mebendazole were only used on more adequate number of animals (Table II). Although the experimental groups each included 80 ewes in milk, the table refers to reduced numbers of animals from which faecal samples could be obtained (also applies to further tables). The dose of 5mg/kg proved nearly as efficient as that of 10 mg/kg in terms of egg count reduction, but a rather high proportion of animals remained positive in the former group.

Table II

Anthelmintic efficacy of mebendazole against gastrointestinal nematodes in sheep, judged by faecal egg counts on the day of (0), and 7 days after (A), the treatment

Dose mg/kg	No. of animals Positive/Total		EPG				Efficiency (%)
			Mean	Max.	Mean	Max.	
	0	A	0		A		
5	67/70	15/58	382.8	1400	13.8	100	96.4
10	68/69	8/71	412.3	1600	12.7	500	96.9
Control	68/70	72/79	337.1	1550	499.4	2400	—

We have, therefore, recoursed to dose rates of 10 and 20 mg/kg in the next trial (Table III). It is noteworthy that the bulk of sheep in each of these groups had been injected with Hőgyes strain of rabies virus before the admin-

istration of anthelmintics and were moribund at the time of faecal sampling after the treatment. It is assumed that their poor condition may account for the marked rise in egg production of worms in the unmedicated sheep.

Table III

Faecal egg counts and drug efficacies in sheep treated with mebendazole (M) or tetramisole (T)

Dose mg/kg	No. of animals Positive/Total		EPG Mean (range)		Efficiency %
	0*	A	0	A	
M 10	28/28	4/28	516.1 (50—2600)	8.9 (0— 100)	98.3
M 20	23/23	2/23	414.0 (50—2600)	4.0 (0— 50)	99.0
T 10	19/20	1/20	520.0 (0—2100)	2.5 (0— 50)	99.5
Control	23/23	22/23	804.5 (50—4400)	1902.3 (0—3600)	—

* 0, day 0; A, 5 days after treatment

Beside the faeces examination, controlled test was also carried out on several of these sheep. As immature worms were found in less than 0.5% of the parasite burdens, these were neglected; mature worm counts and efficacy figures are summarized in the Table IV.

Table IV

Necropsy worm counts and drug efficacies (%) calculated by comparing the mean worm burdens in the treated groups with that of the control group; autopsy was performed 4—7 days after the treatment

Dose mg/kg	No. of animals	Number of worms recovered*								Mean
		H.	O.	T.a.	T.c.	C.	N.	B.	S.	
M 10	11	0	0	0	0	0	22.7	0	63.6	86.4
% eff.:		100	100	100	100	100	94.8	100	32.7	96.3
M 20	14	0	0.7	0	0.7	0	13.9	0	6.8	22.1
% eff.:		100	99.7	100	98.6	100	96.8	100	92.8	99.1
T 10	14	0	0.3	0	0	1.1	0	0	0	1.4
% eff.:		100	99.8	100	100	99.8	100	100	100	99.9
Control	13	16.9	281.1	185.8	502.3	743.1	441.9	37.3	94.6	2303.8

* H, *Haemonchus;* O, *Ostertagia;* T.a., *Trichostrongylus axei;* T.c., *Trichostrongylus colubriformis;* C, *Cooperia;* N, *Nematodirus;* B, *Bunostomum,* S, *Strongyloides*

With a view of practical requirements, a standard dose rate of 5 or 10 mg/kg calculated for the heaviest sheep in flock of ewes in milk was given either individually, or mebendazole was mixed in concentrate ration for a group of

experimental animals. This was consumed by the sheep without any hesitation. The dose rates stated in Table V are obviously to be considered as minimum dosages, more or less exactly only received by the heaviest individuals, whilst sheep of smallest body weight may well have received a dose as high as 6.5 and 13 mg/kg instead of 5 and 10 mg/kg, respectively.

Table V

Faecal egg counts and drug efficacies in ewes treated individually with mebendazole (M) or tetramisole (T) at a standard dose rate. M 10F designates group of ewes receiving mebendazole mixed in the feed

Minimum dose mg/kg	No. of animals Positive/Total		EPG				Efficiency (%)
			Mean	Max.	Mean	Max.	
	0*	A	0		A		
M 5	32/40	1/40	378.7	3700	1.2	50	99.7
M 10	29/39	1/39	244.9	1750	1.3	50	99.5
M 10F	39/50	0/50	294.0	1300	0	0	100
T 10	73/102	3/98	296.6	3400	2.5	100	99.1
Control	41/49	36/49	294.9	1300	385.0	1950	—

* 0, day 0; A, 5 days after treatment

In the last trial of the series, of 580 ewes in 3rd to 5th month of pregnancy 100 animals were marked and individually treated with mebendazole at a standard minimum dose rate of 20 mg/kg. As very low worm egg counts were detected on the day of treatment, subsequent sampling of faeces was omitted, and the ewes were observed only to see if any toxic effect was produced. There could not, however, be discovered symptoms of toxicity in the ewes or any abnormalities in their offspring either. Normal lambing occurred in all the treated animals.

Discussion

As shown by MARSBOOM (1973), mebendazole is a new anthelmintic extremely well tolerated by many species of animals. In a single dose as high as 320 mg/kg, it failed to produce any apparent side effect in the sheep. This agreed also in the light of the present study notwithstanding that some body weight changes were recorded in our acute toxicity trial (Table I). It would certainly be erratic to ascribe the differences in weight "gain" over the one week period in small groups of ewes to a true drug action. The difference is inconsistent and probably due to the disparity of initial mean body weights in the control and treated groups. Apart from the groups of ewes in the pilot

experiment, there could not be shown toxicity in the experiment in which a total of one hundred pregnant ewes were treated at a dose rate of 20 mg/kg.

Tetramisole injected subcutaneously was used as reference drug for the estimation of the anthelmintic activity of mebendazole. Parenteral administration of tetramisole (or levamisole) at a dose ranging from 5.5 to 15 mg/kg was found harmless and effective earlier also by PUCCINI (1968), SINYUKOVA (1970), CHROUST (1972), TURTON (1972) and FURMAGA et al. (1974).

As with tetramisole, similarly high percentage efficacies against common gastrointestinal nematodes were obtained with mebendazole in the present trials. This corroborates the data derived from small scale experiments of GUILHON et al. (1972), TABBAA (1972), and GUILHON and BARNABÉ (1973). Although it is effective at a dose as small as 5 mg/kg, the use of a minimum dose of 10 mg/kg is rather recommended to get a reasonable high proportion of animals with negative faecal egg counts. Further increase of the dosage, say to 20 mg/kg, seems little rewarding except for the elimination of less sensitive species, namely, *Nematodirus* and *Strongyloides* in particular (Table IV), and also for tapeworms and lungworms (TABBAA, 1973). Some resistance of *Strongyloides* to mebendazole was reported also by TABBAA (1972).

The use of mebendazole caused not only inhibition of the egg production in worms but also their substantial removal from the host as indicated by the controlled test (Table IV). Percentage efficacies based on the faecal egg count reduction (Table III) appear to show a good correlation with those judged by necropsy worm burdens. It is evident from these trials that the single administration of the drug provides satisfactory cure, so, dosing sheep on three consecutive days (KUTZER et al., 1974) seems quite unnecessary. As the mebendazole at a dose rate of 10 mg/kg of body weight was readily taken in the feed, it can be regarded as a drug of choice for mass treatment of the sheep. The more so, as it has a large safety index ($LD_{50} > 320$ mg/kg).

Further trials with mebendazole on sheep suffering of severe parasitic gastroenteritis, and testing its efficiency against both immature worms and populations resistant to other benzimidazoles, might contribute to the informations currently available on this promising anthelmintic.

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Summary

Anthelmintic activity as well as the tolerance of mebendazole by pregnant ewes was tested on a total of 786 sheep with mild naturally acquired gastrointestinal nematode infection.

Hungarian merino sheep, mainly pregnant or ewes in milk, 2—5 years old, weighing 45—60 kg, were allocated to groups of 10—105 animals and treated with single oral doses of mebendazole in 10% suspension or mebendazole pulvis was mixed into the feed. As reference drug, tetramisole at 10 mg/kg was subcutaneously injected into groups of animals. Efficiency of the treatment was evaluated by comparing the worm egg counts before, and 4—7 days after, the medication, and also the necropsy worm burdens recovered from the abomasum and the small intestine of treated and control groups of 11—13 sheep each (controlled test). Toxic effect was checked upon by careful inspection for side effects in groups of 10 pregnant ewes dosed with 5—320 mg/kg of mebendazole, in 100 ewes treated with single 20 mg/kg dose of mebendazole at 3rd to 5th month of pregnancy, and by examination for obvious abnormalities in the offspring.

The mean anthelmintic efficacy (%) figures in different experimental groups were as follows:

mebendazole at dose rate of 5 mg/kg: worm egg counts: 100, 96.4, 99.7,
mebendazole at 10 mg/kg: egg counts: 100, 96.9, 98.3, 99.5; worm counts: 96.3,
mebendazole at 10 mg/kg bodyweight mixed into feed: 100,
mebendazole at 20 mg/kg: egg counts: 100, 99.0; worm counts: 99.1,
tetramisole at 10 mg/kg s/c: egg counts: 100, 99.5, 99.1; worm counts: 99.9.

Species of *Nematodirus*, and *Strongyloides* particularly, proved more resistant to the action of mebendazole in comparison with other gastrointestinal nematodes.

No toxicity was seen in any of the treated animals. Lambing occurred normally. Feed mixed with mebendazole was readily consumed by the sheep. The oral LD_{50} value of mebendazole for sheep is over 320 mg/kg.

References

Chroust, K.: Acta vet. (Brno) **41** (1972), 19. Furmaga, S., Gundlach, J. L. and Sobieszewski, K.: Medycyna Weterynaryjna, 1974, 460. Guilhon, J. and Barnabé, R.: Bull. Acad. Vét. **46** (1973), 299. Guilhon, J., Caillier, R. and Hubert, J.: Bull. Acad. Vét. **45** (1972), 445. Kutzer, E., Prosl, H. and Frey, H.: Dt. Tierärztl. Wschr., 1974, 117. Marsboom, R.: Toxicol. Appl. Pharmacol. **24** (1973), 371. Puccini, V.: Acta med. vet. (Napoli) **14** (1968), 851. (cit.: Helm. Abstr. **40** (1971), 4168.). Sinyukova, Yu. P.: Bull. Vses. Inst. Gelm. K. I. Szkjabina **4** (1970), 123. Tabbaa, M.: Feldversuche mit dem Anthelmintikum Mebendazole bei Schafen. Thesis. Hannover, 1972. Turton, J. A.: Res. vet. Sci. **13** (1972), 160. Van Den Bossche, H.: Biochemical effects of the anthelmintic drug mebendazole. In: Comparative biochemistry of parasites (ed.: Van Den Bossche, H). New York, 1972.

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EFFECT OF THYROID STIMULATING HORMONE ON THE CIRCULATING THYROXINE CONCENTRATION IN DUCKS

(SHORT COMMUNICATION)

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There is considerable indirect evidence that thyroidal secretion of thyroxine in birds is controlled by the anterior pituitary gland (ASSENMACHER, 1973). However, until recently it has not been possible to test the ability of avian thyroid- stimulating hormone (TSH) to stimulate thyroxine secretion. In the present report this is investigated.

The experiments were performed in three 12-week-old domestic ducks. Serial 1 ml blood samples were collected into heparinized syringes from an indwelling siliconized rubber catheter (Silastic, Esco Rubber Co., London) in the branchial vein. The plasma was separated, rapidly frozen and stored at

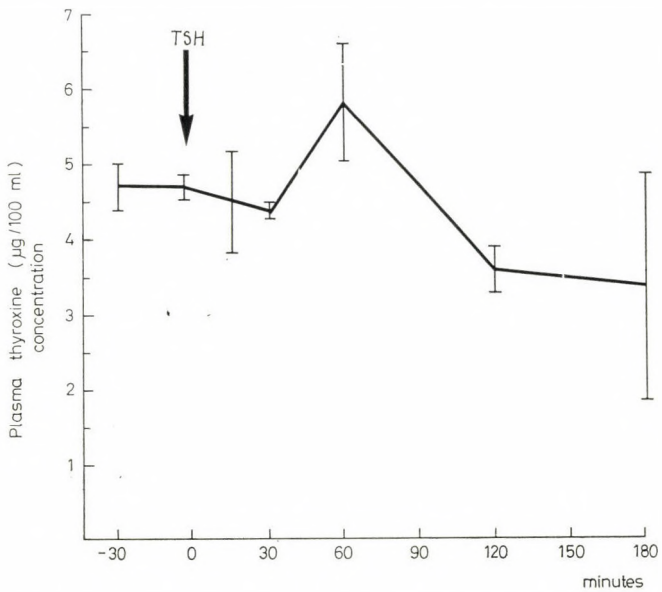

Fig. 1. Plasma thyroxine concentration in domestic ducks following the administration of avian thyroid- stimulating hormone

—20 °C prior to assay. Chicken TSH (200 μg) was injected into the bird *via* the catheter. The preparation of thyrotrophin (SCANES and FOLLETT, 1972) had a potency of 127.8 (88.2—174.4) μg equivalent NIH—TSH—S6/mg in a chick thyroidal ^{32}P-phosphate incorporation assay (GREENSPAN et al., 1956). The plasma thyroxine level was estimated by a competitive protein-binding assay (Thyropac 4 kit, Radiochemical Centre, Amersham).

The effect of avian TSH on thyroxine secretion in the duck is shown in the figure. No very dramatic changes in thyroxine level were observed. In all ducks, however, the thyroxine concentration was elevated for one hour following thyrotrophin administration (significantly, $P < 0.05$ over pre-treatment and initial post-treatment levels). This was equivalent to an increase of $22.7 \pm \pm 9.0$ (S.E.M.)% over the pre-treatment level, or 25.5 ± 14.5 (S.E.M.)% over the initial post-treatment level.

The experiment adds some evidence to the concept that thyroxine secretion in the bird can be influenced by the anterior pituitary gland.

References

ASSENMACHER, I.: In: Avian Biology (Eds. D. S. FARNER and J. R. KING). New York, 1973. GREENSPAN, F. S., KRISS, J. P., MOSES, L. E. and LEW, W.: Endocrinology **58** (1956), 767. SCANES, C. G. and FOLLETT, B. K.: Br. Poultry Sci. **13** (1972), 603.

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BIBLIOGRAPHIA

László P. PELLÉRDY: *Coccidia and coccidiosis*, Second revised and completed edition, common publication of Akadémiai Kiadó (Budapest) and Paul Parey Verlag (Berlin), 1974. 959 pages, 224 illustrations. Price: Ft 550.—.

The first edition of this volume was published under the same title by Akadémiai Kiadó in 1965. The first textbook, 657 pages, was a great success all over the world so that practically all copies were sold out in five years. This and the rapid expansion of knowledge on coccidia stimulated the publishing company Paul Parey to prepare a new edition in collaboration with Akadémiai Kiadó, the original publisher. In 1970, the author was instructed to compile the manuscript.

The text had to be rearranged for the second edition. The first edition was the first attempt on a world scale to present descriptions of all coccidia of all animals (and man), and to deal with the pathological importance of coccidia and coccidiosis prevention in a single volume. Apart from species descriptions, the biological properties of coccidia were discussed in as a great detail as permitted by the contemporary knowledge of the topic, under the heading "general coccidiology".

In the second edition, the original structure of the book could no longer be maintained, owing to limitations put to the expansion of the volume. So many new coccidia were described in the meantime that to include them all, the author was obliged to omit the chapters dealing with general coccidiology and as a result, practically all known species are dealt with in the textbook on nearly 1000 pages.

It should be mentioned that species descriptions make up only one part of the book and these are chiefly of faunistic value. From the point of view of pathology, the chapters dealing in great detail with damages of coccidiosis in domestic and wild animals, pathogenesis of coccidial infections, immunity to, and therapy and prevention of, coccidiosis, are of an outstanding importance. These parts are up-to-date reviews of present knowledge on the topic. Although there was some delay in publication (the author submitted the manuscript to the publisher in February 1972), it seems that the author was able to include newest research results and information even during the period of printing.

Apart from the above changes, the contents of the volume follow the arrangement pursued in the first volume. In accordance with zoological systematics, the descriptions of known coccidian parasites are presented in the sequence from lowest to highest animals. In addition to the morphological description of species, the author takes great care in discussing biological properties and pathological aspects. This is a great help to veterinarians engaged in coccidial diseases, especially of useful animals and poultry: the author has devoted about 50 pages to chicken coccidiosis, about 10 pages each to rabbit and cattle coccidiosis, apart, of course, from detailed descriptions of all coccidian parasites of these hosts. The coccidioses of other useful animals are dealt with proportionally to their importance. It is unusual that the coccidia of man (Homo) and human coccidioses are, in accordance with Simpson's systematics, discussed in the middle of the book at the end of the order *Primates* which had been placed before the order *Edentata*. In this part the reader misses the description of the newest research results on sarcosporidia, even if the author, as in the entire book, is cautious not to state a final position in matters of dispute. The experiments in question indicate that an *Isospora* of man is actually a sarcosporidium (or *vice versa*). Some time after the ingestion of sarcocystis cysts, human volunteers began to pass oocysts in the same manner as similarly infected cats. The author is similarly cautious in discussing the toxoplasma problem. It is now an established fact that the final host of *Toxoplasma gondii* is the cat and that the oocysts passed by it on experimental infection are in every respect similar to the oocysts of the coccidium formerly known as *Isospora bigemina*. In this light, the systematic position of, or validity of, *Isospora bigemina* might well be regarded as doubtful. The reader misses the author's opinion on this point, although it was probably withheld for lack of final evidence at the time of submission of the manuscript.

It is the author's great merit that, despite the nomenclatural confusion still prevailin in the literature, he insisted on keeping the rules of the International Code of Zoological Nomenclature issued in London in 1961. This may not have been easy in several cases in which names formulated with an astonishing arbitrariness had to be left unaltered because of restrictions imposed upon emendation by the Code. Also, it was impossible to avoid the adoption of certain Greek—Latin hybrid names.

It seems that the author avoided to make suggestions for a systematic revision. This reservation seems natural in view of the recent changes in the taxonomic classification of coccidia, still in progress in these days. At all events, systematics is not the main line of the book, being only a means for properly arranging the biological information presented.

One serious shortcoming of the first edition was the lack of a subject index, for lack of space. This failure has been corrected in the second edition in which a detailed and well-elaborated subject index facilitates orientation between topics and groups.

The greater part of the excellent illustration material are the author's own drawings and photos. The reader regrets that more abundant illustration, above all colour photos, could not be included in such a large volume prepared with such an excellent printing technique.

The vast material dealt with in the textbook is completed by a list of several hundred references. This list is, of course, not complete, because the available references would fill a complete volume, but it is nevertheless the most complete bibliography of its kind published so far.

It is expected that both veterinary scientists and practitioners will profit much from this book and we hope that a next edition — probably in co-authorship with other investigators — can be compiled in the not too far future.

T. KOBULEJ

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A kiadásért felel az Akadémiai Kiadó igazgatója — Műszaki szerkesztő: Botyánszki Pál

A kézirat nyomdába érkezett: 1975. III. 17. — Terjedelem: 10,5 (A/5) ív, 47 ábra

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РЕЗЮМЕ

ИЗУЧЕНИЕ ИЗНАШИВАЕМОСТИ ПОВЕРХНОСТИ ПОЛА

Б. КОВАЧ А.

На основании литературных данных и собственного опыта автором подчеркивается тот факт, что из-за несоответствующих условий содержания, в первую очередь из-за пола, среди свиней часто наблюдается заболеваемость конечностей. Для хирургической оценки поверхностного слоя пола сконструирован электрический прибор, при помощи которого, применением рога копыта свиней изучалась шероховатость и изнашиваемость пола. Поверхность пола из разного материала (дерево, бетон, асфальт, пластмасса, резина) терлась 1—5 минут кусочками рога разной поверхности и веса. Из степени изношения рога и образующегося от трения углубления делалось заключение о шероховатости пола и его изнашиваемости. В первой части исследований обнаружено, что на полу шероховатой поверхности (шероховатый бетон, такой же асфальт) рог слишком быстро изнашивается, во второй же части, —что на поверхности пола за данное время возникает определенное углубление (износ). Данные исследований собраны в таблице. Автор рекомендует, чтобы до того времени, пока нет у нас соответствующего метода, за надежную гладкость принималась таковая поверхности скобленного твердого дерева. Что касается изнашиваемости пола при определенном трении наиболее удовлетворяющим считается или таковаяю дерева или твердого асфальта.

ИЗУЧЕНИЕ ПРОДУЦИРОВАНИЯ СЕРОВОДОРОДА ШТАММАМИ BRUCELLA OVIS

П. ШУТКА

Автором изучалась причина разногласия литературных данных о продуцировании сероводорода штаммами Brucella ovis. Методом бумажных полос уксусно-кислого свинца по Хадлесону и пробой МИ—ДИ по Тири автором изучено продуцирование сероводорода 17 собственными штаммами, референтивным штаммом Br. ovis обозначения 63/290 и штаммом Br. melitensis »M 16«. На основании последней пробы все штаммы оказались средними продуцентами сероводорода. Эта продукция сероводорода является бесперебойной, слабой интенсивности и одинакового количества. На основании контрольного эксперимента и раствором сероводорода на воде выявлено, что проба МИ—ДИ примерно в 20 раз чувствительннее, чем метод Хадлесона. Таким образом, считающийся характерным признак продуцирования сероводорода у разных бруцеллезных видов и типов основывается только на количественном различии. В связи с этим возникает вопрос, насколько можно этот признак использовать в дифференциации отдельных видов и типов.

ГИСТОЛОГИЧЕСКОЕ И ГИСТОХИМИЧЕСКОЕ ИЗУЧЕНИЕ НАДПОЧЕЧНИКОВ ГУСЕЙ И УТОК

А. ХАССАН

Изучение надпочечников гусей и уток показало, что субкапсулярная зона корковой ткани как гистологически, так и гистохимически отличается от внутренней зоны. Изнурение адренального холестерола и аскорбиновой кислоты у обоих видов наблюдалось в период половой активности. Надпочечники утки снабжены более плотной соединительнотканой капсулой и интерстицием и соотношение хромафинной ткани больше, чем у гуся.

ИЗУЧЕНИЕ АТНИГЕННОЙ СТРУКТУРЫ ИЗОЛИРОВАННЫХ ИЗ ПОДСОСНЫХ ПОРОСЯТ ШТАММОВ ESCHERICHIA COLI

Я. ВАРГА и АДЕЛ Ф. ФАРИД

Изучены биохимические свойства и определен серотип 222 колибациллезных штаммов, изолированных из тушек 88 подсосных поросят 30 хозяйств, павших в возрасте 1—12 дней. Для изучения антигенов О имелись сыворотки 57 О и ОК, для изучения же антигенов К — все анти-К сыворотки (1—94).

Среди изолированных штаммов 218 оказались Escherichia coli. Больше чем две трети (69,7%) штаммов вызывали бета-гемолиз. На основании других биохимических свойств 15 штаммов принадлежали тоже к роду Escherichia, но они были уреаза-положительными.

При помощи имеющихся сывороток удалось определить серотип 176 (80,7%) штаммов. Больше чем две трети штаммов представляли собой только 7 серотипов.

Наичаще изолированы следующие серотипы: О149 : К91(В) : К88ас(L), О147 : : К89(В) : К88ас(L), О41 : К(А?), О75 : К-, О9 : К(А?) : К88ас(L), О139 : К82(В) и О2 : К. Большинство штаммов содержало некоторый К(В) и К88ас(L), поверхностный антиген; но обнаруживались и штаммы, содержащие термостабильный, вероятно К/А, поверхностный антиген и кроме них антиген, не содержащий К-варианты.

МУКОРМИКОЗ С АСТЕРОИДАМИ У МОЛОДОГО БЫКА

И. ВИТОВЕЦ, П. ВЛАДИК, Ц. ПРОКШ и П. ФРАГНЕР

В двух увеличенных средостенных лимфатических узлах 6-месячного быка обнаружены микотические гранулемы с морфологией элементов грибка, напоминающей представителей рода Mucor. Вокруг некоторых гранулем обнаруживались астероидные образования, показывающие специфическую иммунофлуоресценцию с специфическим антигамма глобулином, меченным изотиоцианатом флуоресцеина. Культивированием выявлено, что гранулемы вызывались видом грибка Mucor pusillus Lindt (1886).

ОПЫТ ВАКЦИНАЦИИ ПРОТИВ ИНФЕКЦИОННОГО ГАСТРОЭНТЕРИТА СВИНЕЙ

III. Вируснейтрализирующий титр антител в молоке привитых свиноматок и его продолжительность во время лактации

Е. МОЧАРИ, Я. БЕНЕДА и Е. ШАГИ

Двадцать семь свиноматок восприимчивого к вирусу заразного гастроэнтерита поголовья крупной фермы привито на 9—27 дней раньше опороса один раз вакциной в нос. Титры вируснейтрализирующих антител в крови и молоке определялись в день опороса и в определенные сроки после него.

Специфические вируснейтрализирующие антитела в сыворотке и молоке свиноматок появились 10 дней после вакцинации и их титры постепенно повышались. К 27-у дню после вакцинации средний титр антител в сыворотке и молоке равнялся 1 : 30 и 1 : 9 соответственно. Эти показатели соответствуют показателям титра антител в сыворотке и молоке свиноматок при естественном заражении вирусом заразного гастроэнтерита в момент, когда эпизоотия уже прекратилась среди подсосных поросят (24 дня спустя после наступления заразы среди свиноматок).

Продолжительность наличия антител в молоке у 9 свиноматок опоросившихся между 19-м и 27-м поствакцинационными днями, изучалась до конца лактации.

ОПЫТ ВАКЦИНАЦИИ ПРОТИВ ИНФЕКЦИОННОГО ГАСТРОЭНТЕРИТА СВИНЕЙ

IV. Вируснейтрализирующей титр антител и их продолжительность в молоке раз и в разные сроки два раза вакцинированных свиноматок

Э. МОЧАРИ, Я. БЕНЕДА и Е. ШАГИ

Авторы вакцинировали 132 свиноматки восприимчивого к инфекционному гастроэнтериту свиней (ИГЭС) поголовья крупного хозяйства раз и два раза с промежутком времени в 2 и 6 недель соответственно живой вирус содержащей вакциной, приготовленной из ослабленной вирулентности вирусного штамма »СКр«. Путем исследования крови через 2—4 недели в течение 6 месяцев следили за появлением и продолжительностью специфических, вируснейтрализирующих антител.

Уровень антител в раз вакцинированных свиноматках достиг пика (средний титр 1 : 24) за время 6 недель. В следующие 4 недели этот уровень понизился еженедельно на 0,5 $\log_2$ значения и потом остановился полгода на данном уровне (средний титр 1 : 16).

В свиноматках, вакцинированных два раза с промежутком времени в 6 недель, уровень специфических антител кульминировал (со средним титром в 1:94) на 8-й неделе после первой вакцинации. В последующие 4 недели этот уровень понизился еженедельно на 1,5 $\log_2$ значения и потом оставался полгода на данном уровне (средний титр 1 : 32)

В животных, вакцинировавшихся два раза, с промежутком времени в 2 недели, уровень специфических антител кульминировал (со средним титром в 1 : 120) тоже на 8-й неделе после первой вакцинации. Этот уровень продолжался в них почти без изменения (в среднем 1: 100) полгода.

На основании этих данных авторы приходят к заключению, согласно которому уровень специфических антител наиболее надежно формируется при двухкратной вакцинации свиноматок с промежутком времени в две недели.

УЛЬТРАСТРУКТУРА СПЕРМИЯ СЕЛЕЗНЯ

I. Головка

М. МАРЕТТА

Изучалась ультраструктура головки спермия селезня. Она состоит из акросомального колпака, акросомы и ядра. Колпачок является терминальным концом головки и обладает гомогенной структурой. Акросома имеет вид иглы и содержит вакуоль. Щель между акросомой и колпачком выполнена зернистой массой. Ядро состоит из грубогранулированного хроматина. Ядерная мембрана прилегает к поверхности ядра. Клеточная мембрана тесно прилегает к акросомальному колпачку и ядру.

УЛЬТРАСТРУКТУРА СПЕРМИЯ СЕЛЕЗНЯ

II. Шейка, промежуточная часть, хвост и концевая часть

М. МАРЕТТА

Иузчалась ультраструктура промежуточной части и хвоста спермия селезня. Шейка включает в себя проксимальную центриоль и примыкающее вещество с электронной плотностью. В промежуточной части можно отличить митохондриальное влагалище, состоящее из 24—30 митохондриев неправильной сферической формы. Ось промежуточной части состоит из дистальной центриоли, из проксимальной части системы осевых фибрилл и наружных фибрилл меньшей электронной плотности, по объему существенно уменьшившихся. Хвост содержит комплекс осевых фибрилл и обволакивающую их аморфную оболочку. Концевая часть состоит только из комплекса осевых фибрилл, распределения $9 + 2$. Клеточная мембрана плотно прилегает к поверхностным структурам хвоста.

ОБНАРУЖИВАЕМОСТЬ АНТИГЕНА К80(В) В ШТАММАХ ESCHERICHIA COLI, ИЗОЛИРОВАННЫХ ИЗ ПОДСОСНЫХ ТЕЛЯТ

Я. ВАРГА и А. Ф. ФАРИД

От 117 подсосных телят авторами изолировано 263 штаммов Escherichia coli и определены их антигены О и К. Обнаружено 33 штамма, которые при изучении в живом состоянии не совсеми антисыворотками (1—94) К реагировали положительно в реакции агглютинации; исключением явились антисыворотки ОК, полученные против типового штамма Е38, О78 : К80(В). Эти штаммы принадлежали к семи разным О-группам (О8, О9, О17, О20, О78 и О153) и на основании К-агглютинации они содержали антиген К80(В). На основании изучения ОК-антисыворотки Е38, О78 : К80(В) однако доказано, что антиген К только штаммов антигенной структуры О78 : К80(В) идентичен с антигеном типового штамма К80(В); поверхностные антигены дальнейших 6 разных штаммов группы О близко родственны ему, но неидентичны с ним.

На основании более тщательного изучения антигена К типового штамма Е38, О78 : К80(В) можно предполагать, что он термолабилен, содержит вероятно поверхностный антиген К(L), ибо после его кипячения 2 1/2 часа при 100°С им не удалось изнурить антиген К гомологической сыворотки ОК. При помощи антигена К(L) в кроликах можно получить антисыворотку и из этой антисыворотки ОК путем абсорбции можно получить чистую антисыворотку К.

ОБУСЛОВЛЕННЫЕ MYCOBACTERIUM AVIUM И НЕТИПИЧНЫМИ МИКРОБАКТЕРИЯМИ ЛИМФАДЕНИТЫ В СВИНОПОГОЛОВЬЯХ

I. Изучение патологии процесса

И. САБО, Ш. ТУБОЙ и А. СЕКИ

На основании анализа литературных данных и собственных наблюдений указывается на изменение эпизоотологии туберкулеза свиней. Приводятся данные гистологического и бактериологического изучения 142 подчелюстных, 225 брыжеечных, 49 перибронхиальных и 4 печеночных лимфоузлов, 19 легких и 12 печеней, собранных из 41 крупнохозяйственного свинопоголовья. В печени и легких туберкулезные изменения не обнаружены и из этих органов микобактерий изолировать не удалось. Из лимфоузлов изолировано 65 штаммов Mycobacterium avium, I штамм группы Руньон II и 49 штаммов группы Руньон IV. Среди 30 штаммов авиум 5 принадлежали в серогруппу Шефер 1, 15 — в серогруппу Шефер II, I — в серогруппу Шефер IV, 4 — в серогруппу Шефер V, I — в серогруппу Шефер VII, 3 — в серогруппу Девис и I — в серогруппу Уатсон. В многих хозяйствах изолировано по 2, 3, даже 4 штамма. При закрытом содержании животных важнейшим источником заражения можно считать подстилку и корм, эвентуально питьевую воду. В таких условиях роль домашней и дикой птиц и почва могут быть только косвенным источником заразы. В хозяйствах, где свиней содержат не в полностью закрытых условиях и близ имеются и приусадьбенные птицы, последние и почва представляют собой важный и опасный источник заразы.

ОБУСЛОВЛЕННЫЕ MYCOBACTERIUM AVIUM И АТИПИЧНЫМИ МИКОБАКТЕРИЯМИ ЛИМФАДЕНИТЫ В СВИНОПОГОЛОВЬЯХ

II. Изучение роли подстилки в возникновении микобактериальных лимфаденитов на промышленных свинофермах

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Среди факторов среды авторами изучалась роль подстилки в возникновении вызываемых микобактериями изменений в лимфоузлах брыжейки и головы свиньи. В образцах подстилки и корма равным образом обнаруживались микобактерии. Результаты экспериментов, проведенных в нескольких хозяйствах, показали, что подстилка играет важную роль в частоте и степени поражений лимфоузлов свиней промышленных ферм микобак-

териями. Поражения в лимфоузлах наичаще наблюдаются среди свиней на подстилке из опилок. У свиней без подстилки поражения лимфоузлов наблюдались тоже чаще, чем у животных на соломенной подстилке. Авторы считают, что происхождение, хранение опилок и частота уборки навоза играют тоже важную роль. Поскольку поражения лимфоузлов наблюдаются и в хозяйствах, где не применяют подстилки из опилок, следует помнить и другие пути заражения. Последние обусловливаются главным образом условиями содержания и факторами среды. Наиболее важным источником заражения является почва, которой загрязняется обычно корм и питьевая вода. Источником заражения свинофермы M. avium может быть близ расположенное неблагополучное птицепоголовье. Кроме этого разные компоненты корма могут быть загрязненными испражнениями неблагополучных диких птиц и таковых приусадьбенных хозяйств. Такое косвенное заражение, разумеется, может наступить не только в данном хозяйстве, но и в кормосмесительных и кормохранительных цехах. В распространении M. avium и нетипичных микобактерий в поголовье непосредственный контакт очевидно играет второстепенную роль, но пренебрегать им нельзя.

СЕРОЛОГИЧЕСКАЯ ПРИНАДЛЕЖНОСТЬ ШТАММОВ ESCHERICHIA COLI ИЗОЛИРОВАННЫХ ПРИ ПОНОСЕ ТЕЛЯТ В ЕГИПТЕ

А. Ф. ФАРИД, Я. МЕСАРОШ, Я. ВАРГА З. С. ЛОФТИ и А. С. АБД. ЕЛ. МАЛЕК

Сто колибациллезных штаммов, изолированных из павших телят в Египте, переслано с целью идентификации. Биохимическим изучением выявлено, что 85 штаммов принадлежали к Escherichia coli. Использованием антисывороток 57 О и ОК тридцать штаммов удалось распределить в группы. Наиболее важными О-группами были 8, 41, 145, 128, 15, 20 и 115.

Поскольку О группы 8 и 20 раньше уже были изолированными в Египте, их можно считать для этой страны наиболее важными. 77% штаммов О-группы содержали антигены К; 4 среди последних представляли собой форму А, остальные — В или L.

ЭКСПЕРИМЕНТАЛЬНОЕ ЗАРАЖЕНИЕ ЯГНЯТ ИЗОЛИРОВАННЫМ ИЗ ОВЕЦ ВИРУСОМ, БЛИЗКО РОДСТВЕННЫМ БЫЧЬЕМУ АДЕНОВИРУСУ ТИПА 2

I. Вирусологические исследования

Ш. БЕЛАК, В. ПАЛФИ и Э. ТУРИ

Из овец, показывающих симптомы пораженности дыхательных органов, изолирован родственный бычьему аденовирусу типа 2 вирус Het/3, которым заражали ягнят, выращенных без молозива. Вирус оказался для ягнят патогенным и путем контакта заражал восприимчивых ягнят. У болеющих животных наблюдались подъем температуры, клинические признаки заболеваемости дыхательных и пищеварительных органов. Среди 9 зараженных ягнят 3 погибли к 1-у, 2-у и 9-у дням после заражения, а остальных убили между 4-м и 13-м днем после заражения. Ягнята выделяли вирус носовой слизью и фекалиями. В сыворотке зараженных ягнят появились нейтрализирующие вирус Het/3 антитела титра 1 : 8—1 : 128. На основании данных эксперимента аденовирусный штамм Het/3 может вызвать у ягнят заболевание дыхательных и пищеварительных органов.

НЕКОТОРЫЕ СВОЙСТВА ВЗАИМОСВЯЗИ ДОЗА АНТИГЕНА — ОТВЕТ АНТИТЕЛ ПРИ ПЕРВОМ ВВЕДЕНИИ БЫЧЬЕГО СЫВОРОТОЧНОГО АЛЬБУМИНА ЦЫПЛЯТАМ

А. НАДЬ, ДЬ. ФЕХЕР и Э. ХОРВАТ

Изучались количественные и кинетические изменения в реакции плазматических клеток в селезенке цыпленка и антител, выявимых количественными реакциями преципитации и пассивной гемагглютинации после введения 10, 50 и 400 мг бычьего сывороточ-

ного альбумина (БСА). Доказано, что в случае максимального количества плазмабластов + плазматических клеток и максимальной концентрации преципитирующих антител взаимосвязь доза—ответ является log-прямолинейной и только максимальные титры пассивной гемагглютинации соответствуют функции log-log-прямолинейной, обычно принятой в литературе.

Кроме этого выявлено, что в данной модели усиление первичного ответа антител, зависящего от дозы антигена, вызывали главным образом антитела типа IgM. Повышение дозы антигена по сути не отразилось на количестве антител типа IgM, но изменило кинетику их продукции.

ЭФФЕКТИВНОСТЬ МЕБЕНДАЗОЛА ПРИ ЕСТЕСТВЕННЫХ ЖЕЛУДОЧНО-КИШЕЧНЫХ НЕМАТОДОЗАХ ОВЕЦ

И. ВАРГА и М. ЯНИШ

Антигельминтиная активность и переносимость мебендазола беременными овцематками изучены на 786 овцах с умеренной естественной инвазией.

Беременные и лактирующие овцематки возраста 2—5 лет, живого веса 45—60 кг, разделенные в группы по 10—105 животных, обрабатывались разовыми дозами мебендазола в виде 10%-ной суспензии или порошка в смеси с кормом. Овцам некоторых групп ради сравнения вводили подкожно по 10 мг/кг тетрамизола. Эффективность обработки оценивалась сравнением количества выделяемых яиц до и 4—7 дней после дегельминтизации у обработанных и контрольных животных; далее вскрытием по 11—13 овец из групп обработанных и контрольных животных каждого критического исследования. За токсическим эффектом следили путем тщательного наблюдения за животными в группах по 10 беременных овцематок, получавших 5—320 мг/кг мебендазола; на 100 овцематках в 3—5-м месяцах суягности, обработанных один раз дозой 20 мг/кг, и отклонениями от нормы у родившихся ягнят.

Эффективность мебендазола в отдельных группах животных была следующей:

при дозе 5 мг/кг количество выделяемых яиц снизилось на 100, 96,4, 99,7%;

при дозе 10 мг/кг количество выделяемых яиц снизилось на 100, 96,9, 98,3%; количество же нематод — на 96,3%;

при дозе 10 мг/кг, когда препарат-порошок скармливали с кормом — 100%;

При дозе 20 мг/кг количество выделяемых яиц снизилось на 100, 99,0; количество же нематод — на 99,1%;

Тетрамизол при дозе 10 мг/кг, подкожно снизил количество выделяемых яиц на 100, 99,5, 99,1%; количество же нематод — на 99,0%.

Виды родов Nematodirus и Strongyloides были более резистентными к мебендазолу, чем виды других родов.

Признаки отравления ни у одного животного не обнаруживались. Окот проходил нормально. Корм с мебендазолом охотно поедался овцами. LD_{50} при введении мебендазола через рот для овец находится выше дозы 320 мг/кг.

ЭФФЕКТ ГОРМОНА, СТИМУЛИРУЮЩЕГО ЩИТОВИДНУЮ ЖЕЛЕЗУ, НА КОНЦЕНТРАЦИЮ ЦИРКУЛИРУЮЩЕГО ТИРОКСИНА В УТКЕ

ДЬ. ПЕТЕШ и К. Г. СКЕНС

Исследования показали, что на секрецию тироксина в птице может влиять передняя доля гипофиза.

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INFLUENCE OF MECHANICAL SHAKING OF THE STOCK SUSPENSION ON THE RELIABILITY OF MICROBIOLOGICAL QUALIFICATION OF FEEDS

By

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(Received November 26, 1973)

Apart from analyses for feed constituents, microbiological examinations have increasingly come to the fore in feed control. By analogy of food examinations, qualitative and quantitative determinations of various microorganisms are being carried out. Counts are as a rule related to 1 g feed.

However, counts determined in identical samples in different laboratories often differ by several orders of magnitude, owing to dissimilar methods of processing.

A standard procedure was evolved in this laboratory more than a decade ago and has been employed ever since for processing of feed samples for microbiological assay. A stock suspension, consisting of 1 g sample in 9 ml sterile saline, is prepared in a test tube, and the tube is rotated 3 or 4 times between the palms of the hand to ensure even distribution.

In the GFR, 10-g feed sample is added to 90 ml diluent, and homogenization is made by mechanical shaking for 15 min. In the USA, homogenization is made either by mechanical mixing for 1—2 min at a very high r.p.m. or by 25 revolutions along the circumference of a vertical circle of 30 cm radius. All these mechanical procedures should ensure an even distribution of viable microorganisms, which originally form chains or aggregations in the test substance.

With an even microbiological status of the sample, the reproduction of the test results is theoretically possible.

Since various feedstuffs are regularly imported to Hungary, checking of the microbiological quality guaranteed by the firm has become necessary and, on the other hand, it seemed worth while to examine whether the introduction of mechanical shaking of test suspensions can increase the reliability of our microbiological assays.

Materials and methods

A total of 30 feed samples were selected at random from batches submitted for routine examinations. Eighteen samples were basic feedstuffs, the rest were compound feeds. A 10-g sample of each batch was, after an appropriate mincing, added to 90 ml sterile saline in a 250-ml Erlenmeyer flask. The sample was suspended in the diluent by shaking with the hand. One ml of each suspension was withdrawn and diluted serially for further examination. Cultures prepared from the dilutions served as controls.

The flask containing the stock suspension was closed with a rubber stopper after removal of the sample, and was placed in a "Labor" shaker in which it was shaken at 150 rev/min for 5 min, then 1-ml samples were withdrawn and the rest was shaken for an additional 5 min. The 1-ml samples shaken for 5 and 10 min were then processed as usual.

Viable germ counts and bacterium flora composition were determined and compared in control samples with those shaken for 5 and 10 min. Total germ counts were determined on meat-containing agar plates adjusted to pH 7.2. Dilutions of 10^4—10^6 were used as inocula and the results were read after incubation for 48 hrs at 26—28 °C. Bacterium flora components were determined by culturing on meat-containing commong agar and on Klimmer's agar for 24 hrs at 37 °C. Mould counts and mould flora composition were determined by culturing on 4% glucose agar, containing the antibiotic additive "Stredipen a.u.v." (500,000 IU streptomycin bas., 300,000 IU penicillin G—procaine, and 100,000 IU penicillin G in the form of K or Na salt). Dilutions of 10^2—10^3 were used as inocula and the results were read after incubation for 6 days at 24—28 °C.

Results

In general, higher germ counts were obtained in samples processed by mechanical shaking than in those shaken by hand only. Also, the germ counts were directly related to the duration of mechanical shaking in the majority of the cases. The germ count "increasing" effect of shaking chiefly came into display in the first 5-min period; it was generally lost when shaking was continued beyond 10 min and was even equivocal with the second 5-min period, *viz.*, the germ counts were lower after 10-min than after 5-min shaking in 26.6% of the cases and identical with the 5-min values in 6.6%.

Rises in total bacterium count were very variable. In general, germ counts were one order higher on mechanical shaking than without it; this increase was less pronounced with basic feedstuffs than with compound feeds (Table I).

Shaking did not alter either the bacterium flora composition or the proportions of the flora components.

Shaking also effected an increase in mould counts, but in this instance practically no correlation with the time of shaking was demonstrable. In the second 5-min period the germ count increased in only 50% of the samples, it did not change in 13.4% and decreased in 36.6%. The degree of increase fluctuated in a very wide range, occasionally attaining a difference of two logarithmic orders. Mould counts also rose to a greater degree in compound feeds than in basic feedstuffs (Table II).

However, while shaking did not alter the composition of the bacterium flora, it did produce certain changes in mould flora components, some of which increased, others decreased, still others remained unchanged (Table III). Mould flora composition fluctuated also with the degree of the applied dilution. The samples originally contained 0 to 6 mould species, whereas on shaking 1 to 7 new species appeared, or 1 to 4 of those originally present disappeared. At higher dilutions (10^3—10^4) of the inoculum, which generally facilitate reading, usually fewer species appeared in the cultures than with 10^2 diluted inocula. Nevertheless, the quantitatively dominant components were still prevalent after shaking, above all the *Penicillium* and the *Aspergillus* species forming long chains of conidia.

Discussion

The present studies were carried out to elucidate whether shaking of suspended feed samples can produce a state in which each viable microorganism present in the sample can serve as a potential colony-forming unit on culturing. Attempts were also made at determining the optimal period of shaking. Finally, it remained to be decided whether the reliability and reproducibility of microbiological feed assays can be improved by this approach.

According to the present studies, mechanical shaking is not the method of choice for attaining this purpose. The germ counts did not unequivocally increase on shaking and decresed rather than rose when shaking was of long duration. This can be attributed to the fact that shaking accelerates the dissolution of certain constituents and enhances the swelling of starch particles, which unfavourably alters the consistence of the sample. Viscous samples are difficult to handle and the sticky medium arrests the bacteria. Thus, only few germs are retained in the thin part of the sample which is still available for mixing by pipetting. Shaking periods not raising viscosity should be determined separately for each type of feed, but it remains to be pursued whether these periods are sufficient to ensure the separation of aggregated microorganisms; they almost certainly are not.

Table I

Distribution of germ counts in feed samples processed with and without mechanical shaking

Sample	Total germ counts in thousands per gramme sample								
	≤ 100	101–200	201–300	301–400	401–500	501–1000	1000–2000	2000–3000	>3000
Not shaken (control)	15	4	1	1	1	3	2	1	2
Shaken for 5 min	7	2	2	1	2	3	7	3	3
Shaken for 10 min	6	3	1	1	2	1	9	3	4

Table II

Distribution of mould counts in feed samples processed with and without mechanical shaking

Samples	Mould counts per gramme sample								
	≤1000	1001–2000	2001–3000	3001–4000	4001–5000	5001–10 000	10 001–20 000	20 001–50 000	>50 000
Not shaken (control)	13	—	3	5	—	7	—	2	—
Shaken for 5 min	4	3	—	4	3	3	6	3	4
Shaken for 10 min	4	3	2	1	3	2	4	8	3

Table III

Changes of mould counts in 30 samples on 5-min and 10-min shaking compared to samples not shaken

Mould counts on shaking for					
5 min			10 min		
unchanged	increased	decreased	unchanged	increased	decreased
		in 1 : 100 dilution			
10 (33%)	12 (40%)	8 (27%)	6 (20%)	15 (50%)	9 (30%)
		in 1 : 1000 dilution			
11 (38.6%)	12 (40%)	7 (28.4%)	12 (40%)	12 (40%)	6 (20%)

As to the value of shaking in improving the reliability of microbiological feed assays, the following aspects should be taken into consideration.

Shaking improved the bacterium count without altering either the flora composition or the proportion of the components. Thus, shaking means no improvement in the qualitative sense, but the findings suggest that the acceptable germ count thresholds should be raised.

Shaking seems to have only disadvantages from the mycological point of view. Increase of mould counts on shaking was chiefly due to the prevalence of species characterized by long chains of conidia. Reliable reading is possible only with high dilutions of the inocula, which means the loss of the species being present at low counts. Some of these may be decisive components in respect of feed qualification. Although new species appeared on shaking in 40 to 50% of the samples, the number of components either remained unchanged or decreased in number in the rest. It should be noted that the new species appearing on mechanical shaking were usually those characterized by long chains of conidia (*Penicillium* spp., *Aspergillus* spp.). In summary, the present findings support the conclusion that mechanical shaking of suspended feed samples does not notably improve the reliability of microbiological feed qualification; it is a surplus operation of doubtful value. Since, however, germ count is considerably influenced by the technique of processing, efforts should be centered on the standardization of microbiological food assays.

Summary

Mechanical shaking of suspended feed samples for 5 to 10 min was usually followed by an increase in the germ counts by about one logarithmic order (range: 1.7—48 times) over those determined in samples not shaken mechanically. Shaking did not alter either the bacterial flora composition or the proportion of the flora components.

Mould counts were also increased by shaking, but in a much wider range (1.3 to 5000 times) than bacterium counts, and the increase was the highest in those samples whose original mould count was low. Shaking often altered the composition of the mould flora, *viz.*, loss of some species and appearance of new ones in the high dilutions, required for reliable reading of the culturing results. Since shaking fails to notably improve the efficiency of microbiological feed control, its use is not recommended for this purpose.

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THE FATE OF MOULD "SPORES" IN THE DIGESTIVE TRACT OF CHICKS

By

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The importance of mycotoxicoses tends to increase with the concentration of animal raising, whereas systemic mycoses affect only certain species to a greater degree than previously when herds and flocks were smaller.

While the aetiological role of certain metabolites is more or less understood in mycotoxicoses, little is known about the pathogenesis of enteric diseases caused by hyphomycetes. Namely, the fate of conidia — endospores — ingested with the feed has been little pursued in the digestive tract, and evidence is also lacking whether these can act as direct causal factors of mycoses or mycotoxicoses.

The problem whether the orally ingested conidia and other reproductive elements of moulds survive in the digestive tract, whether they are eliminated with the faeces in a viable state, was awaiting clarification, being of immediate interest in respect of both microbiological feed control and animal health. The present experiments were performed to obtain more information on this problem.

Materials and methods

Mould strains. Mould strains frequently present in feeds and capable of causing mycosis, or mycotoxicosis, or both, were selected for the study. Although growth ability at 37 °C was regarded as the theoretical criterion of pathogenicity, strains growing only at lower temperatures were also examined. Strains growing at 37 °C: *Aspergillus candidus, A. fumigatus, A. flavus, A. niger, A. ochraceus, Circinella* sp., *Rhizopus* sp. Strains not growing at 37 °C: *Penicillium chrysogenum, P. funiculosum, P. notatum.*

The strains were propagated in 4% glucose agar containing "Stredipen a.u.v." (streptomycin bas. 500,000 IU, penicillin G—procaine 300,000 IU, K or Na salt of penicillin G, 100,000 IU) as antibiotic. Agar plates were prepared in 100-ml Erlenmeyer flasks and surface inoculation was followed by incubation for seven days at the optimal temperature. The rich fungal growth was collected by rinsing with 90 ml sterile saline per flask and "conidium"

counts in the rinsing fluid were determined by the pour-plate technique. The counts ranged between 930 thousand and 300 million, depending on the strain.

Experimental birds. A total of 100 one-day-old broiler hybrid chicks, procured from the hatchery, were used. Ten experimental groups and one control group were formed, each consisting of 10 birds. The chicks were kept in synthetic rat boxes in a well-ventilated room, at 30—32 °C temperature and appropriate air humidity. For the first 6 days, adsorbent paper, later good-quality wood shavings were used as bedding; the litter was exchanged according to requirement.

The birds were fed on a commercial chick starter until 10 days of age, after which they received a chick-rearing formula. The starter was sterilized to prevent health injury by its high germ counts. Fresh drinking water was supplied twice a day and pebbles of appropriate size were also furnished.

Fungus spores were administered at the same time every day by pipetting directly into the crop. The rinsing fluid used for this purpose was stored in the refrigerator and only the required amount was withdrawn from it each time. To prevent experimental error resulting from the deterioration or germination of the conidia in the rinsing fluid, the latter was freshly prepared every five days. Prior to administration the rinsing fluid was diluted to give a spore concentration of 1—2 million/ml. The single dose was 0.5 ml, thus each chick was given a daily dose of 0.5—1 million conidia. Administration on the above schedule was continued for 10 days, followed by a 4-day rest period and another 10-day treatment with extremely large doses, 5—40 million conidia daily, representing concentrations never occurring in feeds.

The birds were continuously examined for any change in general condition, movements, appetite, faeces (consistency, colour, odour) as well as for occasional nervous symptoms. Body weights were recorded at the beginning and on days 10, 24 and 31 of experiment.

The pathological effect of conidia was assessed by comparing body weights of treated and control birds and by postmorten examination of one chick killed in each group on days 1, 3 and 10. Rectal swabs taken from each bird between days 10 and 14 were used for mycological studies. Another chick in each group was killed on day 14. One of the six birds retained in each group for a second treatment was exsanguinated after 10 days of treatment with the extreme doses, and from the rest of the birds rectal swabs were taken on day 10, and five days later. Finally, all birds, including the controls, were killed by bleeding 31 and 32 days after beginning of the experiment. No conidium treatment was made on the day of exsanguination.

To re-isolate the fungus, streak plates were prepared on antibiotic-containing agar, on the first by a large loop from contents of crop, proventriculus, gizzard, the most anterior and most posterior segments of the small intestine,

the anterior segment of the large intestine, from coecum and rectum; later pour-plates were prepared from homogenates in antibiotic-containing agar medium of 2 cm portions of the above organs. Streak plates were also prepared with samples from trachea, lungs, air sacs, spleen, liver, kidneys and heart blood; the latter three samples were also used for culturing on Drigalski's agar. The cultures were incubated for 6 days at 26 °C for mould isolation and for 24 hrs at 37 °C for the isolation of bacteria.

To test the effect of the pH of the medium on the germination of conidia, aliquots of one strain each of *A. flavus*, *Rhizopus* and *Penicillium* were inoculated into 1% glucose broth adjusted to pH 4.0, 4.5, 5.0, 5.5, 6.0 or 7.4 and were incubated at 26 °C. The pH values of intestinal content were determined in various segments with indicator strip.

Results

Clinical observations and postmortem findings

All chicks remained healthy throughout the experiment. This can already be judged from their body weights that ranged between 700 and 1000 g at 31 or 32 days of age. The even growth rate required for reaching this weight category is in itself exclusive of the presence of mycosis, mycotoxicosis or any other disease.

Birds killed by bleeding at different times of experiment did not show any gross lesions.

Microbiological findings

Moulds related to the conidia administered could not be isolated from parenchymatous organs, blood, trachea and lungs. We usually failed to re-isolate the moulds from the digestive tract, except from the crop, of chicks below 10 days of age (Table I).

Effect of the pH of the medium on germination of conidia

With all three strains tested *(A. flavus, Rhizopus, Penicillium)*, germination failed to occur in glucose broth at pH 4.0, 4.5, 5.0 and 5.5. Both germination and mycelium formation did take place, though at a very slow rate, at pH 6.0, whereas growth was abundant at pH 7.4.

Discussion

Only part of the fungus strains tested could be re-isolated, on rare occasions, from the small and large intestines of the chicks. This supports the con-

Table I

Re-isolation of fungi from chicks killed after oral treatment with conidia for 1, 3 and 10 days

Organs	Days	Fungus strains representing the conidia used for oral infection and colony counts obtained in isolation experiments									
		A. candidus	*A. flavus*	*A. fumigat.*	*A. ochrac.*	*A. niger*	*Circinella*	*P. chrysog.*	*P. funiculos*	*P. notatum*	*Rhizopus*
Crop	1	6	9	2	—	—	6	1	2	6	—
	3	—	—	2	—	3	—	1	5	9	—
	10	—	—	—	—	—	6	—	—	—	—
Proventriculus	1	2	—	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	4	—	—
	10	—	—	—	—	—	—	—	—	—	—
Gizzard	1	—	—	—	—	—	3	1	—	—	—
	3	—	—	—	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
Anterior segment of small intestine	1	—	—	—	—	—	4	—	—	—	—
	3	13	—	—	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
Large intestine	1	100	2	—	—	—	3	—	—	1	2
	3	98	—	—	—	—	—	—	3	4	—
	10	—	—	2	—	—	—	—	—	—	—
Coecum	1	1	—	—	—	—	2	—	—	—	—
	3	—	—	—	—	—	2	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—
Rectum (faeces)	1	—	—	—	—	—	1	1	—	—	—
	3	—	—	—	—	—	1	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—

clusion that the orally administered conidia deteriorated in the proventriculus and gizzard of both young and older chicks.

Theoretically, the deterioration of conidia and mycelia reaching the digestive tract by oral route may be due to the following reasons.

The present *in vitro* studies on pH effect and the related literary data have unequivocally shown that an inappropriate pH can be regarded as the main factor responsible for the inhibition of growth and other life functions of conidia. This accords well with the observation that pH values of 5.0—6.0,

5.0, 3.5—4.0, 5.0—5.5, 5.0 and 5.0—6.0 were measured in the contents of crop, proventriculus, gizzard, duodenum, jejunum and rectum of the birds, respectively, at 10 days of age. Since pH values are similar in the stomach and abomasum of non-ruminant and ruminant domestic mammals, respectively, there is reason to postulate that the above conclusion also applies to those animals.

Other factors acting towards the depression of life functions of ingested moulds are the intestinal O_2 and CO_2 tension which are unfavourable for moulds, and other poorly understood reactions of the macroorganism, which develop with progressing age and also depress the growth of moulds.

If germination of the ingested conidia does yet take place, owing either to a greater pH tolerance of the strain or species or to impaired functions of the host, the mycelia become digested in the stomach. Many authors have reported that the mycelia ingested with feeds massively contaminated by non-toxic fungi are undergoing a complete lysis; *e.g.*, the mycelia of *P. roqueforti*, *P. camemberti* and *Oospora camemberti* become digested in humans.

Finally, taking into consideration that re-isolation of the experimentally administered fungus strains was successful only in two cases, the conclusion lies close at hand that the walls of the ingested conidia become lysed by the digestive juices.

The present findings permit some important conclusions in respect of poultry health, which seem to apply to other useful animal species as well.

In view of the practically complete destruction of conidia and other reproductive structures of moulds in the alimentary tract, oral ingestion of even very large amounts of such fungal stages involves practically no risk of mycosis or mycotoxicosis. Accordingly, no mycotoxicosis develops unless the ingested feed itself contains mycotoxins in a toxic concentration, and even the fungi growing readily at body temperature do not cause mycosis unless the mycelia present in the massively contaminated feed do not become digested by the digestive juices, *viz.*, if the conditions prevailing in the alimentary tract are such as to not inhibit the germination of conidia, further development of the ingested mycelia or their access to the blood and lymph circulation. Such conditions, however, occur only in animals with impaired health, lowered powers of resistance, or disorders in the secretion of digestive enzymes and bile, *viz.*, if the gastric mucosa is mechanically impaired by feed particles, bacterium-induced ulceration, *etc.* Mycoses always have a multifactorial aetiology, depending greatly on individual predisposition. They preferably occur in young animals and in animals with deranged constitution and consequent depressed metabolic activity, owing to unnatural keeping conditions. Fungus strains not growing at body temperature are never responsible for mycosis.

Moulds responsible for lung mycosis never cause disease on ingestion with the feed. Chicks infected in the present study from 2 days of age over

20 days with 0.5 to 40 million conidia administered directly into the crop developed neither symptoms nor gross lesions of lung mycosis. The realistic evaluation of microbiological feed analysis, the improvement of the outlook of breeders as well as aspects of guarantee equally demand that it should be emphasized over and over again that the fungus species most frequently responsible for lung mycosis, *viz.*, *A. fumigatus*, *Mucor* species, *etc.*, are ubiquitous and are pathogenic on inhalation only, never on oral ingestion. It follows that isolation from a given feed of *A. fumigatus* or some other fungus pathogenic for the lung is no proof of the responsibility of the fungus for lung mycosis, except if the plaintiff can present authorized evidence that the fungus responsible for the pneumomycosis had not been present in the unit, flock or herd prior to the introduction of the suspect feed or that the onset of the disease was closely associated with the feeding of this diet. Evidence to the first criterion is practically impossible, considering the omnipresence of the incriminated fungi, and fulfilment of the second criterion is far from being easy, since the disease is known to be multifactorial. Related studies in this laboratory also support the conclusion that the fungal contamination of commercial compound feeds is practically never of such a degree as could cause disease in poultry.

Summary

Large doses of spores of various mould species were administered directly into the crop of day-old chicks to obtain evidence whether oral ingestion of the reproductive stages (conidia, endospores) of moulds can cause mycosis or mycotoxicosis in this species. Seven species growing well at the body temperature of the birds and three species not growing at that temperature were tested. Individual daily doses of 500,000 to 1,000,000 conidia were administered for 10 days and after a subsequent 4-day rest period the daily dose was risen to 5—40 million conidia, given for another 10 days. Part of the species could be re-isolated from the digestive tract, above all from the crop, for 3 days, exceptionally for 10 days. Isolation from visceral organs and blood failed throughout. This supports the conclusion that oral ingestion of conidia cannot in itself elicit either mycosis or mycotoxicosis.

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STUDIES ON RELATIONSHIPS OF BLOOD GROUP ANTIGENS AND INCIDENCE OF MAREK'S DISEASE IN VACCINATED AND NON-VACCINATED POPULATIONS OF CHICKEN

By

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In 1949 PAULING disclosed that the presence of a mutant haemoglobin in humans was associated with a resistance to malaria infection. WASHBURN (1968) studied the interaction of a minor haemoglobin component and the incidence of Marek's disease (MD) in chicken. The mutant gene of this haemoglobin proved to be autosomal and codominant; the presence of its homozygote form in the population was parallelled with 20% higher resistance against MD than that of the normal haemoglobins.

In 1968 COLE described that alone on the basis of inoculation with the MD virus, resistant and susceptible lines against MD were selected within a short time (two generations). The percentage of the susceptible birds was 12.9% in the resistant line and 90.7% in the susceptible line. In the original common population the susceptibility was 51.1%.

Recently BRILES and BRILES (1972) have reported on a highly significant advantage of some B blood group genotypes (B^2B^6 and B^2B^7 *vs.* B^1B^6 and B^1B^7) after a natural outbreak of MD.

It is therefore reasonable to suppose that the genes responsible for blood-group characteristics, being also autosomal and codominant, as well as the physiological variability described by COLE, which is determined by genetical constitution, can be related to each other.

The possible existence of a close correlation between a blood type, or a blood-group allele, and the resistance against MD in a population, or rather in a pure line of chicken, would provide an effective method of selection to develop a highly resistant population without infecting the animals for this purpose.

Materials and methods

In 1971, 3364 day-old chicks were settled in an isolated farm of Bábolna Agricultural Combinate. 1809 of the chicks belonged to Line 1, the remainder to Line 2. In each line about half of the animals were vaccinated against MD, the other half remained as controls. At the age of 4 weeks all individuals of both lines were inoculated with the mixture of the blood and organ homogenates from pullets suffering from MD. When the birds were 8 to 11 weeks

old, blood was taken from the whole population for blood typing. Survivors were killed at 14 weeks (98 days) of age. Postmortem findings of these and of birds died by this time have been registered. Statistical significance was computed by the χ^2 method.

Results

Both postmortem findings and blood type were available for 2360 birds. The statistical analysis of the data showed (Table I) that non-vaccinated control birds of Lines 1 and 2 did not differ from each other concerning resistance against MD. However, the vaccinated birds in Line 1 were more resistant than those in Line 2 ($P < 0.05$).

Table I

The distribution of chickens with and without Marek' disease lesions in Lines 1 and 2

	Vaccinated				Control				Total
	Line 1		Line 2		Line 1		Line 2		
	n	per cent	n	per cent	n	per cent	n	per cent	
Chickens with MD lesions died before the age of 98 days	36	5.6	43	7.7	143	21.8	112	21.6	
killed at the age of 98 days	63	9.8	78	13.8	171	26.2	145	28.2	
Chickens without MD lesions	541	84.6	432	78.5	337	52.0	259	50.2	
Total	640	100.0	553	100.0	651	100.0	516	100.0	2360

Line 1 *vs.* Line 2 (vaccinated): $P < 0.05$

Line 1 *vs.* Line 2 (control): non significant

The presence of B_{10} antigen seemed to be advantageous for the resistance (Table II). Thus in Line 1 the percentage of vaccinated chickens with MD lesion was only 6.7% for birds having the B_{10} antigen as compared with the average for Line 1, *viz.* 15.5%. Similarly, of the non-vaccinated controls those having the B_{10} antigen showed relatively low morbidity rates, *viz.* 28.6% *vs.* 48.0% in Line 1 and 32.4% *vs.* 49.6% in Line 2. However, the number of birds possessing the B_{10} antigen was too low for a statistical analysis.

Table II

Percentage of diseased chickens in two lines relating to each blood-group antigen tested

Blood-group antigens	Vaccinated				Control			
	Line 1		Line 2		Line 1		Line 2	
	diseased/ total	per cent	diseased/ total	per cent	diseased/ total	per cent	diseased/ total	per cent
A_1	3/24	12.5	18/73	24.6	33/71	46.5	41/77	53.2
A_3	10/33	30.3	10/51	19.7	28/71	39.5	19/48	39.6
A_4	54/348	15.5	58/329	17.6	186/374	49.6	88/185	47.5
E_5	43/276	15.5	54/296	18.3	138/293	47.2	115/249	46.2
B_1	3/25	12.0	5/25	20.0	18/29	62.0	10/23	43.5
B_4	49/381	12.9	59/320	18.4	184/395	46.6	118/254	46.4
B_{10}	2/30	6.7	9/45	20.0	12/42	28.6	11/34	32.4
Total	99/640	15.5	121/553	21.8	314/651	48.0	257/516	49.6

* Total does not mean the sum of the numbers in the corresponding column, since in each bird more than one antigen may be present.

Among the other blood-group antigens the presence of the B_4 antigen seems to be advantageous. The number of chickens possessing this antigen was sufficiently high in both lines for statistical analysis (Table III). The difference in morbidity rate was significant in three of the four groups of birds.

The presence of blood-group antigens belonging to the A system did not show significant differences in morbidity in any of the groups under study.

Discussion

The present results suggest that a genetical relationship may exist between some B blood-group alleles and the resistance against MD. This is supported in the case of antigen B_4 by the fact that the differences were more definite in the control groups where the interactions were not disturbed by immune response.

Further investigations are needed to detect whether these relationships derive from pleiotropic effect of the blood-group alleles or their linkage to the gene controlling the resistance against MD.

Table III

Occurrence of Marek's disease lesions among chickens positive and negative for B_4 antigen in vaccinated and control groups of Lines 1 and 2

	Vaccinated				Control			
	Line 1		Line 2		Line 1		Line 2	
	No. of birds		No. of birds		No. of birds		No. of birds	
	$B_4(+)$	$B_4(-)$	$B_4(+)$	$B_4(-)$	$B_4(+)$	$B_4(-)$	$B_4(+)$	$B_4(-)$
Chickens without MD lesions	332	209	261	171	211	126	136	123
Chickens with MD lesions	49	50	59	62	184	130	118	139
died before the age of 98 days	33	30	43	35	112	59	81	64
killed at the age of 98 days	16	20	16	27	72	71	37	75
Total	381	259	320	233	395	256	254	262
Percentage of chickens with MD lesions	12.9	19.3	18.4	26.6	46.6	50.7	46.4	53.0
χ^2	5.49		8.91*		8.59*		15.42***	

* $P < 0.05$; *** $P < 0.001$

Summary

The data of postmortem examination and blood typing for 1193 vaccinated and 1167 non-vaccinated chickens belonging to 2 lines were statistically analyzed from the point of view of resistance against Marek's disease (MD).

The presence of two blood-group antigens (B_4 and B_{10}) seems to be associated with some resistance against MD. This relationship was significant for the B_4 antigen only; the number of B_4 individuals were high enough for the χ^2 test.

References

Briles, W. E. and Briles, R. W.: Abstracts of papers read at the 13th Europ. Conf. Anim. Blood Groups Biochem. Polymorph. Vienna, 1972. Anim. Blood Grps. Biochem. Genet. (Wageningen) **3** Suppl. 1 (1972), 82—83. Cole, R. K.: Avian Dis. **12** (1968), 9—28. Pauling, L., Itano, H. A., Slinger, S. J., Wells, I. C.: Science **110** (1949), 543—548 Washburn, K. W.: Poultry Sci. **47** (1968), 561—564.

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STUDIES ON THE THYROID FUNCTION OF BURSECTOMIZED COCKERELS IN VIVO

By

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The function of the bursa of Fabricius in birds has been studied from an immunological point of view for one and a half decades (GLICK et al., 1956, 1964; WARNER and SZENBERG, 1964; ISAKOVIĆ and JANKOVIĆ, 1964). It was earlier supposed to be an endocrine like gland influencing the physiological processes involved in sexuality and reproduction (GLICK, before 1956, see in Science 180: 45—47, 1973). Now the bursa is known to produce antibody-forming immunocompetent B cells (GLICK et al., 1956; BRASWELL et al., 1965; GLICK, 1967, 1968), which in some cases can function as cells competent in cell-mediated immunity, too (COLE et al., 1970; WICK et al., 1970a, b; NILSSON and ROSE, 1972). The relations between the presence or absence of the active bursa and some endocrine functions are also known. Testosterone propionate, for instance, is able to inhibit the development of the embryonic bursa (MAY and GLICK, 1964; WILSON and GLICK, 1966), and the bursa of radiothyroidectomized birds is much smaller in weight than that of controls (SNEDECOR, 1968a, b; SNEDECOR and CAMYRE, 1966; TIENHOVEN et al., 1965). OS Leghorn chickens characterized by an inherited autoimmune thyroiditis (TIENHOVEN and COLE, 1962; COLE, 1966; COLE et al., 1968; WITEBSKY et al., 1969) grew without any symptom of the disease if they had been bursectomized (WICK et al., 1970a, b, c; COLE et al., 1970). However, the symptoms appeared in birds injected with cells of their own bursa (NILSSON and ROSE, 1972), indicating the immunological, and not endocrinological, nature of this phenomenon.

Our aim was to study the relations between the thyroid function and the presence or absence of the functionally active bursa of Fabricius in growing chickens. Furthermore, we wished to throw some light on the relations between the values of the thyroid secretion rate estimated by different *in vivo* methods (PIPES et al., 1958; SINGH et al., 1968a, b).

Materials and methods

Experimental animals

Birds were kept in a laboratory in which the light (12 hours/day) and the temperature (22.5 $\pm$ 1.8°C) were standardized.

Group A. Eleven "Hunnia" broiler cockerels, 10 weeks old, without any pretreatment.

Group B. Seven "Hunnia" broiler cockerels, of the same age, bursectomized surgically after hatching.

Group C. Seven "Naked-Neck Transylvanian" (HUTT, 1949) cockerels, one and a half years old.

Group D. Seven "Bantam" (PUNETT and BAILY, 1914) cockerels, one and a half years old.

Group E. Seven F_1 hybrid male progenies from crosses between Bantam females and Naked-Neck Transylvanian (NNT) males.

Group F. Seven cockerels, progenies from crosses between F_1 females (mentioned above) and Bantam males.

The birds belonging to groups C, D, E and F were examined to compare the validities of the TSR values estimated by different methods. The thyroid function of these adult animals was determined by two methods (see below), one of which can be used for fast growing experimental animals. Reproduction of these experiments, involving 10 experimental days at least, would be impossible because during this time many physiological changes (growth, sexual maturity, *etc.*) occur, while in adults the physiological processes can be considered to be in equilibrium. On the other hand, the more exact method ("direct output" method, SINGH et al., 1968) for the determination of the thyroid secretion rate is too slow for these fast growing birds. Therefore, a less exact but quicker method developed by PIPES et al. (1958), PREMACHANDRA et al. (1959) and PREMACHANDRA (1962) has been used. To conclude the validity of the results, comparison of the values for adults from the two methods was attempted.

Groups A and B were kept in the laboratory from the hatching on. The other groups were brought to the laboratory six weeks before the experiments started. All birds were kept on a standard diet containing 1.1 ppm iodine. The temperature and the illumination were standardized.

Experiment 1

Each bird in groups C, D, E and F was injected s.c. with a dose of 7 μCi/100 g b.w. of carrier-free $Na^{131}I$. Radioactivity above the thyroid region was measured *in vivo* every day according to the principles of REINEKE and SINGH (1955) and PIPES et al. (1958). After suitable correction (for background radioactivity, geometry and isotope decay) the *in vivo* thyroidal radioactivity data (expressed as ratios of the injected dose) were used for calculation of the thyroid hormone secretion rate (TSR) and the kinetic parameters (U, b, k_4' and k_4)* of thyroid hormone secretion. The data measured between the 3rd

*1. U = thyroidal ^{131}I uptake, expressed as a proportion of the injected dose, after extrapolation to zero time (SINGH et al. 1968). 2. b = slope of the semilogarithmic regression line calculated between the time (x) and the logarithm of the thyroidal ^{131}I radioactivity. This characterizes the degree of ^{131}I release from the thyroid (SINGH et al., 1968, 1968a, b). 3. k_4' = thyroidal iodine release-rate constant. k_4 = thyroidal release-rate (turnover rate) constant corrected for recirculation.

Table I

Thyroid indices for animals on methimazole diet

Experimental groups	No. of birds	Age, weeks	Body weight, g	U	b_2 met	b_1 met $+T_3$	^{131}I retention	^{131}I $t_{1/2}$ in days	TSR according to PIPES et al.
A	11	10	1427±37	0.18±0.02	—0.07±0.01	—0.05±0.02	0.87±0.01	5.0±0.6	3 μg/day
B	7	10	1725±79	0.32±0.04	—0.04±0.01	0	0.91±0.03	6.1±0.9	less than 2 μg/day
C	7	79	2172	0.13±0.01	—0.023±0.003	—0.006±0.001	0.94±0.003	15.2±0.9	Approximately 2—3 μg/day
D	6	79	910±36	0.11±0.02	—0.04±0.01	—0.04±0.01	0.83±0.01	5.6±0.7	*
E	7	79	1720±66	0.10±0.008	—0.03±0.004	—0.03±0.007	0.95±0.004	16.1±0.7	*
F	6	79	1097±42	0.13±0.02	—0.05±0.005	—0.05±0.01	0.87±0.008	5.0±0.7	*
Statistical differences between bursectomized (B) and control group: (A)			0.2>P>0.1	0.2>P>0.1	0.4>P>0.2	0.01>P>0.005 highly significant	0.2>P>0.1	0.2>P>0.1	0.05>P>0.01

met = data relating to the methimazole-treated birds (2nd—8th days of Experiments 2 and 3).

met + T_3 = data relating to methimazole + exogenous T_3-treated birds after 1 day of Experiments 2 and 3.

* Impossible to determine exactly, because of the change in the thyroidal ^{131}I release rate. After the release had been inhibited by a given dose of T_3 following a larges dose, the release rate increased again.

and 18th days of the experiment were used in the formulas of SINGH et al. (1967, 1968a, b) in the "direct output" method for the estimation of the TSR and the mentioned thyroid indices.

On the 18th and 19th days the birds were injected s.c. with 0.3 I.U./100 g b.w. TSH, and the *in vivo* measurement of thyroidal radioactivity was continued in every second hour for 72 hours.

Experiment 2

Each bird in groups A and B was injected with a 200 μg/bird dose of carrier-free $Na^{131}I$. The radioactivity of the thyroidal region was measured *in vivo* every day as in Experiment 1. After 48 hours the food and drinking water of the birds were supplemented with 0.1% or 0.05% methimazole, according to the TSR-estimation method elaborated by PIPES et al. (1958). After 8 days the birds were injected with a 2, 3 or 5 μg/100 g b.w./day dose of L-triiodothyronine (T_3) every second day. In practice, on the 8th, 10th and 12th days the birds were injected with 4, 6 and 10 μg/100 g b.w. T_3. A dose which inhibited the decrease of the thyroidal ^{131}I radioactivity was considered equal to or larger than the spontaneous thyroidal hormone release.

Experiment 3

Two months after Experiment 1 individuals of groups C, D, E, F were injected with 300 μg of carrier-free $Na^{131}I$. Their TSR values were estimated as in Experiment 2. The only difference was that these birds were injected with 10 μg/100 g b.w./day T_3 on the 13th, 14th, 15th days, too. The radioactivity data from this period (between the 9th and 15th days of the experiment) were used, together with the data for the earlier experimental period when goitrogen was given alone, for calculation of the regression lines for the "methimazole + T_3" and "methimazole" periods. The regressions of release curves were calculated in the same manner as in the "direct output" method of SINGH et al. (1968) and thyroid secretion indices were also calculated (Table I).

The thyroidal release kinetic indices,* *viz.* k_{4met} and k_{4met+T_3}, were compared with the kinetic indices k_{4c} relating to the spontaneous thyroidal iodine release of untreated birds measured as in Experiment 1.

On the 15th day birds were injected with 0.3 I.U./100 g b.w. TSH and the *in vivo* measurements of thyroidal radioactivity were continued in every

* k_{4c} = constant relating to untreated control birds;
k_{4met} = constant relating to birds fed with methimazole;
k_{4met+T^3} = constant relating to methimazole and triiodothyronine-treated birds;
k_{4TSH} = constant relating to TSH-treated birds;
$k_{4TSH+met}$ = constant relating to birds kept on methimazole diet and treated with TSH.

second hour for 48 hours and the data were used for calculation of TSH and other thyroid parameters concerning the TSH effect. The results of Experiments 1 and 3 are presented in Table II.

Table II

TSR values calculated by the "direct output" method of SINGH et al. (1968a) for adult birds on normal and methimazole-containing diet

Group	Treatment	Normal diet μg T$_3$/100 g b.w./day	Methimazole diet μg T$_3$/100 g b.w./day
C	Control	0.815±0.119	1.883±0.305
C	TSH	5.021±1.220	4.441±1.312
D	Control	1.111±0.236	4.533±1.636
D	TSH	18.974±4.221	11.878±3.311
E	Control	0.645±0.234	1.989±0.640
E	TSH	12.745±3.693	11.380±4.040
F	Control	1.918±0.401	6.298±1.215
F	TSH		18.276±3.886
C	T_3-supplemented*	—	0.579±0.149
C	T_3-supplemented	—	2.802±0.754
E	T_3-supplemented	—	1.414±0.358
F	T_3-supplemented	—	6.213±0.852

* The dose of T_3 (triiodothyronine) see in the text

Results and discussion

The comparable data from the six groups are presented in Table I. The body weights of the broilers are almost the same as those of group E adults. The data for the adults can be divided, but not sharply, into those for groups D and F and to those for groups C and E. The surgical bursectomy did not influence the thyroid function dramatically.

The U values can be considered characteristic of the thyroial iodine uptake (SINGH et al., 1968). These values for the broilers are larger than those for the adults of the different breeds and hybrid combinations, although this difference is statistially not significant. The values for group B are on the average twice those for group A, nevertheless, this difference is again not significant. This indicates that some effect is exerted by the bursa on the uptake function of the thyroid, but this effect can only be studied on genetically selected populations.

The ^{131}I retention and biological half-life ($t_{1/2}$) data differed significantly between groups C + E and D + F, but there was no significant difference between the bursectomized and the control broiler cockerels. The TSR values caculated by SINGH's (1968) method for adults kept on normal and methimazole-containing food are presented in Table II.

Table III

Slopes b of ^{131}I release curves (regression lines) of birds kept on methimazole diet (1st—8th days of Experiments 2 and 3)

b intervals	10-weeks-old broilers		Adult breeds and hybrids			
	A	B	C	D	E	F
—0.01 to —0.05	5	5	7	4	7	5
	(70%)	(45%)	(100%)	(66%)	(100%)	(63%)
—0.05 to —0.10	1	5	—	—	—	3
	(15%)	(45%)	0	0	0	(37%)
—0.10 to —0.15	1	—	—	2	—	—
	(15%)	0	0	(33%)	0	0
—0.15 to —0.20	—	1	—	—	—	—
	0	(10%)	0	0	0	0

χ^2 values: group A: 3.97 (n.s.); groups A+B: 1.17 (n.s.): groups A—F: 3.79 (n.s.); groups C—F: 12.20 (n.s.); but the tendency towards inhomogeneity is higher than in the case of groups A—F.

Statistical comparison of b values by Student's *t*-test:

A	B	C	D	E	F
A	n.s.	n.s.	n.s.	n.s.	n.s.
	B	0.1>P>0.05	n.s.	0.1>P>0.05	n.s.

The distribution of the b values (SINGH et al., 1968) calculated from birds fed with methimazole are presented in Table III. According to the χ^2 analysis, the surgical bursectomy decreased the rate of the secretion. While the b data for group A could be considered similar to those for group D and E adults, for group B they seem to resemble those of groups C and E. On the other hand, the tendency towards inhomogeneity is the largest in groups C—F, it is smaller in groups A—F, and is the smallest in groups A—B. This indicates that studies on the relationship between the bursa of Fabricius and the thyroid function

can only be exact when made on genetically-selected homogeneous populations.

The TSR data estimated by the method of PIPES et al. (1958) seem to be suitable for use as indices of thyroid function influenced by surgical bursectomy. The smallest (2 μg/100 g/day) dose of T_3 applied by us was enough to inhibit the iodine secretion of the bursectomized (B) animals, whereas 3 and 5 μg doses of this hormone were required to do so in control broiler cockerels (A). Unfortunately, this index of thyroid function can be considered a questionable one (REINEKE and SINGH, 1955; REINEKE 1964, 1965; TANABE, 1964; SINGH et al., 1968). We found in two instances in group B that birds which were inhibited with a 2 μg dose of T_3, on the subsequent days began to release iodine from thyroids again when they were injected with 5 μg dose of T_3. The same phenomenon was observed in one case in group A, when 3 μg T_3 proved an inhibiting, and 5 μg a "re-releasing" dose. In adult birds (C—F) this phenomenon was observed more frequently. This aspecific type of iodine-release observed earlier by REINEKE and SINGH (1955) cannot be considered, as it is probably a non-specific disturbance in the intrathyroidal iodine metabolism. The question has remained open as to whether the TSR value estimated by this method and influenced by this phenomenon could be correlated with the

Table IV

Comparison of thyroidal iodine turnover rate constants k_4 determined by the "direct output" method for animals on normal and methimazole diets

Group	Treatment	k_{4c}	k_X	k'_{4met}
C	Control	0.0256±0.0028	0.0260±0.0099	0.0517±0.0061
D	Control	0.0318±0.0057	0.0536±0.0231	0.0854±0.0217
E	Control	0.0207±0.0047	0.0402±0.0087	0.0606±0.0079
F	Control	0.0375±0.0174	0.0709±0.0265	0.1079±0.00207
C	TSH-treated	0.1500±0.0279		0.1511±0.0032
D	TSH-treated	0.4734±0.0423		0.2694±0.0441
E	TSH-treated	0.4406±0.0290		0.3006±0.0400
F	TSH-treated	0.4660±0.0540		0.3098±0.0362
C	met. + T_3			0.0361±0.0217
D	met. + T_3			0.0751±0.0194
E	met. + T_3			0.0585±0.0106
F	met. + T_3			1.1051±0.0102

* $k_X = k'_{4met} - k_{4c}$. Hypothetically, this constant characterizes the iodine release when it is blocked to the degree as described by TANABE and KOMIYAMA (1962). k_{4c} and k'_{4met}: see in the text.

TSR values estimated by the more exact but slower "direct output" method (SINGH et al., 1968). Some conclusions can be drawn from a comparison of the results of Experiments 1 and 3, because the objects of these experiments were the same individuals. The data of the "direct output" method as well as those of Experiment 3 used in calculations such as the "direct output" method, are presented and compared in Tables II, III and IV. As regards the data measured on the methimazole diet, k_4' values were used instead of k_4 because in this case the calculation of the iodine recirculation is not necessary. The data in Table II indicate that the iodine secretion of birds on methimazole diet was much more intensive than of those on normal diet. Exogenous thyroid hormone decreased the thyroid release, but only in the case of group C did the values of TSR (and k_4) approach those measured in animals on the normal diet and in no case was a complete inhibition of iodine release found. According to the hypothesis of PIPES et al. (1958), the hormone release on the methimazole diet must exceed the normal because of the inhibition of recirculation. If this were so, the k_{4c} and k'_{4met} should be the same for a given individual. However, this is not the case.

Table V

Correlations between the individual values of k_{4c}, k_{4met} and k_x

The two indices examined	All birds	Correlations			
		C	D	E	F
$k_{4c} - k'_{4c}$	+0.83	+0.92	+0.73	+0.86	+0.90
$k_{4c} - k'_{4met}$	+0.69	+0.23	+0.94	+0.20	+0.52
		n.s.		n.s.	
$k_{4c} - k_x$	+0.035	—0.30	+0.89	—0.37	+0.007
	n.s.	n.s.		n.s.	n.s.
$k_{4TSH} - k'_{4TSH+met}$	+0.79	+0.92	n.s.	+0.85	+0.77

Indeed, the difference (k_x) between these two indices is nearly the same as k_{4c}. According to the method of TANABE and KOMIYAMA (1962), nearly the same values of TSR can be calculated when using the "direct output" method. The TANABO—KOMIYAMA method is a modification of that of PIPES; the inhibition of iodine release from the thyroid is only partial: the release is inhibited to the normal level and not completely. Our results indicate that when the TANABE—KOMIYAMA method is used similar to k_{4c} but there is very little correlation between k_4 and k_x when calculated for the same individuals. On the other hand, the correlation between k_{4c} and k'_{4met} proved to be high (+0.96

on the average, but different in different groups of animals), showing that although the two indices of the thyroid function are of different nature, they are not independent of each other.

Summary

The effect of surgical bursectomy on some parameters of the thyroid function of growing broiler cockerels was studied.

It has been found that while 2 μg/100 g b.w. dose of exogenous triiodothyronine was completely able to inhibit the thyroid secretion of bursectomized birds, as much as 5 μg/100 g b. w. of the hormone was incompletely able to inhibit that of the controls, when the animals were kept on methimazole diet. The comparison of some *in vivo* TSR-estimation method showed the usefulness of the SINGH—REINEKE "direct output" method and gave a possible explanation of correct TSR data estimated by the technique of TANABE and KOMIYAMA (1962).

References

BRASWELL, E. H., MCDUFFIE, F. C. and GLICK, B.: Poultry Sci. **44** (1965), 1354. COLE, R. K.: Genetics **53** (1966), 1021—1023. COLE, R. K., KITE, J. H. JR. and WITEBSKY, E.: Science **160** (1968), 1357—1358. COLE, R. K., KITE, J. H. JR., WICK, G. and WITEBSKY, E.: Poultry Sci. **49** (1970), 839—848. GLICK, B. In: GOOD, R. A. and GABRIELSEN, A. E. (Eds.): The Thymus in Immunobiology. New York, 1964, pp. 343—359. GLICK, B.: Fed. Proc. **26** (1967), 704. GLICK, B.: Poultry Sci. **47** (1968), 807—813. GLICK, B., CHANG, T. S. and JAAP, R. G.: Poultry Sci. **35** (1956), 224—226. HUTT, V. B.: The Genetics of the Fowl. New York—Toronto—London, 1949. ISAKOVIĆ, K. and JANKOVIĆ, B. D.: Int. Arch. Allerg. **24** (1964), 226—310. MAY, D. and GLICK, B.: Poultry Sci. **43** (1964), 450—453, NILSSON, L. A. and ROSE, N. R.: Immunology **22** (1972), 12—23. PIPES, G. W., PREMACHANDRA, B. N. and TURNER, C. W.: Poultry Sci. **37** (1958), 36—41. PREMACHANDRA, B. N.: Paper presented at XIIth World's Poultry Congress, Sidney, Australia, August 1962. PREMACHANDRA, B. N., PIPES, G. W. and TURNER, C. W.: Poultry Sci. **38** (1959), 795—798. PUNETT, R. C. and BAILEY, P. G.: J. Genetics **4** (1914), 23—29. REINEKE, E. P.: Fed. Proc. **23** (1964), 203. REINEKE, E. P.: Physiologist **8** (1965), 257. REINEKE, E. P. and SINGH, O. N.: Proc. Soc. Exp. Biol. Med. **88** (1955), 203—207. SINGH, A., REINEKE, E. P. and RINGER, K.: Gen. Comp. Endocrinol. **9** (1967), 353—361. SINGH, A. and REINEKE, E. P.: Gen. Comp. Endocrinol. **10** (1968), 296—303. SINGH, A., REINEKE, E. P. and RINGER, K.: Poultry Sci. **47** (1968a), 205—211. IDEM: Ibidem **47** (1968b), 212—218. SNEDECOR, J. G.: Gen. Comp. Endocrinol. **10** (1968a), 277—281. IDEM: Poultry Sci. **47** (1968b), 148—151. SNEDECOR, J. G. and CAMYRE, M. F.: Gen. Comp. Endocrinol. **6** (1966), 276—287. TANABE, Y.: Endocrinol. Japon. **311** (1964), 260—264. TANABE, Y. and KOMIYAMA, T.: Endocrinology **70** (1962), 142—148. TIENHOVEN, VAN A. and COLE, R. K.: Anat. Rec. **142** (1962), 111—118. TIENHOVEN, VAN A., SIMKIN, D., WESKE, J. and BARR, G. R.: Endocrinology **76** (1965), 194—197. WARNER, N. L. and SZENBERG, A.: Ann. Rev. Microbiol. **18** (1964), 253—268. WICK, G., KITE, J. H. JR., COLE, R. K. and WITESBKY, E.: J. Immunol. **104** (1970a), 45—53. WICK, G., KITE, J. H. JR. and WITEBSKY, E.: J. Immunol. **104** (1970b), 54—62. IDEM: Ibidem **104** (1970c), 433—352. WILSON, J. and GLICK, B.: Poultry Sci. **45** (1966), 892—896. WITEBSKY, E., KITE, J. H. JR., WICK, G. and COLE, R. K.: J. Immunol. **103** (1969), 708—715.

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IN VITRO STUDIES ON DOMESTIC FOWL THYROIDS

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The thyroid of fowls is more sensitive to thyrotropine (TSH) than the mammalian thyroid (BERGMAN and TURNER, 1933; BATES, 1962; NEWCOMER, 1967, 1968) and there are intraspecies genetic differences in the degree of this sensitivity (FODOR and PETHES, 1974). Owing to the high sensitivity of chickens, many TSH titration methods have been evolved (BATES et al., 1957; LAMBERG, 1953; FREY and ALBERT, 1959; MESS and HÁMORI, 1961; WAHLBERG, 1953; MESS, 1962; TAKÁCS, 1962; KRAWCZUK et al., 1963; CREEK, 1965), but no *in vitro* assay has been applied to this species. On the other hand, the *in vitro* biotitration of TSH proved to be a fairly good method when mammalian (sheep bovine, porcine, *etc.*) thyroids were used as target. Some of these methods are based on measuring the changes in weight of thyroid slices (BAKKE et al., 1957), others on the iodine uptake (BAKKE and LAWRENCE, 1954a, b) or release (TSUI and OGURA, 1960), by the thyroid and still others on the measurement of the release of hormonal iodine from the glands (BOTTARI and DONOVAN, 1958; BOTTARI et al., 1963). The use of thyroid slices from larger animals would be more practical, for different TSH preparations can be tested on thyroid slices from a given animal. However, because of the heterogeneity of the thyroid gland (WOLLMAN, 1965), the distribution of active follicles within a gland is uneven. In contrast, the chicken gives a fairly homogeneous reponse to TSH, therefore, the chance of working out a more exact TSH assay seemed to be more promising.

The present work was aimed at examining the possibilities of evolving an *in vitro* system in which domestic fowl would be the test animal for TSH biotitration.

Materials and methods

The *in vitro* system employed was a slightly modified version of an earlier system (MORTON and CHAIKOFF, 1943; FRANKLIN and CHAIKOFF, 1943; FRANKLIN et al., 1944).

Experiment 1

Five 1-year-old Bantam (HUTT, 1949) cockerels were injected with 4 μCi/100 g b.w. of carrier-free $Na^{131}I$. After 24 hours the birds were killed, their thyroids were individually sliced and the slices were divided into two which were then weighed. The slices were put into ice-cold Parker's medium 199, washed five times by centrifugation in 40 ml medium and transferred into incubation tubes both of which contained 3 ml of Parker's medium supple-

mented with glucose and water to make it optimum for bird tissues. One of the tubes contained 10^{-3} I.U. of TSH ("Ambinon", Organon) and finally, 5 tubes were used to test the effect of TSH on the release of hormonal iodine, together with 5 tubes containing control slices from the corresponding individuals. During a 6-hour incubation at 40 °C, a gas mixture, consisting of O_2 and 5% CO_2, was bubbled through the medium. The media were changed every hour and measured for ^{131}I radioactivity with an EKCO apparatus.

Experiment 2

Four "Hunnia" broiler cockerels were killed. Each thyroid was sliced, divided into four, these were weighed and washed and placed each into an incubation tube. Each of the 16 tubes contained 1 μCi ^{131}I. Two of the four parts of the sliced thyroid of each bird were incubated individually for two hours as indicated in Fig. 2. The other two parts served as controls. The radioactivities of the thyroid slices were determined after suitable washing in order to determine the degree of iodine uptake. The parallel values were averaged.

Experiment 3

Experiment 2 was repeated, except that every thyroid slice was incubated in the absence of TSH and a 90-min incubation period was employed. The thyroid slices were washed (see, Experiment 1) and measured for ^{131}I radioactivity. They were then incubated again in inactive medium, in the presence and absence of TSH, exactly as in Experiment 2. After this second incubation, the radioactivities of the incubation media were measured and expressed as percentages of the ^{131}I activities of the corresponding thyroid slices as determined between the two incubations.

Experiment 4

"Hunnia" broiler cockerels were injected with 10 μCi ^{131}I/100 g b.w. $Na^{131}I$. After 72 hrs the animals were killed and their lobes of thyroid were incubated twice in inactive media as, described for Experiment 1 each time for 90 min. The lobes of the thyroid of each animal were divided into four, one of which served as the control, the second for testing the effect of TSH in a dose of 1 I.U./tube, the third for testing the effect of methimazole and the fourth for testing the effect of methimazole (0.01%) + TSH. To the medium in tubes 1 and 3 bovine albumin was added to equalize the protein concentration in all tubes. After the first and second incubations the radioactivities of the media and the slices were measured and then the latter were treated with an equal volume of 30% trichloroacetic acid (TCA) solution. The precipitate was washed

once with 30% TCA and measured for ^{131}I radioactivity. The activities of the thyroid slices, the incubation media and the TCA-precipitated proteins were determined, and are given in Fig. 4. The standard error of the mean did not reach 10% in either case.

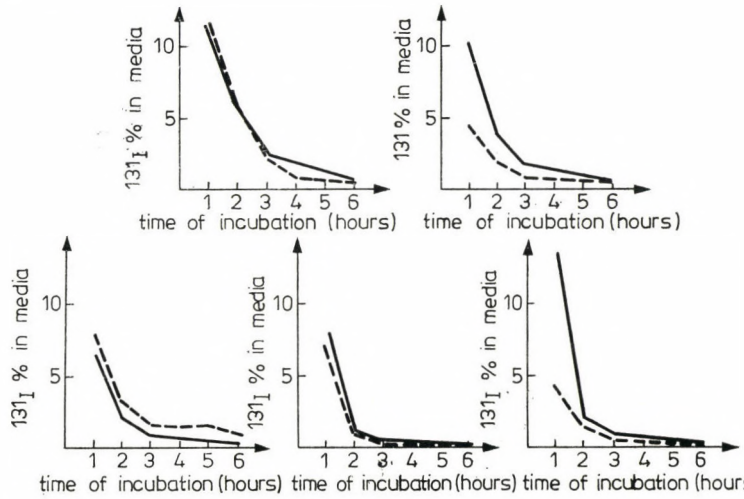

Fig. 1. Demonstration of Experiment 1. The radioactivities of incubation media as a function of the time of incubation and expressed as percentages of the radioactivity of the corresponding thyroid slices. — — — — those parts of the thyroids which were incubated in the absence of TSH; ———— media in which parts of the thyroids were incubated in the presence of a dose of TSH. The output-rate decreased considerably during the 6-hour period. ^{131}I %, the dose of ^{131}I expressed as per cent of *in vivo* injected dose

Results

Experiment 1

As shown in Fig. 1, the rate of thyroidal release of the cockerels is fast *in vitro*, faster than observed in the literature *in vivo*. This can be considered a non-specific release, and is in agreement with the results of MOLNÁR et al. (1956a, b). It is therefore not a suitable index for testing any effect (including that of TSH) influencing the iodine release by the thyroid.

Experiment 2

The results of this experiment are shown in Fig. 2. Although a negative correlation can be observed between the applied dose of TSH and the ^{131}I uptake by the thyroid, as in the experiments of BAKKE and LAWRENCE (1954a, b), it seems that the individual variance in spontaneous uptake is too large to use the uptake as an index of the applied TSH dose.

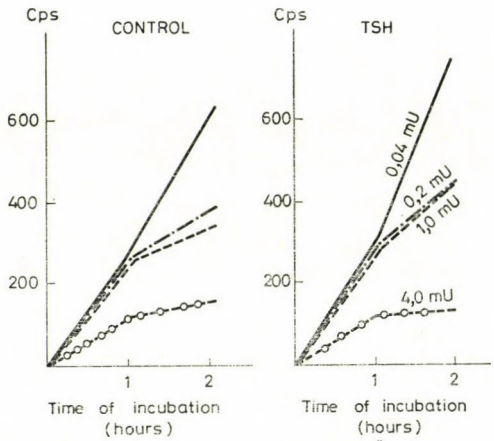

Fig. 2. Demonstration of Experiment 2. The *in vivo* uptake of ^{131}I by thyroid slices in the presence and absence of TSH. Left: data of control thyroid slices; right: data of thyroid slices incubated with TSH. The same type of line demonstrates the thyroid of the same cockerels. ———— bird No. 1; —·—·—·— bird No. 2; — — — — bird No. 3. —○—○—○—bird No. 4. The effects of different doses of TSH were not evaluable under these experimental conditions

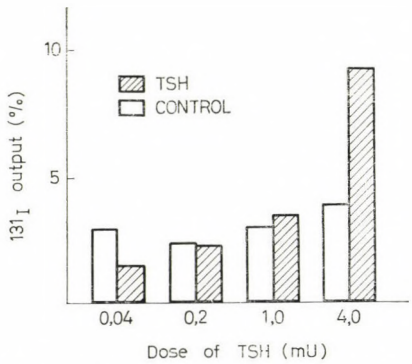

Fig. 3. Demonstration of Experiment 3. In a system similar to that of TSUI and OGURA (1960), the thyroid slices of cockerels seem to be a suitable target for biotitration of different doses of TSH. /// TSH; □ control

Experiment 3

This experiment is reminiscent of the system of TSUI and OGURA (1960). It can be seen in Fig. 3 that the degree of release of ^{131}I taken up *in vitro* is a better index for titration of TSH than the uptake. The individual deviations of the control values were also much smaller. It seems that the method of TSUI and OGURA, after refining and a better adaptation for fowl or chicken, may be a promising bioassay for TSH.

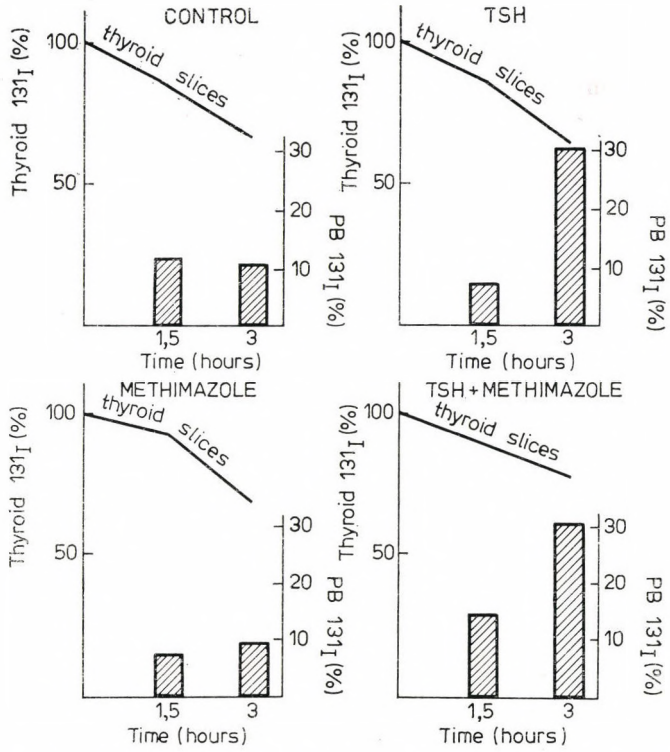

Fig. 4. Demonstration of Experiment 4. While the degree of release of organic ^{131}I is not suitable for differentation between the effects of different TSH doses, the protein-bound (PB) ^{131}I raadioactivities of the incubation media proved to be suitable indices. The theoretical explanation of the sensitivity of this parameter to the presence of TSH is not known yet. Ordinate (left): radioactivity of thyroid slices expressed as percentage of the ^{131}I content before incubation. Ordinate (right): radioactivity of PB ^{131}I expressed as percentage of the radioactivity of the incubation medium. Abscissa: incubation time in hours. Columns-PB ^{131}I radioactivity; Curves: radioactivities of the thyroid slices

Experiment 4

The results of this experiment are demonstrated in Fig. 4. The decrease of the ^{131}I radioactivity of the thyroid was much more intensive than *in vivo* and is not suitable for testing any effect in TSH, just as was found in Experiment 1. However, the release curve declined less rapidly than in Experiment 1, indicating that after 72 hrs there was practically only organified or reinorganified ^{131}I in the gland. This is supported by the fact that methimazole did not affect the degree of release. Surprisingly, however, the radioactivity of the TCA-precipitated fraction of the incubation medium was significantly higher in the presence of TSH than in its absence.

The measurement of the TCA-precipitated radioactivities of the incubation media of thyroid slices of birds treated 3 days earlier with ^{131}I proved the best index for biotitration of TSH in our experiments.

Discussion

Experiments 1 and 2 it have shown that the principles of the BAKKE and LAWRENCE method cannot be used for fowl. MOLNÁR et al. (1956a, b) showed that in mammals, too, the thyroidal iodine release *in vitro* is much greater than that *in vivo*. They worked out a "gravity flow" method, by which this non-specific phenomenon could be avoided and explained it theoretically, too. Nevertheless, for routine work, the BAKKE and LAWRENCE method is widely used when mammalian thyroids are involved.

The principle of TSUI and OGURA (1960) seems to be suitable for application to chicken, too. Considering the higher sensitivity of this species to TSH and the possibilitiy of working on selected homogeneous strains characterized by a given degree of sensitivity to TSH (FODOR and PETHES, 1974), it seems a promising TSH biotitration method.

The sensitivity to TSH of the protein-bound radioactivity of the incubation media of thyroids containing organified iodine is somewhat surprising. Theoretically, it is difficult to explain it in the knowledge of the mechanism of thyroid secretion (PASTAN and WOLLMANN, 1967). A possible explanation would be that TSH *in vitro* has a more intensive effect on the carriers of follicle iodoprotein droplets than on the proteolytic and deiodinating processes. Further studies are in course to explain this phenomenon and to use this promising index in a TSH-biotitration method in practice.

Summary

A search for valuable parameters for biotitration of TSH in an *in vitro* system, with the domestic fowl as test animal species, showed the principle of the TSUI and OGURA (1960) method to be applicable for this species. The protein-bound ^{131}I activity of incubation media of thyroid slices of birds pretreated with ^{131}I was found to be a promising index of the *in vitro* effect of TSH.

References

BAKKE, J. L. and LAWRENCE, N.: Am. J. Med. **17** (1954a), 116. IDEM: J. Clin. Endocr. **14** (1954b), 771. BAKKE, J. L., HEIDEMAN, L. M., LAWRENCE, N. L. and WIBERG, G. C.: Endocrinology **61** (1957), 352—353. BATES, R. W.: The Chick Method of Thyrotropin Essay. In: WERNER, S. C., GARRISON, R. V. (Eds): Thyrotropin. Springfield, Ill., 1973. BATES, R. W., CORNFIELD, J., LASKEY, A. M. and HOWARD, T. M.: Endocrinology **60** (1957), 225—238. BERGMANN, A. J. and TURNER, C. W.: Endocrinology **24** (1933), 656—664. BOTTARI, P. M. and DONOVAN, B. T.: J. Physiol. **140** (1958), Suppl. Proc. Physiol. Soc., 36—37. BOTTARI, P. M., DANOVAN, B. T. and EL-KABIR, D. J.: J. Physiol. **169** (1963), 278. CREEK, R. O.: Endocrinology **76** (1965), 1124—1135. FODOR, A. and PETHES, G.: J. Gen. Comp. Endocrinol. Biochem. **24** (1974), 140—151. FRANKLIN, A. L. and CHAIKOFF, I. L.: J. Biol. Chem. **148** (1943), 719—720. FRANKLIN, A. L., LERNER, S. R. and CHAIKOFF, I. L.: Endocrinology **34** (1944), 165—168. FREY, H. M. and ALBERT, A.: Endocrinology **64** (1959), 304—307. HUTT, F. B.: The Genetics of the Fowl. New York, Toronto, London, 1949. KRAWCZUK, A., DZIERZANOVSKI, E. and PROKOPCZUK, J.: Annales d'Endocrinologie **24** (1963), 864—865. LAMBERG, B. A.: Acta Endocrinol. **18** (1953), 405—420. MESS, B.: In: A kísérletes orvostudomány vizsgáló

módszerei (Ed.: KOVÁCH, A.). Budapest, 1962, Vol. VI, pp. 318—329. MESS, B. and HÁMORI, J.: Acta physiol. hung. **20** (1961), 229—304. MOLNÁR, G. D., ALBERT, A., KEATING, R. F. JR. and OVRIS, A. L.: Clin. Endocr. Metab. **16** (1956a), 927. MOLNÁR, G. D., KEATING, R. F. JR., OVRIS, A. L. and ALBERT, A.: Endocrinology **58** (1956b), 501—511. MORTON, M. E. and CHAIKOFF, I. L.: J. Biol. Chem. **147** (1943), 1—9. NEWCOMER, W. S.: Am. J. Physiol. **212** (1967), 1391—1396. IDEM: Gen. Comp. Endocrinol. **11** (1968), 539—547. PASTAN, I. and WOLLMAN, S. H.: Am. J. Cell. Biol. **35** (1967), 262. SHELLABARGER, C. J.: J. App. Physiol. **6** (1954), 721—723. TAKÁCS, L.: In: Kísérletes orvostudomány vizsgáló módszerei (Ed.: KOVÁCH, A.). Budapest, 1962, Vol. VI, pp. 329—358. TSUI, Sh. and OGURA, H.: Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. **238** (1960), 391—418. WAHLBERG, P.: Acta Endocrinol. Suppl. **23** (1955), 1—79. WOLLMANN, S. H.: Heterogeneity of the Thyroid Gland. In: Current Topics of Thyroid Research (Eds: CASSANO, O. and ANDREOLI, M.). New York, 1965.

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VACCINATION EXPERIMENTS ON PREVENTION OF *E. COLI* DIARRHOEA IN SUCKLING CALVES

I. USE OF ALUMINIUM GEL ADJUVANT

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Gastro-enteritis arising from various causes is responsible for great losses among newborn calves in large cattle farms. In Hungary, on the average 5.2 to 6.8% of all newborn calves die of diseases of digestive organs before two weeks of age (MÉSZÁROS, 1972). The major part of losses is due to diarrhoea caused by *Escherichia coli* bacteria. The disease in general is an enteritis, the septicaemic form in contrast to other countries, being rare. Predisposing factors and *E. coli* strains belonging as a rule to several O groups play a major aetiological role. Formerly, strains belonging to groups O8, O9, O41, O78 and O101 (ÁLDÁSY, 1955, 1959), recently those having the antigenic structures O101:K30(A), O101:K28(A), O101:K(A), O20:K·, O41:K(A) and O8:K28(A) (VARGA and FARID, 1974) have been most frequently isolated from carcases of suckling calves died of enteritis.

The diseased animals are treated with antibiotics, usually by the oral route. Losses are nevertheless great because the *E. coli* serotypes pathogenic for calves are generally resistant to all or most of the commercially available antibiotic preparations. Statistical data of the calf herds in more than 30 cattle farms have shown that, despite antibiotic therapy, 32—43% of the calves with *E. coli* enteritis are dying (KOVÁCS et al., 1972).

Since *E. coli* diarrhoea in the calf herds occurs frequently and the therapy often fails, experiments were performed on prevention by immunization of pregnant cows with *E. coli* vaccines.

Materials and methods

Isolation, biochemical and serological properties of E. coli *strains*

To identify the serotypes responsible for outbreaks, a total of 104 *E. coli* strains were isolated from the carcases of 45 suckling calves in 14 cattle farms. All calves had died with symptoms of *E. coli* diarrhoea before reaching two weeks of age, and the epizootiological course and gross lesions were also characteristic of this condition. Strains were isolated from the small intestine, the mesenteric lymph nodes and, in a few cases, also from other organs. Two to three isolates were obtained from each carcase. The isolates were tested for biochemical behaviour, and were examined serologically with 57 O or OK sera and with all K sera (1–94) except three (VARGA and FARID, 1974). The biochemical tests, preparation of antisera, and agglutination tests were carried out as described by KAUFFMANN (1966) and EWING et al. (1956).

Vaccines

Two monovalent vaccines and one trivalent vaccine were prepared. The fresh isolates selected as vaccine strains were cultured separately for 12 hours in broth $D_{1,5}$ (SCHLECHT and WESTPHAL, 1966). The cultures containing 10^9/ml live *E. coli* bacteria, were checked for purity and K antigen, germ counts were determined, and inactivation was made in 0.15% formalin for 24 hours at 37 °C. The inactivated cultures were tested for sterility and a vaccine containing 5 mg/ml $AlPO_4$ precipitate was prepared. The pH was adjusted to 6.8—7.0 and merthiolate was added for preservation. The two monovalent vaccines were prepared from strains O101:K30(A) and O8:K·, respectively, while the trivalent preparation contained equal amounts of strains O101: K30(A), O8:K28(A) and O78:K80(B).

Vaccinations

Vaccinations were carried out in seven cattle farms, in one each with the two monovalent vaccines and in five with the trivalent preparation. Each cow was vaccinated twice by the subcutaneous route, viz., with 20 ml four weeks before the expected term of calving and again with 20 ml two weeks before term. Unvaccinated cattles of varying numbers, kept in the same house as the vaccinees, served as controls. The vaccinations were performed in the period from December to June.

Sampling, blood and milk tests

Two blood samples and one colostrum sample were collected from each cow. The first blood sample was withdrawn before the first vaccination, the second one week after calving. Considering that the concentration of colostral immunoglobulins rapidly declines (ÁLDÁSY and ERŐS, 1969), the colostrum sample was usually taken on the day of calving or within three days thereafter. From a few vaccinated cows serial samples of colostrum were collected on days 1 to 5, to assess the decrease of antibody level.

Blood sera, and colostral whey obtained by clotting with a milk coagulant and filtration, were tested for O antibodies by an indirect haemagglutination test (IHA), using TAKÁTSY's (1955) Microtitrator method. The sheep erythrocytes used in this test were presensitized with the O antigens of the *E. coli* vaccine strains as proposed by WILSON and SVENDSEN (1971), except that the erythrocytes had been washed in phosphate buffer. The erythrocytes used in tests of samples from cows treated with the trivalent preparation were presensitized with a pool of equal amounts of O8, O78 and O101 antigens (BERCZI et al., 1967).

K antibodies were demonstrated in serum and colostral whey by tube agglutination test (KAUFFMANN, 1966), titrating with the K antigen of each vaccine strain separately.

Results

The serological identification of *E. coli* isolates from calves that died before the vaccination experiment is shown in Table I. 62.4% of all isolates were identifiable with the available sera. The isolates obtained from dead calves in 14 cattle farms belonged to nine O serogroups. All strains possessed a K antigen. A substantial part of the isolates belonged to group O101; these had one of three (or more) different K antigens, *viz.*, O101:K28(A), O101:K30(A) and O101:K(A). The strains of the latter pattern contained K(A) antigens, as they were agglutinable by O serum only after autoclaving for two hours at 2 att, but they were not agglutinable either by any K serum. The antigenic patterns of the strains belonging to serogroup O8 were identified as O8:K27(A) and O8:K28(A).

Isolates from only one farm among the 14 were not identifiable serologically. In the majority of the farms the same serotype was isolated from all dead calves. In only one farm were strains belonging to two different O serogroups responsible for the disease and death of two calves kept in one and the same house.

The antigenically identifiable isolates proved to be resistant to most commercially available antibiotic preparations. The antibiograms are shown in Table II. The *E. coli* strains occurring in calves in the seven vaccinated herds were all resistant to streptomycin, oxytetracycline, Neomycin and chloramphenicol. Some strains were moderately sensitive to furadantine or polymyxin B, but in certain herds all isolates were resistant to each of the above six antibiotics. The antibiotic sensitivity of the isolates from one and the same herd was always uniform and independent of the serological type.

The clinical results of vaccination are shown in Table III. The serological type of the strain used in the monovalent vaccine was always the same as that of the isolate obtained from the given herd. In the trivalent vaccine the K antigen and, if possible, also the O antigen of one of the vaccine strains corresponded to that of the serotype responsible for the disease. Table III shows that trivalent vaccine was also applied in herds in which strains of O21:K(B?) had been isolated. The reason was that the latter strains were agglutinable in living state by OK antiserum to the type strain O78:K80(B) up to the latter's end titre. It was found in earlier studies (VARGA and FARID, 1975) that, although the K antigen of these strains is not identical with K80(B), it is closely related to the latter, so that antibodies to O78:K80(B), appearing in the colostrum of vaccinated cows, are capable of agglutinating strains of O21:K(B?)

Table I

O and K antigens of *E. coli* strains isolated from suckling calves

Serogroup	No. of isolates
O101:K30(A)	27
O101:K28(A)	3
O101:K(A)·	3
O21:K·	7
O20:K·	6
O78:K80(B)	4
O3:K·	4
O7:K·	3
O8:K27(A)	1
O8:K28(A)	2
O17:K·	3
O153:K·	2
Total	65 (62.4%)

K·, not standard K antigen

Table II

Antibiotic sensitivity of *E. coli* strains isolated from calves

Serial No. of herd	Serogroup of isolates	Streptomycin	Oxytetracycline	Neomycin	Chloramphenicol	Furadantine	Polymyxin B
1	O3:K·	—	—	—	—	—	+
2	O101:K30(A)	—	—	—	—	+ (15)	—
3	O101:K28(A)	—	—	—	—	—	+ (15)
4	O21:K·	—	—	—	—	+ (13)	+ (13)
5	O8:K28(A)	—	—	—	—	+ (20)	—
6	O21:K·	—	—	—	—	+ (15)	+ (11)
7	O101:K30(A)	—	—	—	—	—	—

— resistant; + sensitive (in brackets: diameter of inhibition zone in mm)

Table III

Clinical data of vaccination with *E. coli*

Serial No. of herd	Serogroup of isolates	Vaccine strains used	Vaccinated animals		Control animals	
			total No.	died	total No.	died
1	O3:K ·	O3:K ·	81	0	15	3
2	O101:K30(A)	O101:K30(A)	29	2	78	33
3	O8:K · O101:K28(A)		108	2	99	10
4	O21:K ·	O8:K28(A)	60	0	7	3
5	O8:K28(A)	O101:K30(A)	34	9	67	18
6	O21:K ·	O78:K80(B)	25	0	3	1
7	O101:K30(A)		25	0	17	3
Totals			362	13 (3.6%)	286	71 (24.8%)

K ·, not standard K antigen

too. Accordingly, the calves of cows immunized with the trivalent vaccine were expected to be protected even if the causal agent of the disease had been a strain of O21:K(B?). Since the common K antigen, related with the K80(B), was also present as a single surface antigen in strains belonging to other O groups (VARGA and FARID, 1974b), it was expected that the antibodies appearing in the colostrum of cows immunized against strain O78: K80(B) can also protect against strains belonging to other O groups, but possessing the common K antigen.

A total of 362 cows were immunized twice within one month preceding parturition. Among the offspring of the vacciness 3.6%, while among that of control cows kept in the same house 24.8% died of *E. coli* diarrhoea up to 12 days of age. Diseased calves of both vaccinated and unvaccinated cows were treated with antibiotics.

Eighteen of 71 dead calves in the control group and 5 dead calves from the vaccinated group were examined bacteriologically. The serogroups of the isolates from control carcases were in each herd identical with those isolated before the vaccination. In vaccinated herd No. 2, strains of serogroup O20 were isolated from a dead pair of twin calf, although in the same herd exclusively strains of O101:K30(A) had formerly been isolated. The isolates from two dead calves in herd No. 3 had the antigenic pattern O9:K(A), while the strains responsible for disease before the vaccination had been identified as

O8:K. and O101:K28(A). In herd No. 5, calf losses were roughly identical in vaccinated and control group. Unfortunately, only one of the nine dead calves of vaccinated cows was available for bacteriological examination, and the strains isolated from it were untypable with the available antisera. Evidence is therefore lacking whether calf losses in the vaccinee group in this particular herd had in fact been due to *E. coli* bacteria or to some other causal factors.

The antibody titres determined by the IHA test in blood serum and colostral whey of vaccinated cows are shown in Table IV, where all figures represent arithmetical means. The erythrocytes used for testing of samples from herds No. 1 and 2 were presensitized with O3 or O101 antigen, those used for samples of herds No. 3 and 4 with a pool of antigens O8, O78 and O101. Table IV shows that the blood sera of the vaccinees had contained agglutinating antibodies to the O antigens of the *E. coli* vaccine strains already before immunization, but the respective titre levels rose 2 to 6 times after vaccine treatment.

Table IV

O antibody titres in blood and colostrum of vaccinated cows as determined by the IHA test

Serial No. of herd	No. of animals examined	Pre-immunization IHA titres in serum	Titre in	
			blood serum	colostral whey
			at parturition	
1	38	38.4±61.8	45.4± 84.4*	135 ±116.1
2	15	32.6±33.6	141.5±102.4	209.6±204.8
3	59	17.8±20.4	27.3± 27.6	44.4± 43.1
4	30	34 ±23.9	92.3± 88.6	126.8± 98.2

* expressed as reciprocals of dilution

K antibody contents in blood sera and colostral whey from vaccinees are shown in Table V. The values represent arithmetical mean titre, to the K antigen of each vaccine strain separately. One month before calving, the pregnant cows of all herds except one had had no serum antibodies to K antigens. The same applied to randomly collected blood and colostrum samples of control cows, although strains of a given serotype had long been present in one or another herd, causing the death of many calves. Antibodies to antigen K80(B), but never to K28(A), K30(A) or another K(A) antigen, were found in a few colostrum samples from control cows. The monovalent vaccines, prepared either from an O3:K· or an O101:K30(A) strain, evoked a firm immune response, but treatment with the trivalent vaccine was responded in

all, except a few, cases by a notable antibody formation to practically only the strain O78:K80(B). The arithmetical means for K(A) antibody levels stimulated by the trivalent vaccine varied between 0 and 4 in the blood serum and between 0 and 8 in the colostrum. The immune response nevertheless showed great individual variations. In each group treated with the trivalent vaccine there were some cows lacking colostral antibody to any K(A) antigen, while

Table V

K antibody titres in blood and colostrum of accinated cows as determined by agglutination test

Serial No. of herd	No. of animals examined	Vaccine strains	Serum titre before immunization	Titre in	
				serum	colostrum
				at parturition	
1	38	O3:K ·	1.5 ± 1.3	22.8 ± 18.3*	48.4 ± 16.8
2	15	O101:K30(A)	0	2 ± 2.1	15.2 ± 9.6
3	59			2.8 ± 4.1	6.5 ± 5.4
			0	1.5 ± 2	4.4 ± 5.2
				16 ± 13.4	19.4 ± 11.1
4	30			0	1.3 ± 4.
			0	0	4 ± 2.5
				21 ± 18	36.2 ± 2 28
5	9	O8:K28(A)		0	0
		O101:K30(A)	0	0	0
		O78:K80(B)		33.6 ± 27.9	43.2 ± 28.6
6	25			2.6 ± 4.9	7.7 ± 5.9
			0	2.3 ± 3.0	9.1 ± 8.3
				60 ± 10.9	59.3 ± 13.0
7	25			2.8 ± 2.5	8 ± 5.4
			0	0	7.3 ± 3.5
				48.8 ± 22	56 ± 15.4

* tube agglutination titre, expressed as reciprocal value of dilution

K ·, not standard K antigen

other animals had colostral antibody levels of 8—16 to the two K(A) antigens and of 32—64 to the antigen K80(B). Vaccinees of herd No. 5 had no K(A) antibodies whatever, either in the colostrum or in the blood serum.

Even initially high colostral antibody titres were steadily declining after the day of parturition, no K antibodies being found after 3 to 4 days post partum.

Prior to the vaccination experiments, a gamma-globulin prepara pasty consistence was prepared from colostrum in two cattle farms. The preparation was mixed in the milk ration of bucket-fed calves from birth to a few days of age, to increase resistance and prevent *E. coli* diarrhoea. 1 : 20 dilutions of both gamma-globulin preparations agglutinated the *E. coli* serotypes possessing a K80(B) antigen, but antibodies to K(A) antigens were not found in them.

Discussion

Strains isolated from suckling calves that died of *E. coli* diarrhoea generally represent only 8—10 O serogroups. More than half of the strains possess a mucoid K(A) antigen. As a rule, only one serotype occurs in one and the same herd, the simultaneous presence of two or three types being rare. The majority of isolates pathogenic for the calf were found to be polyresistant to antibiotics.

Vaccination of cows with aluminium gel-adsorbed *E. coli* vaccine 4 and 2 weeks before calving was followed by the appearance of specific antibodies to the K antigen of the vaccine strains in the blood and colostrum of the immunized animals. Vaccination presupposes the precise knowledge of the serological type of the strains causing disease in the suckling calf herd. O antibodies were present in blood and colostrum of both vaccinated and unvaccinated cows at parturition, but K antibodies, especially K(A) ones, appeared exclusively in blood and colostrum of vaccinees. The protection acquired by calves on suckling out the colostrum or being bucket-fed with it, is due, apart from certain as yet not understood factors, to specific colostral K antibodies (Briggs et al., 1951; Ingram et al., 1956). The colostral K antibody level is, among others, considerably influenced by the antigenic structure of the vaccine strains. On application of the trivalent vaccine, the K(A) antibody titre was always lower than that developed in response to the K80(B) antigen. Administration of strains of K(A) antigenic pattern in the form of a monovalent vaccine was found to evoke a more firm immune response than their administration in the form of the trivalent preparation; it follows that in those herds in which the disease is caused by a K(A)-containing strain, the use of a monovalent vaccine is more to the purpose. Calf losses in vaccinated herds, if they occurred at all, had always been due to a serotype not included in the vaccine. Accordingly, although vaccination can prevent outbreaks causing major losses, after a certain time a different serotype may appear and become predominant in the herd.

Literary data have been scanty on vaccinations against *E. coli* disease in suckling calves. In experiments in which the vaccine was either prepared from a wrong serotype or was administered without adjuvant, as a rule in a low dose and several months before calving, the results were invariably nega-

tive. Other authors (ULBRICH, 1954; GAY et al., 1964a, b; SALAJKA and ULLMANN, 1971; GAY, 1971), however, reported good results based on preliminary serological exminations and use of an appropriate adjuvant. In view of this and of our own experience, we believe that if the above conditions are observed, the vaccination of cows in advanced state of pregnancy is an effective preventive measure against *E. coli* diarrhoea in suckling calves.

Summary

After preliminary serological studies, vaccination experiments against *E. coli* diarrhoea in suckling calves were carried out with monovalent and trivalent vaccine preparations containing aluminium gel adjuvant.

The *E. coli* strains pathogenic for suckling calves usually represent only 8 to 10 serological groups. The majority of the strains possess a mucoid K(A) antigen, and many of them are polyresistant to antibiotics.

Specific K antibodies to the vaccine strains appeared in the blood and colostrum of cows immunized twice during the last month preceding calving. The protection conferred on the calves by suckling out, or bucket-fed with, the colostrum, is due to the presence of K antibodies therein. Efficient vaccination presupposes the precise knowledge of the *E. coli* serotypes causing disease in the calf herd. Vaccination of the cows on an appropriate schedule can prevent the incidence of *E. coli* diarrhoea in suckling calves, if the requirements of environmental hygiene are also met.

References

ÁLDÁSY: P.: Kórokozó coli-baktériumok típusainak meghatározása. Thesis. Budapest, 1955. IDEM: Magyar Állatorvosok Lapja (MÁL), 1959, 195. ÁLDÁSY, P. and ERŐS, T.: MÁL, 1969, 183. BERCZI, I., STIPKOVITS, L., BEREZNAI, T. and ANTAL, T.: MÁL, 1967, 399. BRIGGS, C., LOVELL, R., ASCHAFFENBURG, R., BARTLETT, S., KON, S. K., ROY, J. H. B., THOMPSON, S. Y. and WALKER, D. M.: Brit. J. Nutr. **5** (1951), 346. EWING, W. H., TATUM, H. W., DAVIS, B. R. and REAVIS, R.. W.: Studies of the Serology of the *Escherichia coli* Group. Communicable Diseases Center, Atlanta, Georgia, 1956. GAY, C. C.: Ann. N. Y. Acad. Sci. **176** (1971), 336. GAY, C. C., MCKAY, K. A., BARNUM, D. A.: Canad. Vet. J. **5** (1964a), 248. IDEM: Ibidem **5** (1964b), 297. INGRAM, P. L., LOVELL, R., WOOD, P. C., ASCHAFFENBURG, R., BARTLETT, S., KON, S. K., PALMER, J., ROY, J. H. B. and SHILLAM, K. W. G.: J. Path. Bact. **72** (1956), 561. KAUFFMANN, F.: The Bacteriology of *Enterobacteriaceae*. Copenhagen, 1966. KOVÁCS, J., MAGYAR, K. and ROMVÁRY, A.: MÁL, 1972, 558. MÉSZÁROS, J.: MÁL, 1972, 135. SALAJKA, E. and ULMANN, L.: Acta Vet. (Brno), Suppl. **2** (1971), 83. SCHLECHT, S. and WESTPHAL, O.: Zbl. Bakt. I. Orig. **200** (1966), 241. TAKÁTSY, GY.: Acta microbiol. hung. **3** (1955), 191. ULBRICH, F.: Zbl. Vet. Med. **1** (1954), 603. VARGA, J. and FARID, A. F.: Acta vet. hung. **XXIV** (1974), 421. IDEM: Ibidem **XXV** (1975), 61. WILSON, M. R. and SVENDSEN, J.: Am. J. Vet. Res. **32** (1971), 891.

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STUDIES ON *ESCHERICHIA COLI* STRAINS ISOLATED FROM LETHAL CASES OF RABBIT MUCOID ENTERITIS

By

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Mucoid enteritis, or *Escherichia coli* enterotoxaemia, is a major enteric disease of rabbit flocks in Hungary (Vetési, 1970). Extreme multiplication of enteropathogenic *E. coli* strains in the large and in the small intestine, endotoxins absorbed from the digestive tract, giving rise to toxic shock phenomena (Greenham, 1962; Vetési, 1970; Glantz, 1970; Weber and Manz, 1971; Prohászka, 1972; Savage and Sheldon, 1973), as well as disorders of water, mineral and carbohydrate metabolism (Vetési and Kutas, 1973; 1974), all play a role in the pathogenesis and the fatal outcome of the disease.

Injury of the intestinal mucosa by mild to severe coccidial infection has also been incriminated to predispose for the disease by facilitating the absorption of endotoxins released on disruption of the extremely large amount of *E. coli* organisms in the digestive tract of growing and adult rabbits (Löliger et al., 1969). Non-enteropathogenic *E. coli* strains have also been shown to cause disease when supervening to intestinal coccidiosis (Weber and Hoffmann, 1973).

A preceding history of mild to severe diarrhoea in attendants of rabbit flocks has been disclosed several times in our studies on minor outbreaks of *E. coli* enterotoxaemia. It therefore seemed worthwhile to examine the *E. coli* strains isolated from outbreaks for O serogroups which might be pathogenic also for man.

In recent years *E. coli* strains belonging to certain O serogroups other than dyspepsia-type have been found to cause a dysentery- or cholera-like condition in adult humans (Rowe et al., 1970; Gorbach, 1970; Evans et al., 1973; Sojka, 1973). Such *E. coli* strains have been demonstrated in diseased or dead domestic mammals and in certain zoo amphibians.

On the other hand, the O78 strains playing an important role in calf diarrhoea and bacteraemia due to *E. coli*, has been shown to give rise either to dyspepsia (Linzenmeier and Metz, 1960) or to a cholera-like disease (Sack et al., 1971) in infants.

Related experimental studies in Hungary (Ralovich et al., 1974) have confirmed that certain enteropathogenic *E. coli* strains can produce diarrhoea not only in infants, but also in adults, and are demonstrable in the faeces occasionally as long as for 20 days.

Materials and methods

Sixty-five *E. coli* strains, isolated from the intestinal contents of 58 rabbits (52 growing and 6 suckling animals) were typed. The rabbits either had died spontaneously in *E. coli* enterotoxaemia, or had been killed in its terminal stage. Eight strains originated from the small intestine, the remaining 57 either from the coecum or the colon (Table I).

Apart from the 11 dyspepsia serogroups, search was also made for other strains which might cause disease in humans. The data of the isolates are shown in Tables II and III.

Table I

Occurence in rabbits of *E. coli* strains pathogenic for humans

	No. of animals examined	No. of isolations	Test materials		Origin of test materials							
					Caecum		Colon		Jejunum		Ileum	
			No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.
Growing rabbits	52	10	59	12	49	10	3	1	4	1	3*	1
Suckling rabbits	6	1	6	1	5	1	—	—	1	—	—	—
Total	58	11	65	13	54	11	3	1	5	1	3	—

* bacteriological examination was only performed on ileum contents

Table II

The 11 *E.coli dispepsiae* type strains used in slide agglutination tests

Polyvalent	International reference strain	Antigenic structure
I	F41	O26:60B:—
	Aberdeen 1064	O55:59B:6
	E990	O86:61B:—
	Stoke W	O111:58B:—
II	Guanabara M 194	112a,c:66B:
	537/52	119:69B:6.
	Cigleris (56/54)	128:67B:2
III	227	124:72B:32
	Canioni (2745/53)	125:70B:19
	E611	126:71B:2
	4932/53	127a:63B:—

Serological examinations

Typing for the 11 dyspepsia serogroups was carried out according to the standard prescriptions employed in the Institute of Public Health in Hungary.

Antisera were prepared to 49 standard type strains supplied from the National Strain Collection (National Institute of Hygiene, Budapest). 24-hour

Table III

The 49 *E. coli* strains to which O sera were used in tube agglutination tests

Designation	Antigenic structure O:K:H	Designation	Antigenic structure O:K:H
U9/41	2:1L:4	Bi7327/51	53:.:3
U4/41	4:03L:5	F8962/41	58:.:—
Bi7458/41	6:2acL:1	F10167a/41	60:.:—
G3404/41	8:8L:4	P12a	73:.:31
Bi8337/41	10:5L:4	E3b	75.::5
Bi626/42	12:5L:—	E38	78:.:—
Su4321/41	13:11L:11	E49	79:.:40
F7902/41	15:14L:4	H17a	83:.:31
F11119/41	16:1L:—	H308b	93:.:8
F10018/41	18ab:76B:14	H520b	105:.:8
3219/54	18ac:77B:7	H521a	106:.:33
F8188/41	19:.:7	1411/50	112a, b:68B:18
P7a	20:17L:—	Guanabara M 194	112ac:66B:.
E14a	22:13L:1	W26	1114:.:32
E39a	23:18L:15	W27	115:.18
E47a	25:19L:12	W30	117:.:4
F41	36:60B:—	178/54	129:.B:11
K1a	28a,b:.:—	N282	133:.:.
Katwijk	28a,c:73B:.	Coli Pécs	135.::.
P6a	32:.:19	1111/55	136:.:.
E40	33:.:—	C771	142:.:.
P11a	42:.:37	4608	143:.:.
H702c	44:74L:18	1624	144:.:.
U19/41	51:.:24	1385/3	145:.:—
		E519—66	148:.:—

agar cultures were washed off, boiled for 2.5 hours at 100 °C, and suspensions adjusted to 2.10^9 germs/ml were administered to rabbits as proposed by EDWARDS and EWING (1962). Cross-agglutination tests were performed with *E. coli* O antigens prepared from 142 standard reference strains. Serum absorption and the production of polyvalent sera were performed according to the methods of EWING (1956) and EWING et al. (1956).

The isolations were carried out on eosin—methylene blue agar medium. Colonies representing all morphological appearances were transferred to agar

slants, incubated for 24 hours at 37 °C, washed off, boiled and tested for O agglutination, using polyvalent O sera. The results were read after an incubation for 24 hours at 37 °C.

The strains producing O agglutination were further tested by monovalent and absorbed sera and only those were classified which showed the same agglutination titre in absorbed serum as the corresponding standard reference strain.

Glucose decomposition was determined in peptone water, using Andrade indicator. Haemolytic activity was tested in agar media containing bovine or sheep blood.

Assays for antibiotic sensitivity were carried out after preliminary incubation in indole-urea medium for 3—5 hours; strains grown on agar medium were tested with the "Resitest" disc set (HUMÁN, Budapest).

Results

The results are summarized in Table IV. Thirteen of the 65 isolates belonged to one or another of the serotypes shown in Tables II and III. Two strains of the 13 could be classified into the dyspepsia group (serogroups O86 and O125), the remaining 11 strains were of types which can presumably play a role in human disease. Fifty-two strains could not be typed with the available sera.

None of the isolates showed haemolytic activity. The antibiotic sensitivity data are tabulated below:

Antibiotic	Percentage of sensitive strains
Chloramphenicol	98
Neomycin	98
Polymyxin B	100
Streptomycin	93
Tetracycline	35
Ampicillin	60
Colistin	66
Gentamycin	100
Kanamycin	95
Nalidixic acid	100

Discussion

Of the 65 strains isolated from the intestinal tract (above all the coecum) of suckling and growing rabbits, 13 (20%) could be classified into O serogroups, including strains pathogenic for humans. Two strains each isolated from a growing rabbit were identified as human *E. coli dyspepsiae* strains (O86 and O125).

E. coli strains belonging to the dyspepsia group have been isolated from rabbits in other countries among others by MATTHES (1969), who reported isolation of an O128:K67 (B12) strain from 70% of rabbits diseased in dysentery; he also found an O55:K59 (B5) strain. The O128 strain caused septicaemia in the diseased animals.

The causal role of an O128 strain in rabbit enteritis has also been reported by NIKKELS (1972), as well as by SADED and EL AGRONDI (1963). Occurrence in rabbits of the *E. coli dyspepsiae* strains O86 and O125 was reported earlier by WEBER and HOFFMANN (1973).

Eleven of our isolates belonged to four of the 49 O serogroups pursued; out of them three were found to belong to groups O2, O8 and O142, whereas two to O18a, c.

Serotype O2 and O18 strains have been demonstrated also by others (WEBER and MANZ, 1971) in the intestine of rabbits that died of or killed for, enteritis; furthermore, type O2, O8 and O18 strains were found in dysenteric young rabbits suffering also from coccidiosis (WEBER and HOFFMANN, 1973).

CZIRÓK et al. (1973) showed the incidence of serotype O2 and O18 strains in human enteric diseases. However, pathogenicity was only ascribed to the strain of serotype O18 as the incidence of O2 strain in clinical cases was comparable to that of healthy humans. Strain of serotype O2 was also demonstrated in normal rabbits (WEBER and MANZ, 1971). Others (LOVE et al., 1972) isolated an O142 strain from infants with gastroenteritis.

Intestinal flora studies on healthy rabbits showed that normally both the large and small intestine contain very few *E. coli* organisms, if any (SMITH, 1965; MATTHES, 1969; PROHÁSZKA, 1972); low to high coliform counts in suckling and growing rabbits have been regarded as temporary components of the intestinal flora (MATTHES, 1969; CHRIST-VIETOR, 1973).

Aoccrding to present knowledge, *E. coli* enterotoxaemia in rabbits is due either to the direct causal role of enterotoxin-producing *E. coli* strains (LÖLIGER et al., 1969; VETÉSI, 1970; VETÉSI et al., 1974), or to any other *E. coli* serotype, if large amounts of endotoxin released from the cells invade the circulation, owing to injury of the intestinal mucosa by some other pathogenic agent (*e.g.*, coccidia) (LÖLIGER et al., 1969; WEBER and HOFFMANN, 1973).

The present findings strongly suggest that *E. coli* strains occasionally involved in the pathogenesis of intestinal or extra-intestinal diseases in man

Table IV

Distribution in O groups of *E. coli* isolates

	No. of		Serogroups of			
	animals	samples	O2		O8	
	examined		A	S	A	S
Growing rabbits	52	59	1	3	2	2
Suckling rabbits	6	6	—	—	1	1
Total	58	65	1	3	3	3

could act as the main causal factor of rabbit enterotoxaemia. Among the 29 attendants employed in the large rabbit farm studied, two were found to excrete serogroup O2 and O18 strains and one was passing a type O8 strain. It was first recognized by MATTHES (1969) that rabbits from flocks with an enteric *E. coli* disease may transmit the infection to humans.

Many strains among our rabbit isolates were resistant to tetracycline, as was also found recently with *E. coli* strains from humans (CZIRÓK et al., 1973).

Summary

Sixty-five *E. coli* strains isolated from the intestinal tract of 6 suckling and 52 growing rabbits with *E. coli* enterotoxaemia were examined for the occurrence of serotypes known to be pathogenic (above all enteropathogenic) for humans.

Thirteen strains (20%) were found to belong to one or another of the serogroups pursued (Tables II and III). Two of the 13 strains were identified as *E. coli dyspepsiae* (serogroups O86 and O125), the remaining belonged to other serotypes pathogenic for man.

Remarkably, only 35% of the examined strains were sensitive to tetracycline.

Of the attendants in charge of the rabbit flock studied, 17% were found to excrete the strains responsible for *E. coli* enterotoxaemia in the flock.

References

CHRIST-VIETOR, M.: Untersuchungen über die Darmflora von Jungkaninchen in Abhängigkeit von Alter und Ernährung. Inaug. Diss. Giessen, 1973. CZIRÓK, É., MADÁR, J., MILCH, H. and GYENGÉSI, L.: Egészségtudomány **17** (1973), 275. EDWARDS, P. R. and EWING, W. H.: Identification of *Enterobacteriaceae*. Minneapolis, 1962. EWING, W. H.: Ann. N. Y. Acad. Sci. **66** (1956), 61. EWING, W. H., TATUM, H. W., DAVIS, B. R. and REAVIS, R. W.: Studies on the Serology of the *Escherichia coli* Group. Atlanta, 1956. EVANS, D. J., DOLORES, J. R., EVANS, D. G. and GORBACH, SH. L.: Inf. Immun. **8** (1973), 725. GLANTZ, P. J.: Canad. J. Comp. Med. **34** (1970), 47. GORBACH, S. L.: New Engl. J. Med. **283** (1970), 44. GREENHAM, L.

typed with O serum in animals and test materials

the isolates									
O18ac		O86		O125		O142		Total	
A	S	A	S	A	S	A	S	A	S
2	2	1	1	1	1	3	3	10	12
—	—	—	—	—	—	—	—	1	1
2	2	1	1	1	1	3	3	11	13

A, No. of animals yielding *E. coli* of the corresponding serogroup; S, No of samples yielding *E. coli* of the corresponding serogroup

W.: Vet. Rec. **74** (1962), 79. LINZENMEIER, G. and METZ, H.: Med. Klin. **55** (1960), 1151. LOVE, W. C., GORDON, A. M., GROSS, R. J. and ROWE, B.: Lancet **2** (1972), 355. LÖLIGER, H. CH., MATTHES, S. I., SCHUBERT, H. J. and HECKMANN, F.: Dtsch. tierärztl. Wschr., 1969, 16, 38. MATTHES, S. I.: Zbl. Vet. Med. B. **16** (1969), 563. NIKKELS, R. J.: Z. Versuchstierk. **14** (1972), 358. PROHÁSZKA, L.: Zbl. Bakt. Hyg. I. Abt. Orig. A. **221** (1972), 314. RALOVICH, B., EMŐDY, L., BARNA, K., BARASCH, GY., MÁRTON, E. and TERNÁK, G.: Zbl. Bakt. Hyg. I. Abt. Orig. A. **226** (1974), 82. ROWE, B., TAYLOR, J. and BETTELHEIM, K. A.: Lancet **1** (1970), 1. SACK, R. B., GORBACH, S. L., BANWELL, J. G., JACOBS, B., CHATTERJES, B. D. and NITRA, R. C.: Inf. Dis. **123** (1971), 378. SADEK, J. and EL AGRONDI, M. A.: J. Arab. vet. med. Ass. **23** (1963), 11. SAVAGE, N. L. and SHELDON, W. G.: Canad. J. Comp. Med. **37** (1973), 313. SMITH, H. W.: J. Path. Bact. **89** (1965), 95. SOJKA, W. J.: Canad. Inst. Food Sci. Technol. J. **6** (1973), 52. Standard Methods Nat. Inst. of Public Health. Budapest, 1969. VETÉSI, F.: Magyar Állatorvosok Lapja (MÁL), 1970, 465. VETÉSI, F. and KUTAS, F.: Acta vet. hung. **XXIII** (1973), 381. IIDEM: Ibidem **XXIV** (1974) 303. VETÉSI, F., KUTAS, F. and SEMJÉN, G.: MÁL, 1974, 675. WEBER, A. and MANZ, J.: Berl. Münch. Tierärztl. Wschr., 1971, 441 WEBER, A. and HOFFMANN, R. : Berl. Münch. Tierärztl. Wschr., 1973, 374.

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EXPERIMENTAL INFECTION OF LAMBS WITH A SHEEP ADENOVIRUS ISOLATE CLOSELY RELATED TO TYPE-2 BOVINE ADENOVIRUS

II. POSTMORTEM AND HISTOPATHOLOGICAL EXAMINATIONS

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As reported in a previous paper (BELÁK and PÁLFI, 1974) and in the first paper of this series (BELÁK et al., 1975), infection experiments were carried out with a virus strain (Het/3) isolated from ewes showing respiratory symptoms. The isolate was found to be antigenically related to type-2 bovine adenovirus. Seven colostrum-deprived lambs were infected intranasally and intratracheally, further two lambs were exposed to infection by contact and four lambs served as control. Three lambs died 1, 2 and 9 days, respectively, after intranasal and intratracheal infection, the remaining four animals of the group were killed between 4 and 9 days postinfection (p.i.), the two contacts on days 7 and 13 of the experiment.

The scheme of the experiment as well as clinical and virological observations were described in the first paper of this series. Postmortem and histopathological findings are reported in this paper.

Materials and methods

All lambs, except the one which died one day after infection, were examined for gross and microscopic lesions. The following organs were used for histopathological examination: lungs, nasal and tracheal mucosa, retropharyngeal, peribronchial and mesenteric lymph nodes, spleen, liver, kidney and — in three cases — brain. The organ specimens were fixed in 10% formaldehyde, embedded in paraffin and the sections were stained with haematoxylin and eosin.

Results

Postmortem findings

The lamb that died on the second day p.i. (No. 1640) showed changes only in the lungs and retropharyngeal lymph nodes. A few sharply delineated lobules at the caudal surface of the apical and cardial lobes showed a brownish-red discoloration and were somewhat more compact than the rest. A small amount of turbid discharge was found on the cut surface of the affected areas. The

retropharyngeal lymph node was swollen and slightly soft, and fluid was abundantly discharged from the cut surface.

The animals killed at 4 and 7 days (Nos 1593, 1591, 1636) and that died on day 9 p.i. (No. 1638) showed essentially similar lesions, differing slightly in severity. The entire lung showed a slight enlargement and was generally somewhat more compact than normal. Slightly compact, brownish-red lobules were found on the cut surface, in regions of the apical, cardial and accessory lobes, above all nearby bifurcations of the tracheal bronchus and other bronchi, as well as in the slightly indented areas beneath the pleura. The cut surfaces of the changed parts, especially the minor bronchi, discharged a turbid, viscous fluid on compression.

In the animal killed 13 days p.i. (No. 1590), the change no longer involved entire lobules. However, collapse of the lung after removal from the carcase was of a lesser degree, and the entire organ was more compact and elastic compared to normal. The same was found in the lungs of the two contacts (Nos 1637 and 1589) killed on days 7 and 13 p.i.

No gross lesions were found in the nasal cavities of the lambs killed 2 and 3 days after intranasal and intratracheal infection (Nos 1640 and 1590). The nasal mucosa of the lambs killed on days 4 and 7 (Nos 1593, 1591 and 1636), of the one died on day 9 (No. 1638) and of the two contacts (Nos 1647 and 1589) were swollen, exhibited reddening in places, and were coated by a viscous, vitreous discharge. At postmortem examination at 4, 7, 9 and 13 days p.i., lambs No 1593, 1591, 1636, 1638, 1590, 1637 and 1589 showed considerable enlargement and greyish-white discoloration of the retropharyngeal, peribronchial and, especially, of the mesenteric lymph nodes; the cut surface had an indefinite structure and was less juicy than normally.

The mucous membrane of the small intestine was slightly swollen, showing in places red spots with indefinite margins (lambs No 1593, 1591, 1636 and 1638).

No gross lesions were found in other organs of the infected animals, and the controls showed no abnormality whatever on postmortem examination.

Microscopic lesions

Two days after infection (lamb No. 1640), single lobules within the changed areas showed collapse of alveoli in the entire region of the lobule (Fig. 1). The lumina of many bronchioli and a few alveoli were filled by exudate, partially or completely. The main elements of the exudate were degenerated, desquamated epithelial cells and neutrophilic granulocytes. The small vessels of the alveolar septa were markedly dilated. Subpleural oedema was found nearby single changed lobules localizing immediately beneath the pleura. Outside the changed lobules only a few bronchioli contained small amounts

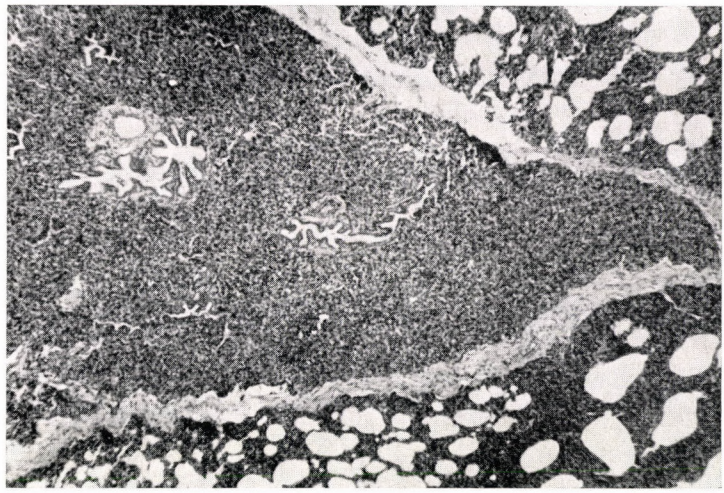

Fig. 1. Atelectasia in a pulmonary lobule; approx. × 40

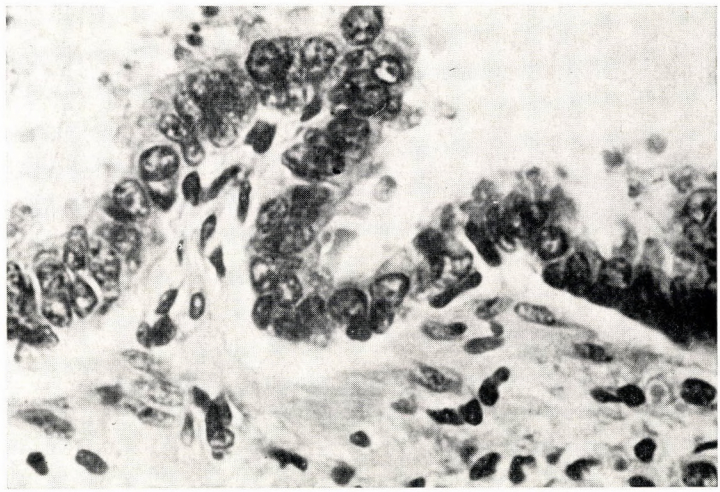

Fig. 2. Swollen and proliferating epithelial cells in a bronchiolus; approx. × 690

of similar exudation. No mucosal lesions were found in the large bronchi or in the nasal mucosa.

Four, seven and nine days after infection (lambs Nos 1593, 1591, 1636 and 1638), lesions were also found in regions adjacent to the grossly visible areas of atelactasia. Many cells of the mucous epithelium lining the small bronchi had swollen nuclei, with a marked granulation of the karyoplasm and a conspicuous nucleolus. In some bronchioli, especially in those of the lamb that died on day 9 (No. 1638), the proliferating epithelial cells exhibited stratification (Fig. 2). Neutrophilic granulocytes and lymphocytes made sporadically

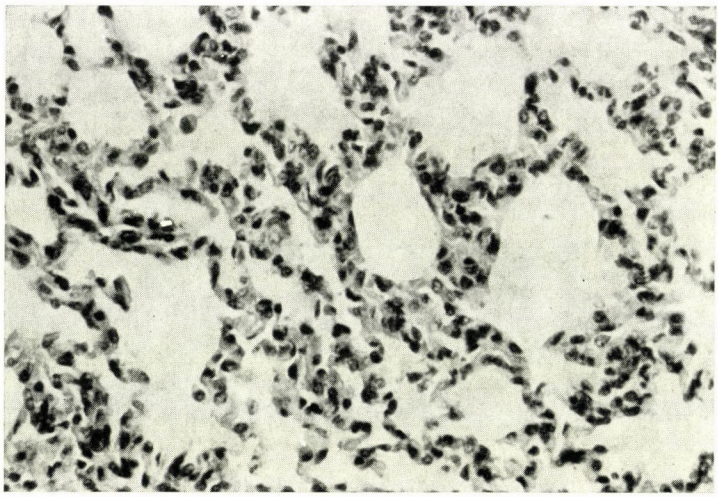

Fig. 3. Widened alveolar septa; approx. × 275

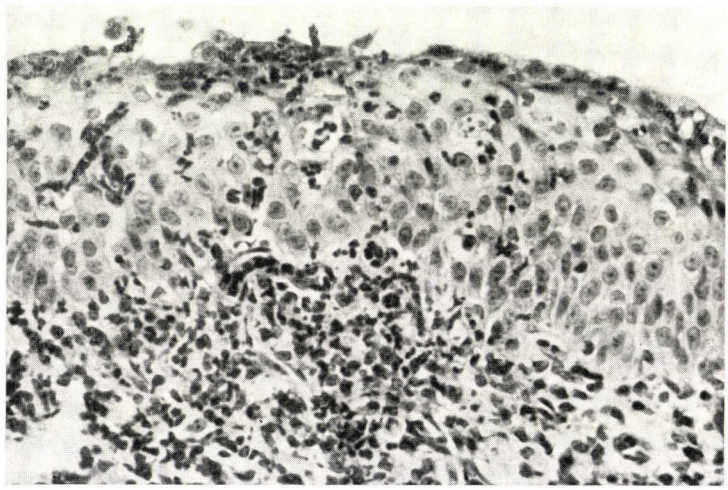

Fig. 4. Deeply seated chiefly neutrophilic infiltration in nasal mucosa. The inflammatory cells also invaded the epithelial layer; approx. × 275

appearance between the epithelial cells. An exudation chiefly consisting of desquamated, degenerating epithelial cells and granulocytes and of a small amount of homogeneous, acidophilic substance, was found in the lumina of a few bronchioli and alveoli. In the atelectatic and adjacent areas, the alveolar septa had markedly widened, owing to swelling of the alveolar epithelium and capillary endothelium cells, as well as to the appearance of many macrophages (Fig. 3).

The nasal mucosa of the above four animals showed epithelial cell degeneration in a few circumscribed areas. In two cases (lambs No. 1636 and 1638), even necrosis of the epithelium was found in large areas, accompanied by a severe inflammatory cellular reaction — chiefly neutrophilic granulocytes — in the propria and submucosa. A similar cell infiltration, affecting the involved areas to various degrees and pushing in places also between relatively preserved epithelial cells, was found in the propria of the nasal mucosa of the other two animals (Fig. 4). The glandular epithelial cells in the propria were generally swollen and proliferating (Fig. 5). One animal (No. 1636) even showed a marked proliferation of adventitial cells around vessels of the propria (Fig. 6). In all four cases, marked vasodilatation and oedema were found in the propria.

Tracheal lesions were not found in the above group of lambs.

Lesions of the retropharyngeal, peribronchial and mesenteric lymph nodes were essentially of the same type and severity in all four cases. The normal nodular structure lost definition, owing to enlargement of secondary lymph nodes and thickening of the myeline bundles. Above all, the reticulum cells showed an intensive proliferation. The distended lumen of the sinuses contained many swollen, proliferating and desquamated reticulum and endothelial cells as well as neutrophilic granulocytes and lymphocytes. One lamb (No. 1636) had a small necrotic focus in the peribronchial lymph node; a few neutrophilic granulocytes and more reticulum cells formed a ring-like infiltration around the focus (Fig. 7).

A slight proliferation of reticulum cells was found in all four cases.

Renal lesions occurred only in the glomeruli and tubuli. The capillary endothelial cells of the glomeruli as well as the nuclei of epithelial cells coating the parietal surface of Bowman's capsule were markedly swollen (Fig. 8), and in some cases a homogeneous eosinophilic substance was accumulated in the cavity of Bowman's capsule (Fig. 9). The epithelial cells of the primary contorted tubules were also swollen, a coarse granulation appeared in the karyoplasm and markedly basophilic inclusion-like bodies, surrounded by a pale halo, were seen in the nuclei of some cells showing hyperchromatosis of nuclear membrane (Fig. 9). Proliferation of tubular epithelium was found in places, the desquamated epithelium cells filling occasionally almost the entire tubular lumen (Fig. 10). The interstitial tissue showed no change.

In the liver, the mucous epithelium cells of the minor biliary ducts were swollen and in places proliferating (Fig. 11). The Kupffer cells were generally the swollen.

One lamb (No. 1591) had glia cell foci localizing distant from vessels in the cerebral cortex and Ammon's horn.

Various organs were examined for cell inclusions. Nuclear inclusions were found above all in organs of the animal killed on day 4 and in lesser numbers in the organs of those killed, respectively died on days 7 and 9. Inclusions

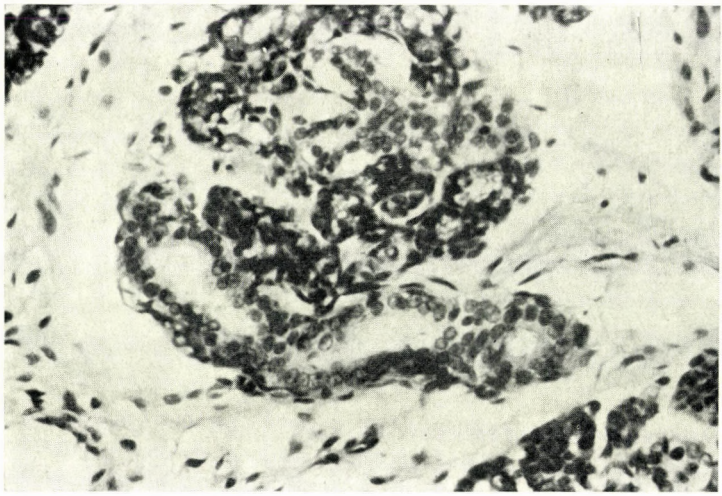

Fig. 5. Swelling and proliferation of glandular epithelium cells in nasal mucosa; approx. × 275

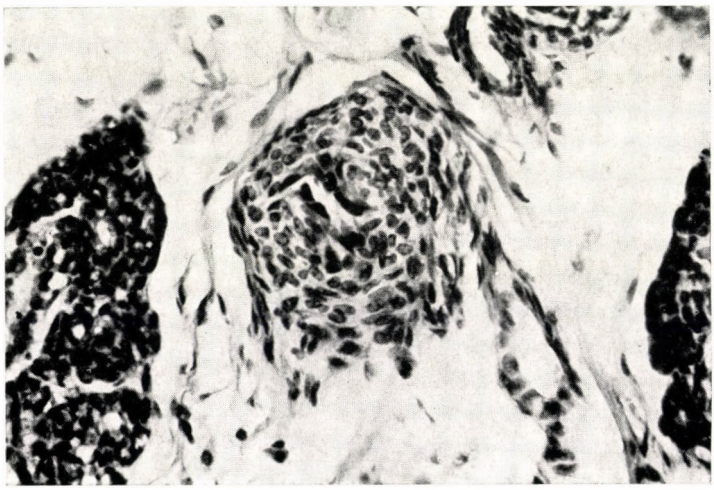

Fig. 6. Proliferation of adventitial cells around a vessel in the propria of nasal mucosa; approx. × 275

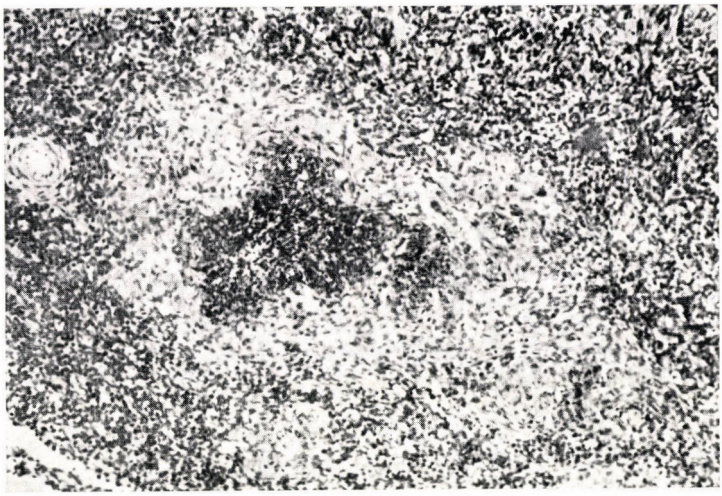

Fig. 7. Necrotic focus surrounded by reticulum cells in peribronchial lymph node; approx. × 110

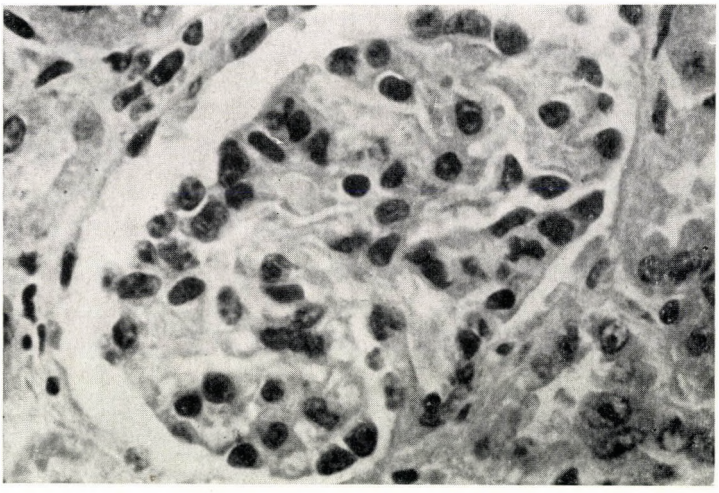

Fig. 8. Marked swelling of nuclei in glomerular epithelium cells; approx. × 690

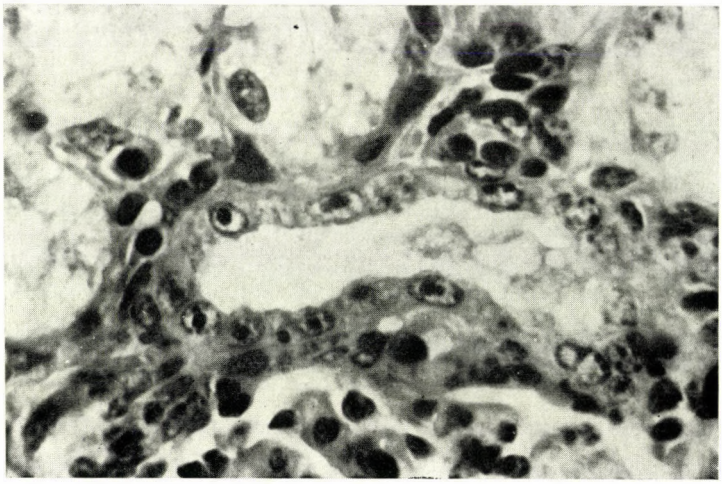

Fig. 9. Inclusion-like bodies in nuclei of renal tubular epithelium cells; approx. × 690

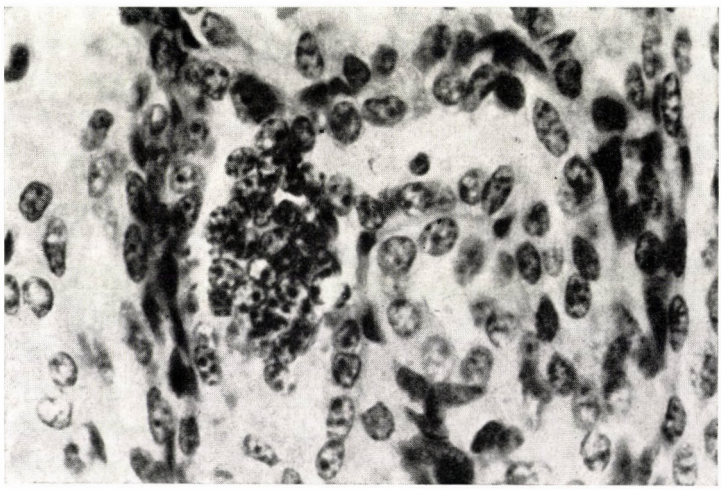

Fig. 10. Proliferating and desquamated epithelial cells in lumen of renal tubule; approx. × 690

were mainly lokalized in the proliferating and desquamating bronchiolar epithelium cells (Fig. 12), alveolar epithelium cells and macrophages in retropharyngeal and peribronchial lymph nodes. The nuclei of the above cells often resembled nuclei in cell cultures infected with the Het/3 adenovirus, *viz.* the karyoplasm of the enlarged nucleus had a markedly granular appearance (Fig. 13). The small granules took on a pale acidophilic stain. In other cases, larger basophilic granules were present in the nuclei (Fig. 14), and in some cells with a hyperchromatic nuclear membrane a central basophilic nuclear inclusion was seen surrounded by a pale halo (Fig. 15). In epithelium cells of the nasal mucosa, inclusions were chiefly found at 7 and 9 days (Fig. 16).

The lamb killed after 13 days showed widening of the alveolar septa over large areas, owing to marked proliferation of macrophages. The cells of the bronchiolar mucous epithelium were swollen and in places stratified (Fig. 17). Changes in other organs were of similar type as above. Nuclear inclusions were only found in a few cells of the nasal mucosa.

Of the two lambs exposed to infection by contact, that killed on day 7 (No. 1637) showed very slight lung lesions, whereas the one killed on day 13 (No. 1589) showed practically the same pulmonary changes as the animal killed 13 days after intranasal and intratracheal infection. The nasal changes were the same as in the lambs killed 7 and 9 days after experimental infection, except that they were more severe in the contact animal killed on day 13.

Discussion

Lambs infected intranasally and intratracheally, or exposed by contact to infection, with Het/3 virus had characteristic, generally slight lesions chiefly in the respiratory organs as well as in lymph nodes draining the respiratory and intestinal tracts. The gross lung lesions were usually circumscribed, the microscopic lesions were less focal in distribution.

The initial pulmonary processes corresponded essentially to exudative inflammatory lesions, involving generally only the minor bronchioli and a few alveoli. The grossly visible circumscribed areas of atelectasia, involving single lobules, developed as a sequel to bronchiolar occlusion by exudative processes. Apart from the latter, regressive, and later also proliferative, processes were taking place in the mucous epithelium cells of bronchioli. Epithelial proliferation was not regularly found, only in circumscribed areas in some of the animals. Thickening of the alveolar septa owing to proliferation of macrophages and swelling of alveolar epithelium and capillary endothelium were grossly visible. The proliferative process involved large lung areas, until finally it became the dominant lesion. In the upper respiratory tract mucosa, initial hyperaemia and exudative changes were followed by superficial regressive changes of

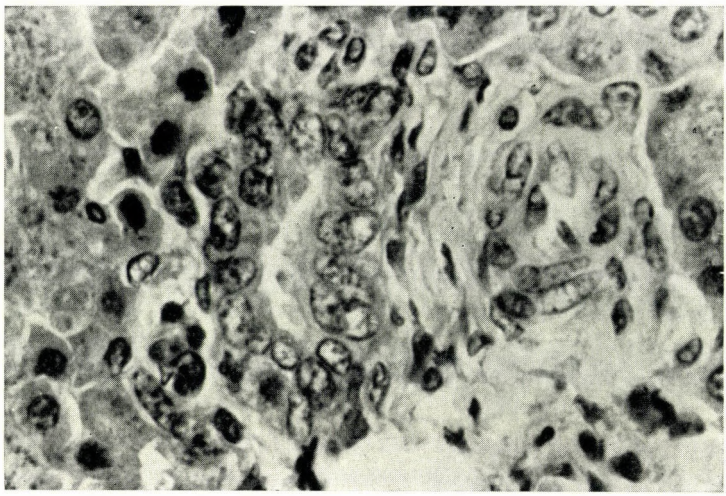

Fig. 11. Swollen and proliferating epithelial cells in a minor biliary duct; approx. × 690

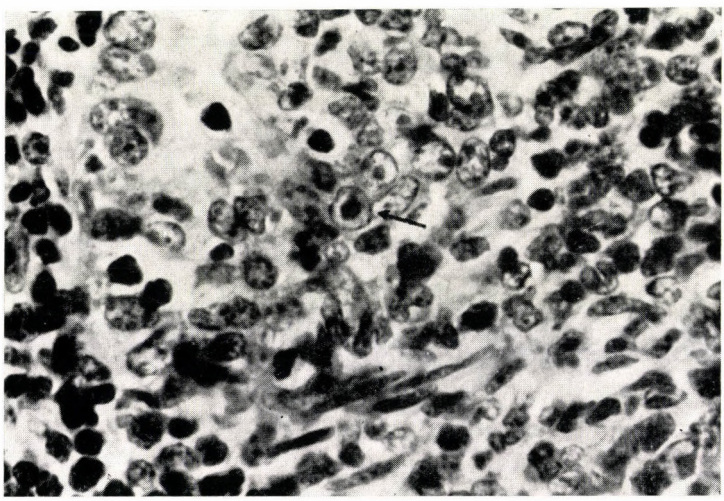

Fig. 12. Intranuclear inclusion in proliferating epithelium cells of a bronchiolus; approx. × 690

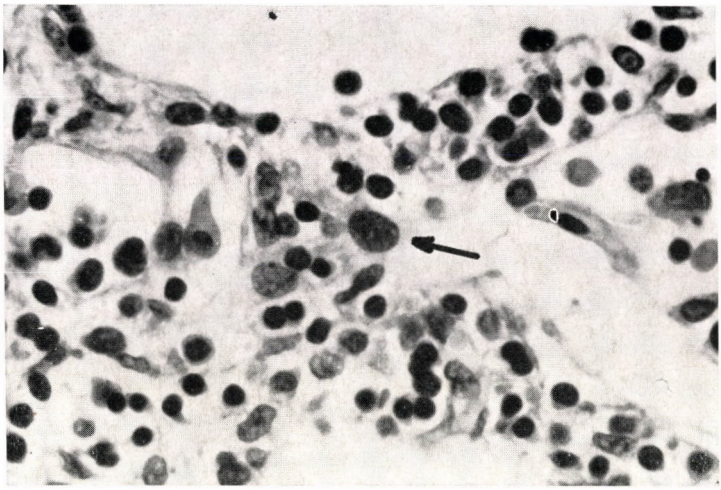

Fig. 13. Developing intranuclear inclusions in reticulum cells of a lymph node; approx. × 690

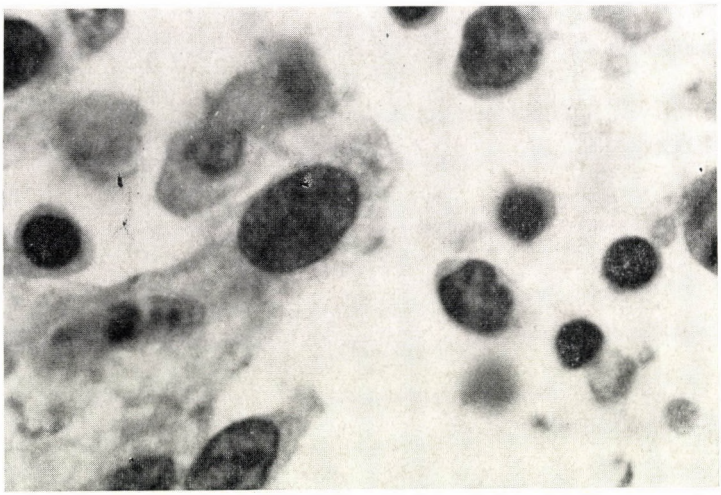

Fig. 14. Major basophilic granules in the karyoplasm of a lymph node reticulum cell. Note developing intranuclear siincluon; approx. × 1800

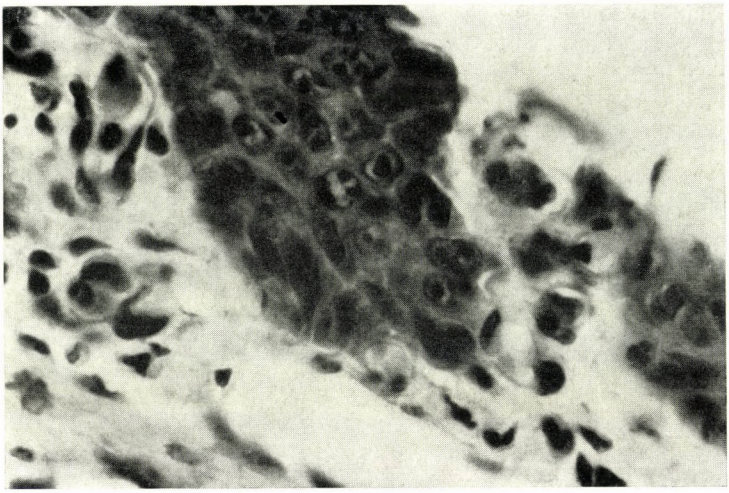

Fig. 15. Fully developed intranuclear inclusions in nasal mucous epithelium cells; approx. × 690

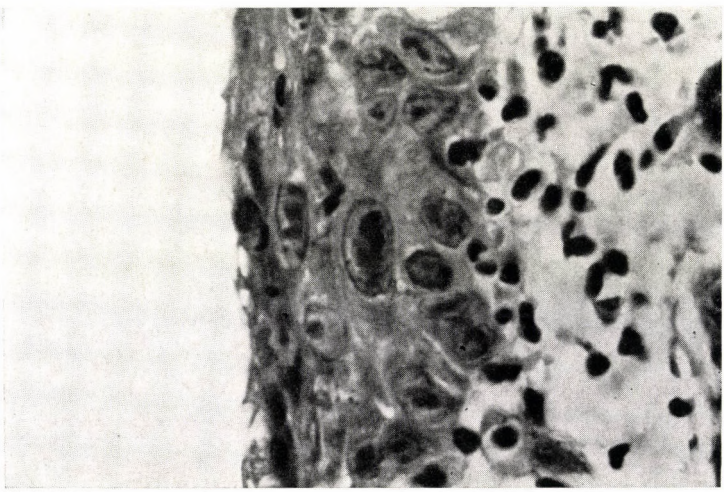

Fig. 16. Intranuclear inclusions in nasal mucous epithelium cells; approx. × 690

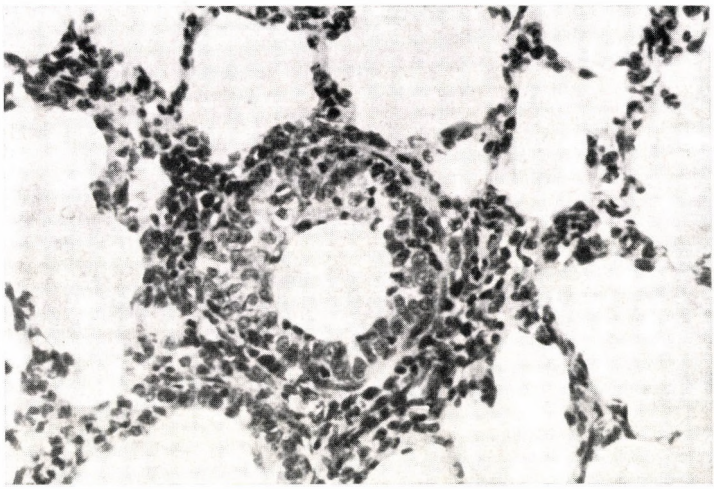

Fig. 17. Epithelium cell proliferation in a bronchiolus; approx. × 275

varying severity in the mucous membrane, appearing above all in circumscribed areas in association with reactive inflammation in deeper tissue layers. The respiratory and intestinal lymph nodes showed a distinct acute, later subacute, inflammation predominated by proliferative phenomena. Circumscribed focal necrosis of a lymph node was found in one lamb.

The pulmonary, upper respiratory and lymph node changes generally corresponded to the lesions usually found in viral infections. The intranuclear inclusions found in largest numbers 4 days p.i. in the mucous epithelium cells of bronchioli, alveoli and nasal cavity as well as in reticulum cells of lymph nodes can be regarded as a characteristic change.

Among organs other than respiratory, changes were regularly found in the kidney. The endothelial cells of the renal glomeruli were swollen and those of the contorted tubuli generally showed a regressive change or, in places, proliferation. Further examinations are in progress to elucidate whether the inclusion-like bodies found in the nuclei of many tubular epithelium cells were genuine intranuclear inclusions. The renal interstitial tissue showed no change.

Cerebral involvement — a few glia cell foci — was found in a single lamb.

The liver generally showed activation of the RHS cells and in some cases epithelium cell proliferation in the minor biliary ducts.

The gross and microscopic lesions caused under experimental conditions by the ovine adenovirus isolate Het/3 (a strain related to bovine type-2 adenovirus) support the clinical observation that the isolate is pathogenic for sheep.

As far as we are informed, no similar infection experiments have previously been reported in sheep.

Summary

Infection experiments were performed with an adenovirus strain (Het/3) isolated from sheep showing respiratory symptoms. The isolate, which is related to type-2 bovine adenovirus, was administered intranasally and intratracheally to seven colostrum-deprived lambs and two further lambs were exposed to infection by contact.

The infected animals developed circumscribed epithelial cell lesions and reactive inflammation in the nasal mucosa, exudative bronchiolitis and consequent atelectasia in the lungs, followed by circumscribed interstitial pneumonia in large areas and a slight proliferation of bronchiolar epithelium cells. A mild vascular wall injury was found in part of the renal glomeruli, tubular nephrosis of varing severity was seen and, in places, there was proliferation of tubular epithelial cells. The brain showed no characteristic change. Characteristic intranuclear inclusions were found in epithelium cells of bronchiolar, alveolar and nasal mucosa as well as in reticulum cells of lymph nodes. Inclusion-like structures were also seen in nuclei of some renal tubular epithelium cells.

The postmortem and histopathological findings weigh in favour of the clinical observation that the virus strain Het/3 is pathogenic for sheep.

References

BELÁK, S. and PÁLFI, V.: Arch. ges. Virusforsch. **46** (1974) 366. BELÁK, S., PÁLFI, V. and TÚRY, E.: Acta vet. hung. **XXV** (1975), 91.

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STUDIES ON THE DIAGNOSTIC VALUE OF CELL INCLUSIONS IN CANINE DISTEMPER

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Since early in this century Lentz (1907) and, independently, Standfuss (1908) had first described the presence of eosinophilic bodies in both the nucleus and the cytoplasm of nerve cells taken from the brain of dogs that died of canine distemper. The inclusion-forming properties of canine distemper virus as well as the frequency of occurrence of inclusion bodies have been extensively studied (Lentz, 1909; Sinigaglia, 1912; Sanfelice, 1915; Nicolau, 1935; Green and Evans, 1939; MacIntyre et al., 1948; Ribelin, 1953; Lauder et al., 1954; Campbell, 1957; etc.). Sinigaglia (1912) was the first to demonstrate inclusions in organs other than the nerve system, *viz.*, in the lung (in epithelial cells of small and medium large bronchi) and conjunctival epithelial cells.

Two types of acidophilic inclusions have been described, *viz.*, cytoplasmic and nuclear. Cytoplasmic inclusions, which represent the more frequent type, occur above all in mucous epithelium cells, chiefly of the urinary bladder and of the respiratory tract, as well as in glia cells and ganglion cells. Intranuclear inclusions have been found in lining and glandular epithelium cells, glia and ganglion cells.

Authors generally agree that inclusion bodies are characteristic of canine distemper, but disagree in respect of their frequency and diagnostic importance. In the present study we made attempts to reconcile the conflicting views and to refine pathological diagnostic work on canine distemper.

Material and method

The test material was collected from the carcases of 60 dogs sent for pathological examination from the Animal Clinic of this University with the diagnosis of canine distemper from 1st October 1971 to 1st March 1972. Part of the dogs had died spontaneously, part had been killed at the owner's request. Postmortem examination showed that seven of the 60 dogs had had a disease other than canine distemper (Rubarth's disease, toxoplasmosis, etc.) so that only 53 cases were included in the evaluation. Forty-four dogs (83%) were younger than 6 months (2—6 months), five (9%) were less than one year old, and only four (8%) were older animals, *viz.*, aged 1.5, 2, 4 and 10 years. Thirty dogs died spontaneously and 23 had been killed. In most cases, case history was not available apart from the cilinical record.

In every case, organ specimens were taken at necropsy from the third eyelid, trachea, lung, stomach (from the fundus area), urinary bladder, kidney

(part of renal pelvis included), liver and brain (from six different regions). Less often specimens were also collected from small intestine, pancreas, parotid gland, tongue, spleen, bone marrow, adrenals, peribronchial lymph nodes and spinal cord (lumbal section). All organ specimens were fixed in 10% formalin, embedded in paraffin, and the sections were stained with haematoxylin and eosin.

A specimen taken from the urinary bladder of an exterminated dog was used for electron-microscopic examination. The specimen was fixed first in 5% glutaraldehyde, then in 1% osmium tetroxide, dehydrated in step-graded ethanol, fixed in Durcupan, and cut into ultra-thin sections. The electron-micrographs were prepared with a TESLA BS 613 electron-microscope.

Organ specimens from dogs died of diseases other than canine distemper were used as control.

Results

Gross lesions

The greater part of the dogs spontaneously died of canine distemper (21 of 30) exhibited a catarrhal-purulent pneumonia, involving a varying number of lobules or entire lobes, and an acute or subacute catarrhal-purulent conjunctivitis. Occasionally, acute gastro-enteritis, slight swelling of the spleen, liver dystrophy, acute or subacute tonsillitis, acute pharyngitis, purulent pleuritis and "hard pad disease" were found in association with the above changes. One among further three dogs had acute gastro-enteritis and "hard pad disease", the second had only a subacute purulent conjunctivitis, the third serous pericarditis with sub-epicardial and myocardial haemorrhages. The remaining six dogs did not exhibit gross lesions.

It should be noted that four of the 21 carcases exhibiting catarrhal-purulent pneumonia also showed gross lesions indicative of toxoplasmosis, which was confirmed later by histological evidence.

Among the 23 emergency-slaughtered dogs 15 had practically no gross lesions, six animals had catarrhal-purulent pneumonia, involving an entire lobe as well as acute or subacute purulent conjunctivitis, and two dogs had acute gastro-enteritis.

Microscopic lesions

Frequency of occurrence of intracellular inclusions

Inclusions of the type described as characteristic of canine distemper were found in all the 53 dogs. Fifty-one animals (96%) had inclusions in organs other than the brain and the spinal cord; in two cases (one spontaneous death,

one emergency slaughter) inclusions were found only in the central nervous system. Eighteen of the 51 dogs (34%) had inclusions exclusively in extracerebral organs, whereas 33 (62%) had them in both the brain and other organs. Inclusions were found in brain cells of 35 dogs (66%), in two of these cases (4%) in the brain alone (Table I).

Table I

Frequency of occurrence of inclusions in organ systems of distemper dogs

		No. of cases in which inclusion bodies were found		
	Total no. of dog carcases examined	only in brain	in brain plus other organs	only in other organs
	53 (100%)	2 (4%)	33 (62%)	18 (34%)
No. of cases carrying inclusions	in brain	35 (66%)		
	on other organs		51 (96%)	

The frequency of both intranuclear and intracytoplasmic inclusions varied greatly in all organs studied, regardless whether the dog had died spontaneously or had been killed. As no notable difference was found between the frequencies of occurrence in any organ or organ system in relation to natural or artificial death, this distinction has been omitted further on.

Inclusions were found in the sequence of frequency in urinary bladder (73%), bronchi (72%), pulmonary parenchyma (68%), third eyelid (60%), renal pelvis (58%), trachea (49%), stomach (45%), and much less often in the other organs studied, including brain and spinal cord (Table II).

The distribution of the inclusions in organs showed great individual variations. Some carcases had them in all organs studied, others either in one

Table II

Indidence of inclusions in different organs of distemper dogs

	Brains and spinal cord								Other organs															
											lung													
	ependyma	striated body	Ammon's horn	brain cortex	c.quadrigemina	cerebellum	medulla oblongata	spinal cord	thirdeyelid	trachea	bronchioli and med. bronchi	alveolar epith. cells	urinary bladder	renals pelvis	stomach	liver	small intestine	pancreas	spleen	adrenal	parotid gland	peribronchial lymph node	tongue	bone marrow
No. of histologically examined cases	53							3	53								37	19	20	9	5	3	7	1
Inclusions present No.	15	4	17	3	5	14	3	0	32	26	38	36	39	31	24	9	4	4	3	4	4	2	0	0
Inclusions present %	28	7	32	6	9	26	6	—	60	49	72	68	73	58	45	17	—	—	—	—	—	—	—	—

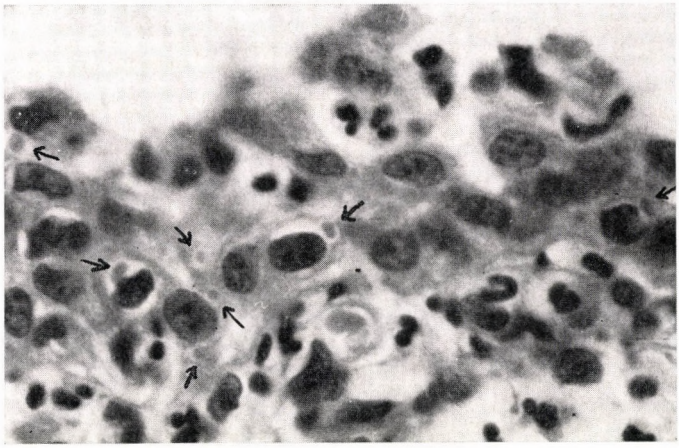


Fig. 1. Round and oval intracytoplasmic inclusions (arrow) in conjunctival epithelium cells of third eyelid. Note infiltrating granulocytes. H. and E., approx. ×1000

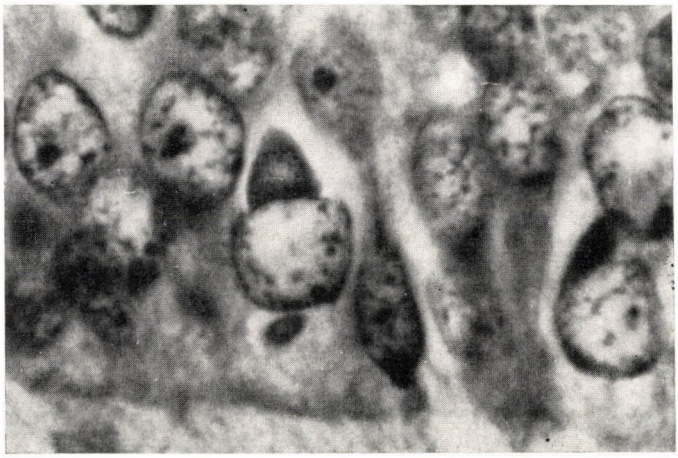


Fig. 2. Juxtanuclear minor oval and larger pyramid-shaped intracytoplasmic inclusion bodies in a bronchial columnar epithelium cell. Note the vacuolized inner structure of the pyramid-shaped body. H. and E., approx. ×2500

or in another single organ. Incidence in a given organ ranged from one or a few cells to 60%, or, infrequently, even 80% of the cells.

Localization of intracellular inclusions in organs

Cytoplasmic inclusions were found in various cell types of each organ as follows: in single cells of stratified column arlining epithelium (Fig. 1); cells of glandular acini and lining cuboidal cells in glandular outlets of the third

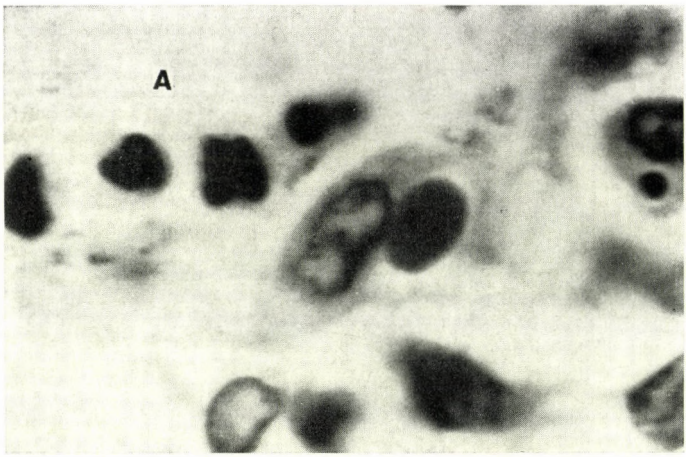


Fig. 3. Homogeneous, oval-shaped intracytoplasmic inclusion in a pulmonary alveloar epithelium cell. A few neutrophilic granulocytes in the alveolar lumen (A). H. and E., approx. ×2500

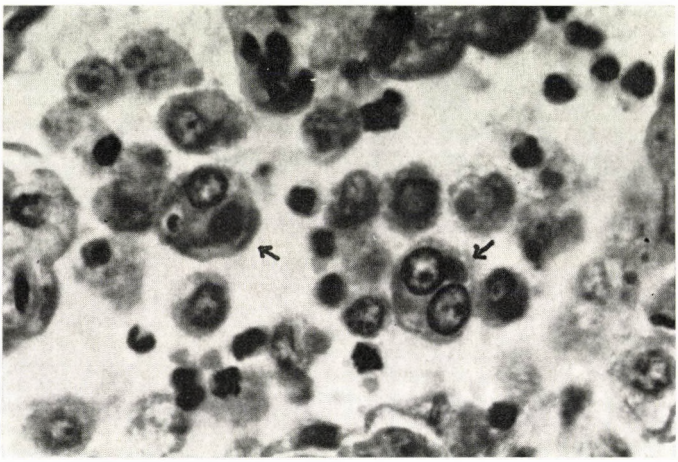


Fig. 4. Intracytoplasmic inclusions (arrow) in desquamated pulmonary alveolar epithelium cells admixing with the catarrhal-purulent exudation, and in giant cells arising in the alveolar epithelium. H. and E., approx. ×1000

eyelid; in villous epithelial cells of trachea and bronchi (from main bronchi to terminal bronchioli) (Fig. 2); in pulmonary alveolar epithelium cells (Fig. 3) — occasionally in desquamated ones admixing with the catarrhal-purulent exudation, or in giant cells arising from pulmonary alveolar epithelium (Fig. 4); in urothelium cells of urinary bladder and renal pelvis (Fig. 5); mainly in chief cells and accessory cells of fundus glands in the stomach (Fig. 6), less often in columnar cells lining the gastric sulci, and in cuboidal or columnar cells of minor or major hepatic biliary ducts.

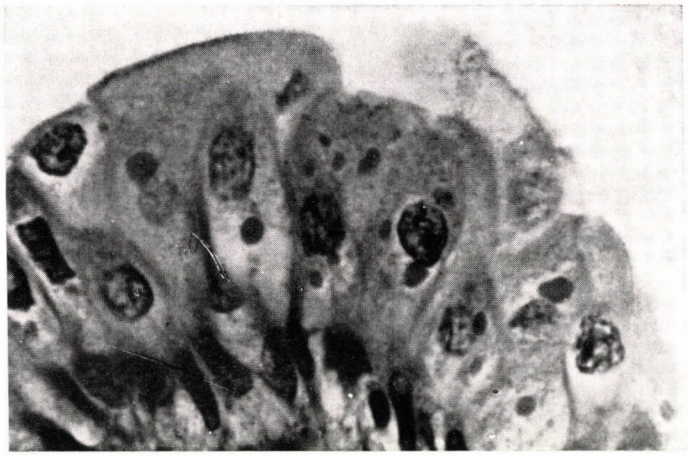


Fig. 5. Pleomorphous cytoplasmic inclusions in lining epithelium cells of the urinary bladder. H. and E., approx. ×1000

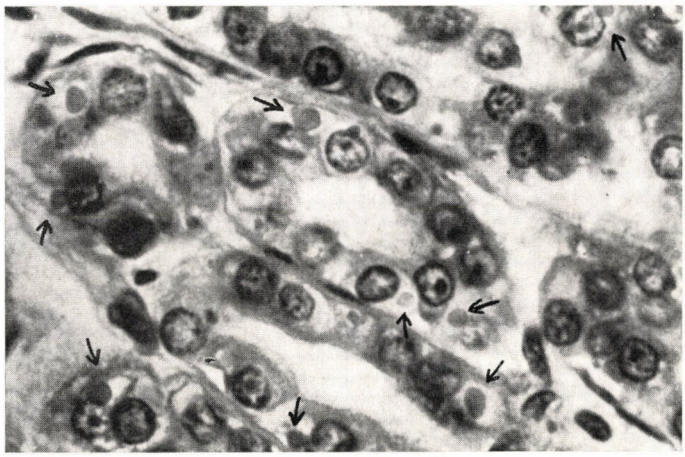


Fig. 6. Intracytoplasmic inclusions (arrow) in glandular epithelium cells of fundus cells. H. and E., approx. ×1000

Among the organs less often studied, inclusions were found in cells of the crypts of Lieberkühn in the small intestine; in lining columnar cells of major and minor pancreatic ducts; in cuboidal cells of intermediate tubuli and columnar cells of intralobular salivary tubules of the parotid gland. We failed to find intracytoplasmic inclusions in the lingual mucous epithelium.

Intranuclear inclusions were found in organs other than the brain in only four of the 53 dogs, always in association with intracytoplasmic inclusions. One of the four dogs had intranuclear inclusions in alveolar epithelium cells,

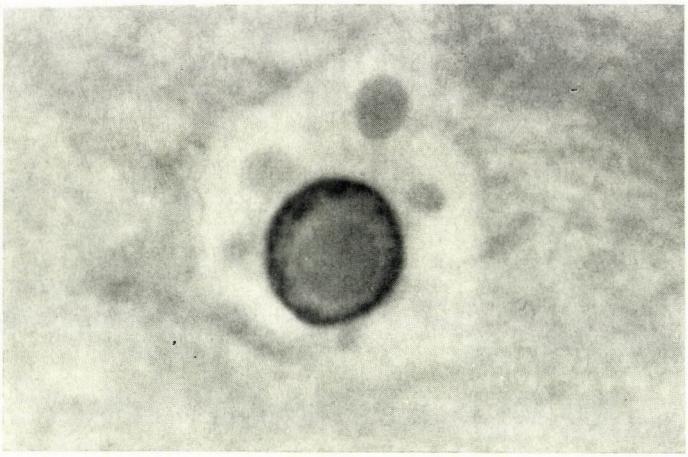


Fig. 7. Intracytoplasmic and intranuclear inclusion bodies in a cerebral astrocyte. H. and E., approx. ×2500

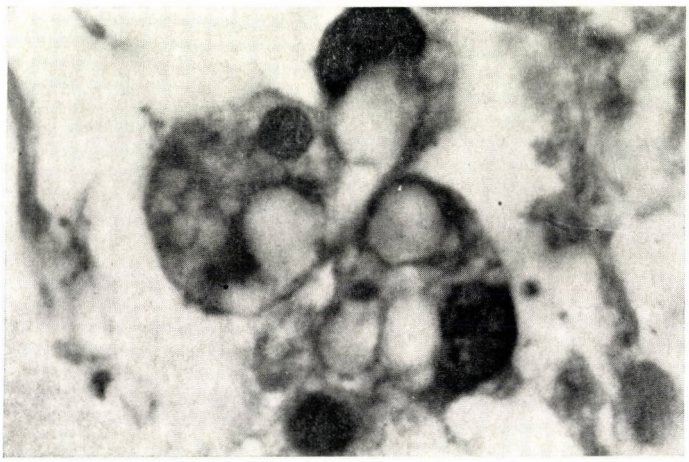


Fig. 8. Intracytoplasmic inclusion in a cerebral granular glia cell. Of the two granular glia cells nearby a small capillary vessel, that carrying the inclusion, has its nucleus out of the plane of section. H. and E., approx. ×2500

two dogs had them in epithelial cells of the urinary bladder and one in chief cells of fundus glands of the stomach as well as in a few cells of pancreatic exocrinic glands. The intranuclear inclusions occurred sporadically in cells of fundus glands and pancreas, somewhat more frequently in urinary bladder urothelium, and appeared in fair numbers in the alveolar epithelium cells.

Apart from epithelial cells, both intracytoplasmic and intranuclear inclusions were in a few instances found in reticulum cells of spleen and peribronchial lymph nodes as well as in histiocytes of the pulmonary interstitial tissue.

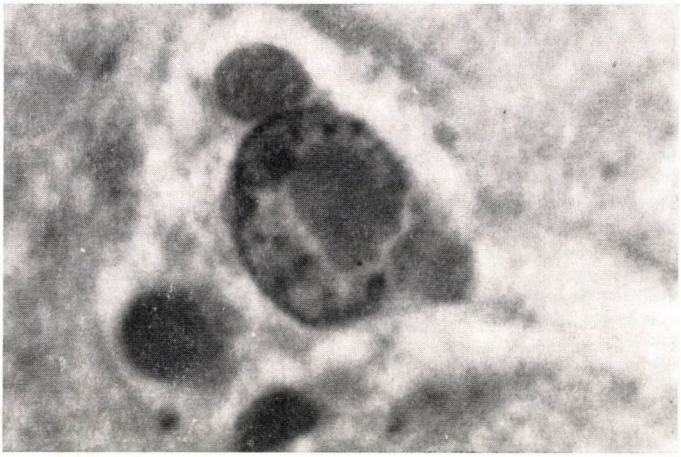


Fig. 9. Two vacuolized intracytoplasmic inclusions and one large, homogeneous nuclear inclusion in a single nerve cell of the cerebral cortex. Two glia cells are seen in a nearby location. H. and E., approx. ×2500

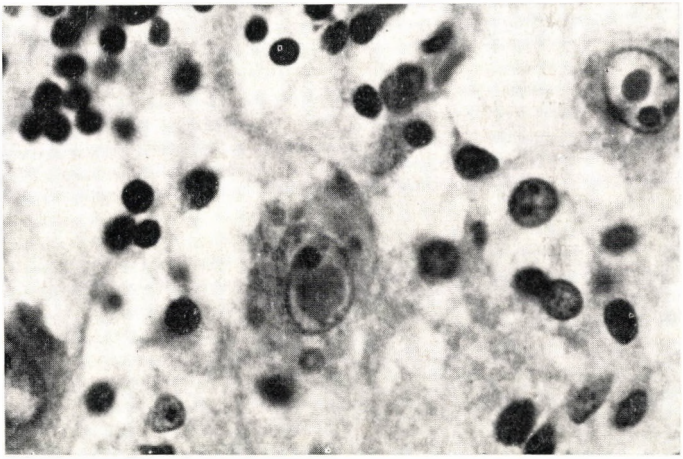


Fig. 10. Inclusion bodies in cerebellar Purkynje cells. One cell contains several small intracytoplasmic inclusions and a large one in the nucleus, whereas in the other cell only one intranuclear inclusion is seen. H. and E., approx. ×1000

In the brain both types of inclusions occurred in nerve cells and in various types of glia cells, usually in ependyma cells and astrocytes, less often in other, *e.g.*, granular glia cells. In 25 (71%) of the 35 cases in which inclusions were found in the brain, these appeared in ectodermal astrocytes localizing in various brain regions. Cells were found to carry one or the other type of inclusion, or both types simultaneously (Fig. 7). Other types of glia cells (above all granular ones) were found to carry exclusively a cytoplasmic inclusion in five

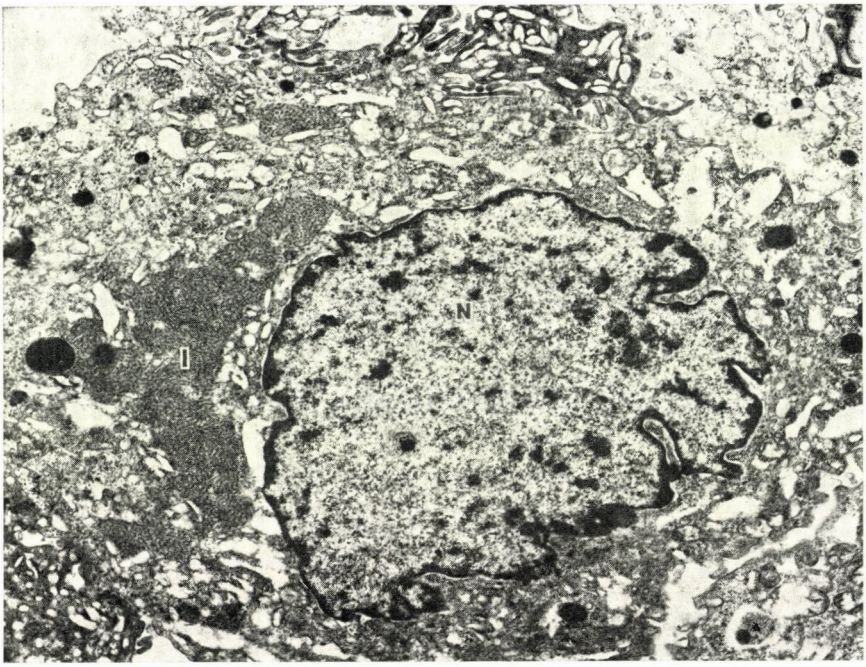


Fig. 11. Electron micrograph of a pyramid-shaped intracytoplasmic inclusion (I) nearby the nucleus (N). ×9260

instances (Fig. 8). In 15 cases (43%) lining ependyma cells of the cerebral ventricles were found to carry chiefly intracytoplasmic inclusions, rarely intranuclear ones. Nerve cells of various types were found to contain inclusions in 14 cases (40%), above all in the region of Ammon's horn, and, somewhat less often, in the cortex cerebri (Fig. 9), cerebellum (Fig. 10) and other brain regions. The greater part of the nerve cells contained both types of inclusion, the minor part either one or the other type.

Morphological appearance of inclusions

The intracytoplasmic inclusions were 1 to 15 μm in diameter and most of them took on a homogeneous vivid red or strawberry-red eosin stain, easily distinguishable from the brick-red colour of the erythrocytes. Vacuole structures were occasionally seen in some of the larger inclusion bodies. The inclusion bodies were variable in shape, being predominatly round or oval, but cylindrical, dumbbell-shaped forms, or irregular forms carrying processes were also found. Some inclusions were of a rectangular or pyramid shape, and localized close by the nucleus, with the broader end against it. Many inclusions were either surrounded by a pale halo, or a pronounced rarefication of the

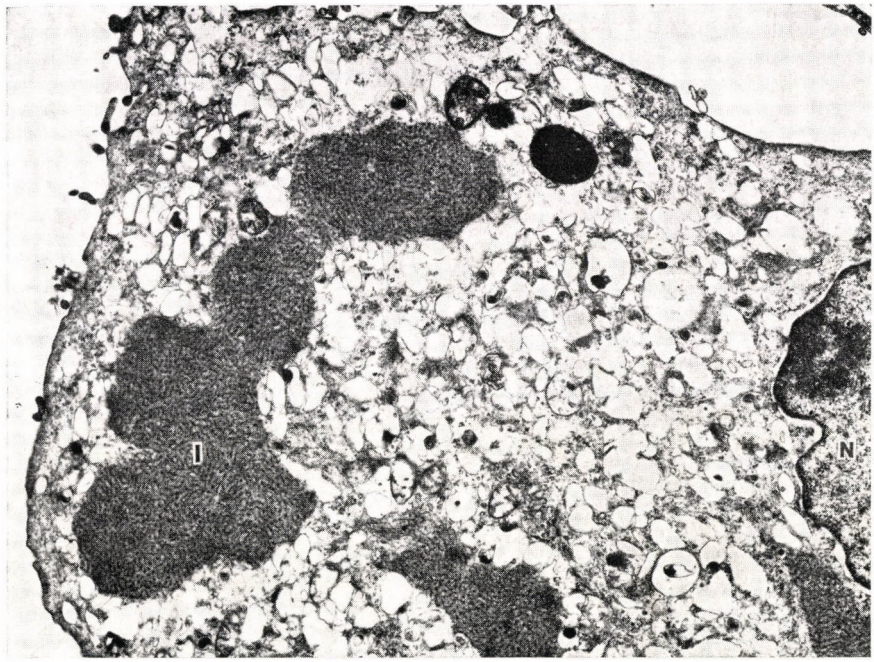


Fig. 12. Electron micrograph of intracytoplasmic inclusion (I) in urothelium cell. The area corresponding to the inclusion is electron dense and shows a net-like structure. ×9260

cytoplasm was apparent around them. Some cells contained several inclusions each.

Most nerve cells carrying a cytoplasmic inclusion showed various degrees of injury.

Like the cytoplasmic inclusions, most intranuclear inclusions were either round or oval, but irregular forms also occurred. Part of the nuclei carrying inclusions were swollen and the nucleolus was pushed to one side by the inclusion. Most intranuclear inclusions were surrounded by a pale halo. The staining properties were similar to those of intracytoplasmic inclusions, except that the intranuclear inclusions found in the brain, above all those localizing in astrocytes and nerve cells, took on less stain, assuming a light red, occasionally a greyish-violet, shade instead of purple or raspberry red.

Electron-microscopic observations

The specimen used for electron-microscopic examination was taken from the urinary bladder of a dog killed for canine distemper when 3 months old. The animal had contracted the disease by natural infection and, according to the case history, had not been treated with either specific antiserum or vaccine.

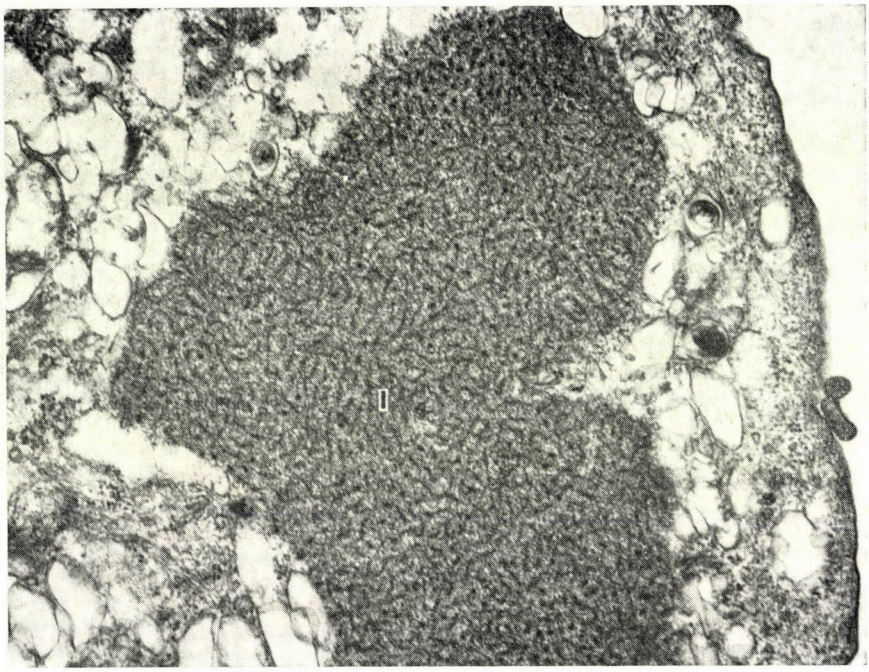


Fig. 13. Detail of (I) from Fig. 12. Note thread-like structure and absence of a limiting membrane around the inclusion body. ×25,200

Ultra-thin sections prepared from urinary bladder urothelium showed homogeneous, well-circumscribed electron-dense structures in the areas corresponding to the inclusions seen by light microscopy (Figs 11 and 12). The cytoplasmic inclusions, though apparently having no limiting membrane (Fig. 13), were sharply demarcated against the neighbouring cell areas. At a lower power of magnification, the cytoplasmic inclusions had an irregular filamentous structure (Fig. 13). At a higher power, each thread corresponded to a tubule, 18 nm in diameter (Fig. 14). The tubules consisted of an electron-dense core and a less dense halo. According to literary data, such tubules are characteristic of the nucleocapsid of canine distemper virus. The nucleocapsid network was embedded in a coarse, granular ground substance. The epithelial cells carrying inclusions, above all the superficial urothelium cells, had markedly vacuolized cytoplasms.

Discussion

The intracytoplasmic and intranuclear inclusions generally regarded as characteristic of canine distemper were found in all dogs that died spontaneously, or were killed in various stages of the disease. Accordingly, demonstration of

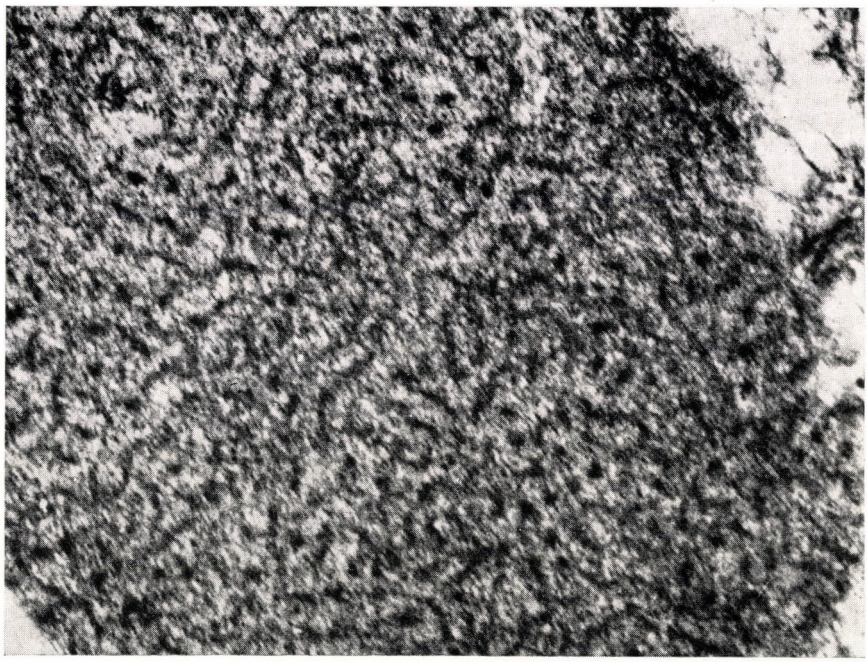


Fig. 14. Detail of Fig. 13. The delicate tubules (nucleocapsids) 18 nm in diameter correspond to the threads seen at the lower power. × 56,000

inclusion bodies as employed by us may be considered an efficient diagnostic procedure even if our results have not been verified by other specific methods, such as virus isolation and immunofluorescence technique. Nevertheless, it may occur that inclusions cannot be demonstrated in any organ by the applied method, especially after a prolonged course of canine distemper.

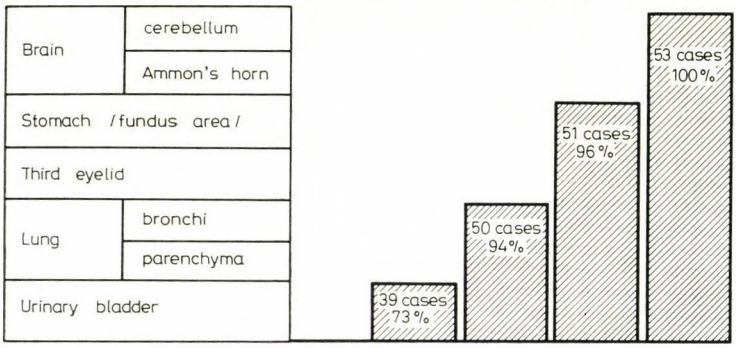
We have no experience concerning the earliest and latest times when inclusions bodies can be found in canine distemper. Certain authors concluded from chiefly practical observation that inclusions were present in the brain from the onset of the illness to about the end of the third month (FISCHER, 1965); in other organs, inclusions were found up to 59—60 days after infection (APPEL, 1969; FAIRCHILD et al., 1971). The length of the period during which inclusions are demonstrable depends to a great extent on the applied method.

The efficiency of the method employed by us seems to be considerably influenced by the site or organ from which the specimens are taken. Although the chances of detecting inclusions are in principle increasing with each organ additionally tested, this tendency cannot be pursued too far in routine diagnostic work. The scrutiny of our findings in this light clearly shows that the best results can be expected from the simultaneous examination of sections from urinary bladder, lung, third eyelid, stomach as well as cerebellum and Ammon's

horn. Examination of the urinary bladder alone would have disclosed in the present study the presence of inclusions in 73% of the positive cases. Additional examination of the lung would increase efficiency to 94%, with the third lid and stomach also included, to 96%, and if findings in cerebellum and Ammon's horn are additionally taken into consideration, the results are the same as obtained with the full range of organs tested in the present study (Table III). I believe that the examination of the above four organs and two brain areas can still be reconciled with the possibilities and limits of routine diagnostic work.

Table III

Diagnostic value of the demonstration of inclusion bodies in various organs of distemper dogs* (The numerical and percentual values inscribed in the columns apply to the organ systems covered by the latter)



* 53 carcases were studied

The risk of diagnostic error cannot, however, be obviated in studies based on the above organ combination; it is, therefore, recommended that specimens should be always collected from other organs also, which can be made use of when demonstration fails in the routine combination.

In carcases stored longer, maceration of the urinary mucous epithelium by contents of the bladder may interfere with the histological evaluation. In such cases sections should be prepared from the renal pelvis as well, because inclusions are nearly always found in this localization.

In the third eyelid, intracytoplasmic inclusions often could not be demonstrated except in glands. It is therefore advisable to take specimens from areas nearby the cartilaginous base, part of which should be included in the sections. In stored carcases inclusions were generally demonstrable as long as the histological orientation in the organs was not disturbed by antolysis. Staining should be made with ethanol-soluble eosin of good quality.

The exclusive use of haematoxylin-eosin preparations may arouse doubts in respect of the specificity of inclusions. Although in the light of the present findings and our related experience, such doubts are largely unfounded, we too, have certain reservations. The development in dogs of similar inclusion bodies seems to be possible with certain other respiratory diseases, above all, with viral diseases. In this field further examinations are under preparation. At the present state of knowledge the problem seems to be of theoretical rather than practical importance. It is expected that for reasons of specificity, immunofluorescence techniques will gradually supersede the simple histological approach to the diagnosis of canine distemper.

Electron-microscopic studies performed as complementary examinations have shown that the ultrastructural details of the inclusion bodies corresponded in every respect to the characteristics of canine distemper infection as shown in cell cultures (NORRBY et al., 1963; KOESTNBR and LONG, 1970) and in specimens from diseased or dead dogs (RICHTER and MOISE, 1970). The network of nucleocapsid tubules, 18 nm in diameter, was demonstrated also in this study in localizations corresponding to light-microscopic inclusions in urinary bladder epithelium cells. It follows that the Feulgen-negative, RNA-containing (LAUDER et al., 1954) inclusion body, showing the antigenic properties of canine distemper virus in immunofluorescence tests (MOULTON and BROWN, 1954; APPEL, 1969), contains the incomplete canine distemper virus itself in a certain stage of the disease (NORRBY et al., 1963; KOESTNER and LONG, 1970; RICHTER and MOISE, 1970). The electron-microscopic demonstration of the inclusion bodies is a specific procedure, but not yet available for routine use. It is, however, an important complementary procedure in experimental studies and is expected to disclose more information on the nature of intranuclear inclusions which is still not understood in all details.

Acknowledgements

The author is indebted to Miss V. NÉMETH, Mrs. E. PETHES and Mrs. J. NAMÉNYI for excellent technical assistance.

Summary

The frequency of occurrence, morphology and diagnostic value of inclusion bodies in canine distemper were studied by a light-microscopic histological method. Various organs of 53 dogs, of which 30 had died spontaneously and 23 and been killed in various stages of the disease, were evaluated in haematoxylin and eosin sections prepared from formalin-fixed and paraffin-embedded specimens. Inclusions were variably present in the different organs, but were found in all the 53 cases studied, localizing in the brain in nuclei and/or cytoplasm of nerve cells and various types of glia cells, and in other organs in accessory and glandular epithelial cells. The inclusions were predominantly intracytoplasmic in the extracerebral localizations.

The highest incidence (73%) was found in urothelium cells lining the urinary bladder; with the lung included, the frequency rose to 94% and if the stomach, third eyelid and brain (cerebellum and Ammon's horn) were also taken into consideration, inclusions were found in all carceses examined, so that demonstration in still further organs did not alter the result. Examination of the above four organs and two brain areas is therefore recommended for routine histological diagnostic work. If in long-stored carcases the urothelium has been macerated, the lining epithelium of the renal pelvis should be examined instead.

The ultrastructure of intracytoplasmic inclusions localizing in mucous epithelium cells of the urinary bladder is described on the basis of complementary electron-microscopic examinations. The inclusion bodies were found to consist of a network of 18 nm wide tubules, characteristic of the nucleocapsid of canine distemper virus.

References

APPEL, M. J. G.: Amer. J. Vet. Res. **30** (1969), 1167. CAMPBELL, R. S. F.: Brit. Vet. J. **113** (1957), 143. FAIRCHILD, G. A., STEINBERG, S. A. and COHEN, D.: Cornell Vet. **61** (1971), 214. FISCHER, K.: Path. Vet. **2** (1965), 380. GREEN, R. G. and EVANS, C. A.: Amer. J. Hyg. **29** (1939), 73. KOESTNER, A. and LONG, J. F.: Lab. Invest. **23** (1970), 196. LAUDER, I. M., MARTIN, W. B., GORDON, E. B., CAMPBELL, R. S. F. and WATRACH, A. M.: Vet. Rec. **66** (1954), 623. LENTZ, O.: Zbl. Bakt. Orig. **44** (1907), 374. IDEM: Zschr. Hyg. Infektionskrankh. **62** (1909), 63. MACINTYRE, A. B., TREVAN, D. J. and MONTGOMERIE, R. F.: Vet. Rec. **60** (1948), 635. MOULTON, J. E. and BROWN, C. H.: Proc. Soc. Exp. Biol. Med. **86** (1954), 99. NICOLAU, S.: Compt. Rend. Soc. Biol. **119** (1935), 269. NORRBY, E., FRIDIIG, B., ROCKBORN, G. and GARD, G.: Arch. ges. Virusforsch. **13** (1963), 335. RIBELIN, W. E.: Amer. J. Vet. Res. **14** (1953), 96. RICHTER, W. R. and MOIZE, S. M.: Path. Vet. **7** (1970), 346. SANFELICE, F.: Zbl. Bakt. Abt. I. Orig. **76** (1915), 495. SINIGAGLIA, G.: Ref.: Berl. Tierärztl. Wschr., 1912, 775. STANDFUSS, R.: Arch. Wissensch. Tierheilk. **34** (1908), 109.

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VACCINATION EXPERIMENTS ON PREVENTION OF *E. COLI* ENTERITIS IN WEANLING PIGS

I. USE OF ALUMINIUM GEL ADJUVANT

By

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(Received December 12, 1974)

Enteritis caused by *Escherichia coli* is a frequent disease of weanling pigs, often causing great losses in large-scale farms. The animals develop the disease as a rule within 1 or 2 weeks after weaning, either in the form of gastro-enteritis or as oedema disease.

Although the epizootological course, symptoms and gross lesions of the condition are well-known (SZABÓ, 1958, 1964; SOJKA, 1965; NIELSEN et al., 1968; SVENDSEN, 1974), its aetiology and pathogenesis are still not fully understood. Management failures and feeding errors predispose for the disease and strains belonging to given *E. coli* serotypes as well as their toxins have been identified as the direct causal agents. Isolates from the small intestine of carcases are as a rule haemolytic strains of the groups O138, O139, O141 or of other O groups (SZABÓ, 1962, 1965; SÖDERLIND, 1971; SEMJÉN and PESTI, 1973). The enteropathogenic *E. coli* strains which, by extreme multiplication, become predominant in the small intestine, produce the enterotoxin (SMITH and HALLS, 1967; GYLES, 1971; PESTI and SEMJÉN, 1973) responsible for enteritis, whereas the endotoxins of *E. coli* bacteria play a role in the aetiology of the oedema disease, presumably along with allergic processes (NAGY et al., 1968; NIELSEN and CLUGSTON, 1971; WITTING, 1971).

As the losses due to the *E. coli* enteritis of weanling pigs could not be notably reduced by antibiotic therapy, experiments were conducted on the possibilities of vaccine prophylaxis.

Materials and methods

The experiments were performed in a closed pig unit. The piglets were weaned at 4 weeks of age and losses usually began to occur at 6—8 days after weaning.

Isolation and biochemical and serological testing of strains

To identify the antigenic structure of the *E. coli* strains responsible for the disease, carcases of weanlings that died of gastro-enteritis in the chosen batch were examined bacteriologically before vaccination. Two or three coliform

strains were isolated from the small intestine and mesenteric lymph nodes of each carcase. The isolates were tested for biochemical properties and the *E. coli* isolates were examined for O and K antigens. Fifty-seven O or OK antisera and all K sera (1—94) were available for identification (VARGA and FARID, 1974). The biochemical and agglutination tests as well as the preparation of antisera were carried out as proposed by KAUFFMANN (1966).

Composition and application of the vaccine

The vaccine contained equal amounts of formalin-inactivated 12 to 16-hour broth cultures of the strains O41:K(A), O138:K81, O141:K85, and O147:K89, 88ac, adsorbed to 5 mg/ml $AlPO_4$ and preserved with merthiolate. Two pig groups, weaned at an interval of one month, were vaccinated, and a control group of almost equal size was set up with each. The vaccine used for group II contained, instead of the original strain, the one having the antigenic pattern O138:K81,88ab. Each animal was vaccinated twice with a 4-ml subcutaneous dose on each occasion, *viz.* when 2—3 weeks old and one week later, before weaning.

Collection and examination of blood samples

Paired sera were used for examination of the antibody response. Blood samples were taken from both experimental and control pigs at the first vaccination and one week after weaning. O antibodies were demonstrated by an indirect haemagglutination test (IHA) (WILSON and SVENDSEN, 1971), using red blood cells presensitized with a pool of equal amounts of O antigen extract from each vaccine strain. K antibodies were demonstrated with KAUFFMANN's (1966) tube agglutination test, independently with the K antigen of each vaccine strain.

Results

The 12 pigs that died in the weanling group before the time of vaccination were all found to have gastro-enteritis at postmortem examination. Grossly visible oedema was not found either in the stomach, or in the mesentery. Most isolates from the small intestine and mesenteric lymph nodes were pure cultures of haemolytic *E. coli* strains. The 30 isolates belonged to four serological groups, having the antigenic patterns O41:K(A), O138:K81, O141:K85 and O147:K89, 88ac. Based on preliminary serological examinations, the vaccine was prepared to contain all four *E. coli* serotypes responsible for disease in the herd. Table I shows the results of vaccination; they were practically identical in Groups I and II. Losses were about 50% lower among the vaccinees, compared to controls kept under the same conditions of management. Isolates from the

control pigs had, apart from a few *E. coli* strains not typable with the available sera, the same antigenic patterns as the isolates from preceding groups of weanlings. Although losses among the vaccinees were also due to gastro-enteritis, strains of O147:K89, 88ac antigenic pattern were found only in two carcases. The other isolates were non-haemolytic strains belonging to serogroups other than enteropathogenic for the pig.

Table I

Efficiency of aliminium-adsorbed *E. coli* vaccine in prevention of *E. coli* enteritis in weanling pigs

Vaccinated group	Vaccinated pigs		Control pigs	
	Total No.	Died	Total No.	Died
I	263	8	273	17
II	240	10	262	19
Total	503	18 (3.6%)	535	36 (6.7%)

The sera from control pigs contained neither O nor K antibodies to any vaccine strain at either sampling time. The same applied to the first samples of the vaccinees, while the second samples showed IHA titres ranging from 1 : 8 to 1 : 32 against the O antigens of the vaccine strains. K antibodies appeared only to strains O138:K81, 88ab and O147:K89, 88ac, at titre levels of 1 : 64 to 1 : 128. The antibodies agglutinating the two strains reacted exclusively with the K88ab or K88ac component, to judge from the absence of reaction with variants not containing the antigen K88.

Discussion

The blood of suckling pigs aged 2—5 weeks contained neither O nor K antibodies to the *E. coli* strains responsible for disease and for losses after weaning, although these strains had been present in the herd for a long time. SHARPE (1965) arrived at the same conclusions with other serotypes.

Two immunizations with an aluminium-adsorbed polyvalent *E. coli* vaccine stimulated the formation of specific O antibodies in pigs aged 2—5 weeks. K antibodies were, however, only formed to K88 antigens, probably because the latter are of protein nature (STIRM et al., 1967).

Vaccination can only confer protection if the antibodies also appear locally, in the intestinal wall. The protective effect is associated chiefly with

type-specific K antibodies (SALAJKA, 1971) and is of an antibacterial rather than antitoxic character (SMITH and LINGOOD, 1971; SMITH, 1972).

As the vaccination failed to evoke an immune response to K(B) and K(A) antigens, immunity is apparently only developed to serotypes possessing the K88 antigen component. Thus, although losses could be reduced by 50%, the practical use of the vaccine preparation is not recommended for the time being.

Acknowledgement

The authors are indebted to veterinarians Dr. B. BERECZ, Dr. S. CSECSERITS, for carrying out the vaccinations and blood samplings.

Summary

On the basis of preliminary bacteriological and serological examinations, prophylactic vaccination of suckling pigs against *E. coli* enteritis affecting them in the weanling age was attempted with an experimental preparation containing *Escherichia coli* strains of O41:K (A), O138:K81, 88ab, O141:K85 and O147:K89, 88ac antigenic pattern and $AlPO_4$ as adjuvant.

Vaccination on two occasions before weaning was followed by a specific O and K serum antibody response to the vaccine strains. K antibodies were, however, formed exclusively to K88 antigens and neither K(B), nor K(A) antibodies were demonstrable in the serum samples of the vaccinees. Losses amounted to 3.6 and 6.7% in the experimental and control groups, respectively.

As the vaccine stimulated a firm immune response exclusively to the K88 antigen, it can presumably only protect against strains carrying this component and should not be, therefore, used in the field in its present form.

References

GYLES, C. L.: Ann. N. Y. Acad. Sci. **176** (1971), 314. KAUFFMANN, F.: The Bacteriology of Enterobacteriaceae. Copenhagen, 1966. NAGY, Z., BERCZI, I. and BERTÓK, L.: Zbl. Vet. Med. B. **15** (1968), 504. NIELSEN, N. O. and CLUGSTON, R. E.: Ann. N. Y. Acad. Sci. **176** (1971), 176. NIELSEN, N. O., MOON, H. W. and ROE, W. E.: JAVMA **153** (1968), 1590. PESTI, L. and SEMJÉN, G.: Acta vet. hung. **XXIII** (1973), 227. SALAJKA, E., ULMANN, L., HORNICH, M. and SARMÁNOVÁ, Z. Acta vet. (Brno) Suppl. **2** (1971), 65. SEMJÉN, G. and PESTI, L.: Acta vet. hung. **XXIII** (1973), 219. SHARPE, H. B. A.: Res. vet. Sci. **6** (1975), 490. SMITH, H. W.: J. Med. Microbiol. **5** (1972), 345. SMITH, H. W. and HALLS, S.: J. Path. Bact. **93** (1967), 531. SMITH H. W. and LINGGOOD, M. A.: J. med. Microbiol. **4** (1971), 467. SOJKA, W. J:: *E. coli* in domestic animals and poultry. Commonwealth Agricultural Bureaux, 1965. SÖDERLIND, O.: Zbl. Vet. Med. B. **18** (1971), 569. STIRM, S., ØRSKOV, J. and MANSA, B.: J. Bacteriol. **93** (1967), 731. SVENDSEN, J.: Nord. Vet. Med. **26** (1964), 226. SZABÓ, I.: Magyar Állatorvosok Lapja (MÁL), 1958, 295. IDEM: Ibidem, 1962, 93. IDEM: Adatok a sertések *E. coli* okozta megbetegedéseihez. Thesis. Budapest, 1965. VARGA, J. and FARID, A. F.: Acta vet. hung. **XXIV** (1974), 421. WILSON. M. R. and SVENDSEN, J.: Am. J. vet. Res. **32** (1971), 891. WITTING, W.: Mh. Vet. Med., 1971, 74.

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RADIATION ELICITED CHANGES IN BURSA OF FABRICIUS AND THYMUS DURING THE ONTOGENY OF CHICKENS

By

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(Received December 20, 1974)

A great number of data have shown the influences exerted by radiations on lymphoid organs of mammals and birds (MOSSER et al., 1970; PORA et al., 1970; POTOP et al., 1967; SATO et al., 1969; WEBER et al., 1969). The thymus is especially sensitive and its reaction is involutional (TESSERAUX, 1959). Both thymus and bursa of Fabricius are very rich in nucleic acids (FREEMAN, 1971; PORA et al., 1969) and highly sensitive to irradiation.

However, to our best knowledge, there are no data on changes in the protein metabolism under such actions in the thymus and the bursa of Fabricius of chickens. The present investigations are concerned with this topic.

Materials and methods

Experiments were performed on Studler—Cornish tetralinear hybrid chickens, fed on a standard concentrate fodder, appropriate to their age. For X-ray irradiation, a single dose of 100 R was used, applied to the whole organism, in open field.

Chickens 3 days and 3 weeks of age were irradiated to obtain information about the age-dependence of the effects; each experimental group was parallelled by a control group. The animals (8 at each time) were killed by decapitation at 4 and 24 hrs and at 3, 8 and 14 days after irradiation.

The following determinations were made from the thymus and the bursa of Fabricius: total protein; Robinson-Hogben's method (1958) as modified by Korpaczy (1958); RNA and DNA; SPIRIN's method (1958) as modifed by ABRAHÁM et al. (1965); free amino acid nitrogen (RAC, 1959).

Statistical evaluation of the results was done with Student's "t" test, after eliminating the aberrant values, following Chauvenet's criterion.

Results

The irradiation of 3-day-old chickens (Table I) did not elicit any changes in the total protein content of the thymus and bursa of Fabricius during the

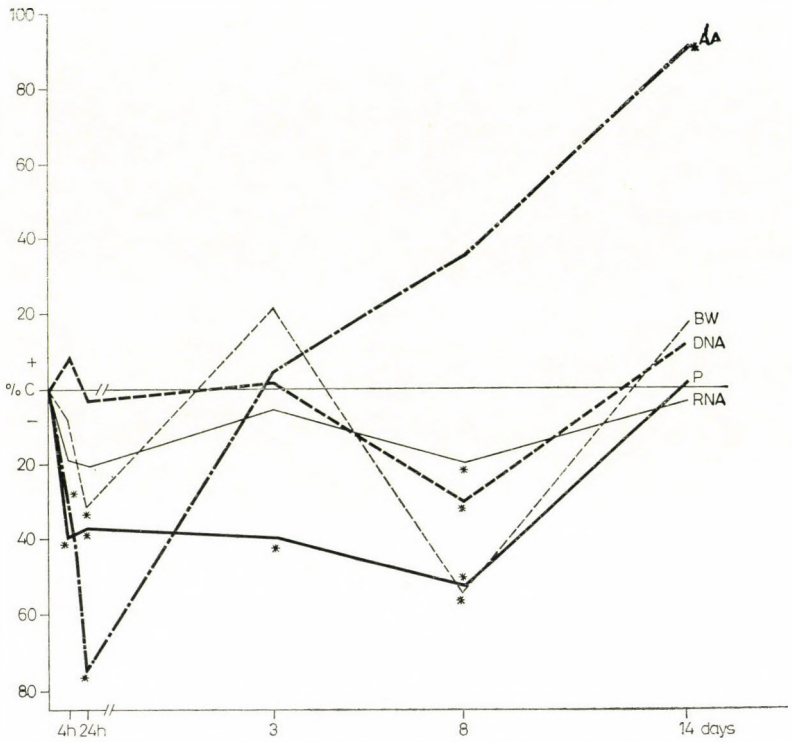

Fig. 1. Percentage differences in RNA, DNA, total protein (P), total free amino acids (AA), and organ weight (BW) in the bursa of Fabricius of chickens irradiated with a dose of 100 R as compared to the controls (C). * statistically significant

first 3 days. By the 8th day, the protein content of both organs had increased, but the value was again normal at 14 days. There was no change in the quantity of RNA as compared to the controls. The DNA content increased both in the thymus and in the bursa of Fabricius, but by the 14th day it returned to the control level.

A common trend for both organs is a large and rapid decrease amino-acid nitrogen after irradiation; this was more pronounced in the thymus. Later on, an increase occurred: at 14 days the values were normal in the bursa of Fabricius, and exceeded the control level in the thymus.

The glands had not changed in weight by the 14th day, when an increase was observed. The increase in body weight was hindered in irradiated chicks.

In chickens irradiated at three weeks of age (Table II) changes occurred which were characteristic of the state of involution of the thymus and bursa of Fabricius, *viz.*, total protein, RNA, DNA, and amino-acid nitrogen contents as well as the weights of the organs and of the body decreased. Minimal values

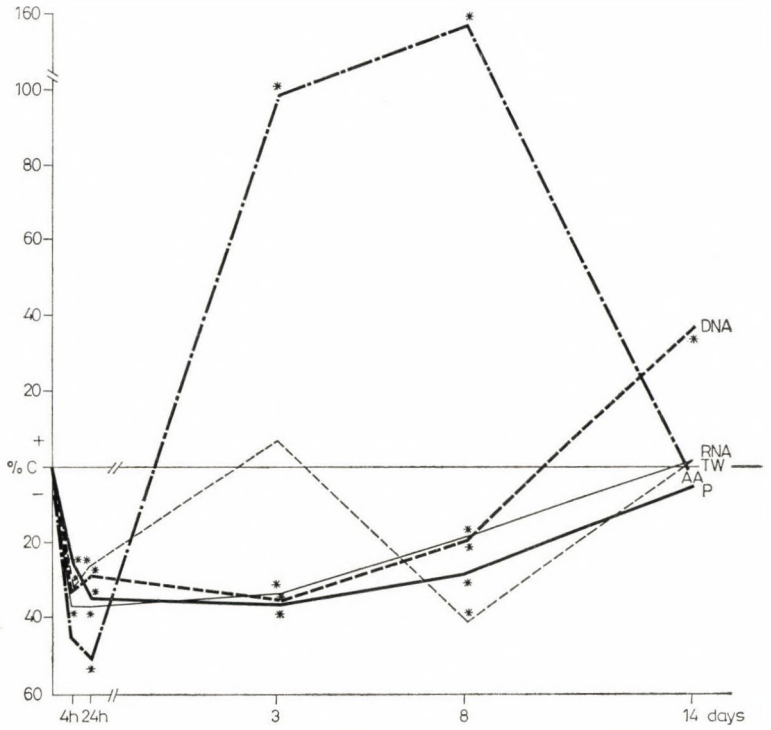

Fig. 2. Percentage differences in RNA, DNA, total protein (P), total free amino acids (AA), and organ weight (TW) in the thymus of chickens irradiated with a dose of 100 R as compared to the controls (C). * statistically significant

were generally reached at 8 days. The changes in were general more pronounced in the thymus than in the bursa of Fabricius. Recovery was observed at 14 days after irradiation (Figs 1, 2).

Discussion

Our first conclusion is that the radiosensitivity of chickens is age-dependent. Thus, the irradiation leads to moderate and late modifications in the lymphoid organs of chickens three days old at the time of irradiation, but sharp and immediate changes occur if 3 weeks old chickens are irradiated.

FREEMAN (1971) showed that chickens are mature by about 3 weeks after hatching. Until this age the adrenal potential is at a minimal physiological level, and this determines a low level of blood glucocorticoids. As SELYE (1971) has shown, the adaptation of the organism to environmental factors

is achieved through the corticoid hormones, and thus is dependent on the maturation of the hypophysis-adrenal axis.

A large difference appears between the two organs concerning their sensitivity to irradiation, the thymus being much more sensitive than the bursa of Fabricius. Literary data (PORA et al., 1970) suggest that the thymus is the most radiosensitive organ. This high sensitivity seems to be due to the high sensitivity of lymphocytes against X-rays (MILCU et al., 1965), and by the large DNA content of the thymus. It was shown that the radiosensitivity of tissues is proportional to the DNA content of their nuclei (RATIU, 1971).

The modifications occurring in chickens irradiated at 3 weeks after hatching point to a state of stress. The involutional phenomena are reversible, due to the small dose which we used. Similar doses were used by other authors to have transient effects on these and other parameters (RATIU, 1971; VASCULESCU et al., 1965); the explanation given by these investigators is that in such conditions no depolymerization of DNA occurs, but only an inhibition or retardation of its turnover.

The loss of DNA content in the thymus of chickens irradiated at 3 weeks has immunological consequences. It is known (GOOD et al., 1964; SATO et al., 1969) that antibody production is suppressed by X-ray irradiation.

In the 3 week-old chickens, the involution of the thymus and of the bursa of Fabricius is shown by the loss of weight of these organs, and by the decrease of the protein, RNA, DNA and free amino acid content. The later modifications are apparent from 4 until 8 days following irradiation. The loss of RNA results from an inhibition of the polymerase (ABRAHAM et al., 1971; FOX et al., 1972), and an enhancement of the activity of lysosomal enzymes (AMBELLAN et al., 1967; WOKAER, 1970). The decrease of these two organs in weight is a consequence of a loss of cells in them, and of the inhibition of mitoses which occurs independent of the nature of the agent (COMSA, 1959).

In 3-day-old irradiated chickens no involution of the glands took place, neither concerning their weight, nor the investigated metabolic parameters. Even increase in weight was observed, indicating a stimulatory effect. However, then a phenomenon common to both ages appeared: the free amino acid nitrogen decreased 4 hrs after irradiation, and this change was even more pronounced at 24 hrs. We observed the same phenomenon in previous investigations, using physical (cold) (GIURGEA et al., 1974), or hormonal (hydrocortisone) (TOMA et al., 1974), or ACTH (GIURGEA et al., 1974) stress agents. There are data (KENDYSH et al., 1969) showing that, after irradiation, a great release of free amino acids takes place from the radiosensitive organs into the blood; these are directed towards the liver for being used in gluconeogenesis.

Events occurring in the bursa of Fabricius and thymus under the action of any external factor are the result of the same modifications of the adrenal function, on which the lymphoid organs are highly dependent. This

Table I

Changes in 3-day-old chickens
superficially irradiated with 100 R X-rays

		Control		4 hrs		24 hrs		3 days		Control		8 days		Control		14 days	
		BF	T	BF	T	BF	T	BF	T	BF	T	BF	T	BF	T	BF	T
Total protein (P),	$\bar{x}$	15.3	23.9	14.6	22.1	19.8	23.6	14.0	21.1	8.8	17.7	12.7	26.8	12.4	17.1	13.9	20.7
mg/100 g	ES±	0.88	0.71	1.30	1.21	2.57	1.29	1.36	1.47	0.80	0.86	0.90	1.67	1.62	2.39	1.30	0.78
	--	8	8	8	7	8	8	6	8	8	8	6	8	8	8	8	7
	±%	—	—	−5	−8	+29	−2	−9	−17	—	—	+43	+51	—	—	+11	+20
	P	—	—	—	—	—	—	—	—	—	—	<0.001	<0.001	—	—	—	—
RNA, mg/g	$\bar{x}$	6.0	5.6	5.5	5.1	6.3	4.4	6.6	6.2	6.3	5.6	7.3	5.0	8.0	8.8	8.5	9.0
	ES±	0.28	0.53	0.02	0.28	0.28	0.60	0.13	0.34	0.23	0.62	0.66	1.78	0.17	0.47	0.49	0.37
	n	8	8	7	8	8	8	8	8.	8	8	6	8	7	8	8	8
	±%	—	—	−9	−8	+5	−21	+11	+12	—	—	+16	−7	—	—	+6	+1
	P	—	—	—	—	—	—	<0.02	—	—	—	—	—	—	—	—	—
DNA, mg/g	$\bar{x}$	6.4	9.6	6.9	13.5	7.6	11.6	6.2	11.2	6.8	10.5	9.5	9.2	6.8	11.3	7.0	12.7
	ES±	0.38	0.90	0.32	0.67	3.59	0.58	0.18	0.69	0.27	1.21	0.86	0.87	0.25	0.64	0.34	0.42
	n	8	8	8	8	7	6	8	8	8	8	8	8	8	8	8	8
	±%	—	—	+8	+40	+19	+21	−4	+16	—	—	+40	−13	—	—	+2	+12
	P	—	—	—	<0.01	<0.02	0.10 P <0.05	—	—	—	—	<0.01	—	—	—	—	—
Free amino acid	$\bar{x}$	46.9	37.2	11.3	4.6	9.9	19.6	26.6	5.6	19.9	28.0	11.12	28.1	3.4	2.8	3.0	12.1
nitrogen (AA),	ES±	14.79	2.38	3.59	0.77	3.18	2.38	7.61	1.07	1.90	4.64	1.60	0.73	0.98	0.69	0.75	2.07
mg N/1000 g	n	5	8	7	8	7	8	6	7	8	8	7	7	8	7	8	7
	±%	—	—	−76	−88	−79	−48	−37	−85	—	—	−45	—	—	—	−12	+331
	P	—	—	<0.02	<0.001	<0.001	<0.001	—	<0.001	—	—	<0.01	—	—	—	—	<0.001
Organ weight,	$\bar{x}$	39.3	64.8	44.3	83.6	40.2	77.0	49.7	53.2	98.7	102.2	95.6	99.0	121.5	156.1	164.3	199.8
mg	ES±	5.45	6.09	2.06	7.80	3.85	6.73	5.44	5.10	14.8	13.82	10.28	6.38	13.82	23.04	18.08	31.27
	n	8	8	8	8	8	8	8	7	8	8	8	8	8	8	8	8
	±%	—	—	+12	+28	+2	+18	+26	−18	—	—	−4	−8	—	—	+35	+28
	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	<0.02

$\bar{x}$ Values are means; ES±, standard error; n, number of animals; ±%, differences in per cent of the controls; BF, bursa of Fabricius; T, thymus

Table II

Changes in 3-week-sold chickens superficially irradiated with 100 R X-rays

		Control		4 hrs		24 hrs		3 days		Control		8 days		Control		14 days	
		BF	T	BF	T	BF	T	BF	T	BF	T	BF	T	BF	T	BF	T
Total protein (P), mg%	$\bar{x}$	23.7	30.7	14.6	22.8	15.0	20.0	14.4	19.4	15.1	18.4	7.1	13.3	17.8	23.4	18.2	22.3
	ES±	3.20	1.82	0.99	1.55	0.45	1.17	0.57	0.97	0.79	0.65	1.18	1.03	1.23	0.38	1.28	1.07
	n	8	8	8	8	8	8	7	8	8	7	8	8	8	7	8	8
	P	—	—	<0.01	<0.01	<0.01	<0.01	<0.01	<0.001	—	—	<0.001	<0.001	—	—	—	—
RNA, mg/g	$\bar{x}$	7.1	9.8	5.8	6.2	5.7	6.5	6.8	6.5	7.4	7.9	6.0	6.5	7.6	8.0	7.4	8.2
	ES±	0.80	0.34	0.18	0.17	0.09	0.22	0.23	0.31	0.23	0.19	0.29	0.12	0.18	0.54	0.09	0.21
	n	8	8	8	8	8	8	8	8	8	7	8	8	8	8	8	7
	P	—	—	—	<0.001	—	<0.001	—	<0.001	—	—	<0.01	<0.001	—	—	—	—
DNA, mg/g	$\bar{x}$	9.0	23.5	9.9	15.8	8.8	16.7	9.3	15.2	7.9	14.2	5.6	11.5	8.5	13.0	9.5	17.9
	ES±	0.77	0.64	0.41	0.41	0.16	0.68	0.12	0.73	0.11	0.51	0.68	0.69	0.33	0.75	0.37	0.87
	n	7	8	8	8	8	8	8	8	6	7	7	8	8	8	8	7
	P	—	—	—	<0.001	—	<0.001	—	<0.001	—	—	<0.01	—	—	—	—	<0.001
Free amino acid nitrogen (AA), mg N/100 g	$\bar{x}$	7.9	8.6	5.5	4.7	1.9	4.2	8.3	17.2	7.0	6.13	9.6	15.5	3.9	7.48	7.5	7.3
	ES±	0.65	0.46	0.53	0.67	0.52	0.58	1.01	1.21	0.99	0.43	1.49	1.27	0.49	1.68	0.66	0.57
	n	8	8	8	8	8	8	6	8	8	8	8	8	8	8	8	8
	P	—	—	<0.02	<0.001	<0.001	<0.001	—	<0.001	—	—	—	<0.001	—	—	<0.001	—
Organ weight, mg	$\bar{x}$	370.3	492.0	325.6	332.2	256.0	365.7	454.6	529.5	971.0	820.0	451.1	485.27	0.888	0.940	1.049	0.960
	ES±	39.37	41.84	27.45	24.66	25.21	40.43	36.17	65.24	6.00	6.00	41.48	4.85	0.12	0.01	0.10	0.03
	n	8	8	6	5	8	8	8	8	8	7	8	8	7	5	8	6
	P	—	—	—	<0.01	<0.02	<0.01	—	—	—	—	<0.001	<0.001	—	—	—	—

$\bar{x}$, values are means; ES±, standard error; n, number of animals; BF, bursa of Fabricius; T, thymus

view is supported also by experiments made on animals irradiated after adrenalectomy: in this case no thymus involution occurred (MILCU et al., 1965). TÖRŐ et al. (1968) have shown that in irradiated rats a stimulation of the adrenals takes place. The high reactivity of the thymus against adrenal secretion is explained by the presence of specific receptors for hydrocortisone/cortisone (ABRAHAM et al., 1971); it is probable that there are such receptors in the bursa of Fabricius, too.

Our results support the view of PORA and TOMA (1969) that the thymus reflects, by its involutional reactions, the pathological or trophic-adaptative states of the organism. The bursa of Fabricius may have a similar property. It seems, however, that the organ which has the main role, at least in a certain ontogenetical stage, is still the thymus.

Summary

X-rays elicit age-dependent modifications in the thymus and the bursa of Fabricius of chickens. Applied at the age of 3 days, no involutional reaction is elicited, while at 3 weeks typical involutional phenomena appear during the first few hours after irradiation. With the dose applied all the changes were reversible both in the thymus and in the bursa of a Fabricius. They were more pronounced in the thymus, which is much more sensitive to X-rays than the bursa of Fabricius.

References

ABRAHAM, A. and PORA, E. A.: Rev. Roum. Biochem. **23** (1965), 656. ABRAHAM, A. and SEKERIS, C. E.: Biochem. Biophys. Acta **247** (1971), 562. AMBELLAN, E. and ROTH, I. S.: Biochem. Biophys. Res. Comm. **28** (1967), 244. COMSA, J.: Physiologie et physiopathologie du thymus. Paris, 1959. FREEMAN, B. M.: In: Physiology and Biochemistry of the Domestic Fowl. London, New York, 1971, pp. 575. FOX, K. E. and GABOUREL, I. D.: Endocrinology **90** (1972), 1388. GIURGEA, R., and MANCIULEA, S.: St. cerc. biol. s. zoologie (1974) (in press). GIURGEA, R. and TOMA, V.: Zbl. Vet. Med. (1974) (in press). GOOD, R. A. and GABRIELSEN, A. E.: The thymus in Immunobiology. New York, London, 1974. KENDYSH, J. N., FEDOTOV, V. P., MOROZ, B. B. and DUBROVIN, A. D.: Radiobiologiya **9** (1969), 31. KOVÁCH, A.: A kísérletes orvostudomány vizsgáló módszerei. Budapest, Vol. II, 1958. MILCU, S. and LUNGU, A.: Hormonii si viata. Bucuresti, 1965. MOSSER, G., GOOD, R. A. and COOPER, M. D.: Int. Arch. Allergy **39** (1970), 62. PORA, E. A. and ABRAHAM, A. D.: Radiobiologia. Bucuresti, 1970. PORA, E. A. and TOMA, V.: Ann. Endocr. Paris **30** (1969), 519. POTOP, I., JUVINA, E., BINER, J. and MREANA, G.: St. cerc. endocr. **18** (1967), 411. RAC, I.: Čas. lék. čes. **98** (1959), 120. RATIU, H. I.: Actiunea dozelor mici de radiatii röntgen asupra acizilor nucleici din sistemul nervos central. Thesis. Bucuresti, 1971. SATO, K. and SUZUKI, K.: Poultry Sci. **48** (1969), 1195. SELYE, H.: Hormones and Resistance. Berlin, Heidelberg, New York, 1971. SPIRIN, A. S.: Biokhimia (Moscow) **23** (1958), 656. TESSERAUX, H.: Physiologie und Pathologie des Thymus. Leipzig, 1959. TOMA, V. and GIURGEA, R.: Zbl. Vet. Med. **21** (1974), 506. TÖRŐ, I., BACSY, E., ÖKRÖS, I., VADÁSZ, GY. and RAPPAY, GY.: Acta med. hung. **25** (1968), 355. VASCULESCU, T., VALEANU, A. and PAPILIAN, V. V.: Oncologia si Radiologia. Vol. IV, 1965. WEBER, W. T. and WEIDANZ, W. P.: J. Immunol. **103** (1969), 537. WOKAER, R.: Mécanismes d'actions intracellulaires des hormones. Paris, 1970.

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OBSERVATIONS ON AN OUTBREAK OF *KLEBSIELLA* MASTITIS IN SOWS

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Lake and Jones (1970) drew attention to the role of *Klebsiellae* in the causation of post-parturient disease in the sow. The purpose of this paper is to describe an additional outbreak in Buckinghamshire and to present information on the economic effect and clinical course of the disease.

Materials and methods

Investigations were made into an outbreak of disease in a closed herd of 80 sows whose progeny was fattened to pork. The sows were kept in a dry sow house (stalls) and were moved to farrowing quarters (crates) one week before farrowing. The farrowing accommodation was cleaned before entry of the next group of pigs. After 3 weeks the sows were moved to multi-suckling pens, weaned at 5 weeks and kept in service pens with boars until at least 3 weeks after first mating when they were moved to the dry sow house.

Results

Groups of females, usually between 6—14 in number, farrowed at 3 weekly intervals. In late December 1971, there occurred the sudden death of four sows from a batch of 8 sows. The sows farrowed normally but up to 1 week earlier than was normal. The piglets suckled but the sows died suddenly within 24 hours of parturition. A routine was adopted on this farm whereby the animals that did not eat within 8 hours of farrowing received antibiotics (streptomycin/penicillin). No response to this treatment was noted. None of these sows was available for examination. The next group of farrowing sows in mid-January was also affected but there were no sudden deaths. From the records, one sow farrowed 13 of which 9 were dead, another sow 12 of which 8 were dead and the last of the three farrowed 4 live piglets and 7 dead. The batch of 7 sows in February farrowed normally in terms of litter size and number born dead and the farmer thought that the problem had disappeared.

The six sows that farrowed in early March were affected. In this group, two sows gave birth to dead litters of 8 and 9 piglets. Two sows gave birth to litters of 10 and 8 piglets, all born alive and one sow produced a litter of 5 live and 7 dead piglets. The last sow produced a litter of 11, of which 4 were dead.

The sow which produced the litter of 8 dead piglets died suddenly two days after farrowing and was examined at postmortem. There was considerable serous fluid in the thorax and abdomen but this was the only abnormality visible other than in the udder. All the 14 glands of the udder showed a marked congestion with focal areas where this colour change was very marked. No samples were taken for laboratory diagnosis because of the time lapse between death and postmortem.

The late March—early April group of sows were also affected and at this point clinical examination of the entire herd was made. Two sows had farrowed one week early in the stalls. One sow had produced 9 stillborn piglets and the other 8 stillborn and 4 live piglets which were subsequently lain upon. Examination of these sows revealed no abnormalities of the udder or vaginal discharge.

No stock had been purchased since October, when a new boar was purchased. Water supplies were all mains supplies, proprietary food was used from a single supplier, and the animals were bedded on shavings and sawdust. Examination of the 10 sows and gilts in the farrowing house revealed information which is recorded in Table I. Characteristically, the sows had elevated temperatures varying between 39.2 and 41.2 °C, all sows appeared dull, were not inclined to stand and were anorectic. All the piglets appeared undernourished, were stairy-coated and were very poor in quality. None of the sows appeared to have a vaginal discharge but the mammary glands of 7 of the sows were affected to some degree (see Table I). The affected glands were extremely hard, very reddened and hot to the touch. Most glands were dry but when it was possible to draw off some fluid this was very thin and grey in colour. All 9 sows with piglets had varying degrees of agalactia, and the piglets were lighter in weight at 3 or 4 days of age than at birth. The sows had been treated with penicillin/streptomycin but there was no improvement in conditions of sows or piglets. Artificial feeding of the piglets was instituted.

The litter records revealed that 10 out of the previous 34 litters had been completely lost and that 137 of 412 piglets born were stillborn or had died during the first week of life. Rectal, vaginal and milk swabs were taken at this point and samples from the prepuce were also obtained from the boars used on the farm. These swabs were negative for pathogens and it was then decided to slaughter one of the affected sows. A sow (No. 5) was then anaesthetized with pentobarbitone sodium, exsanguinated and subjected to autopsy.

Profuse, pure growth of *Klebsiella* was obtained from all glands which

Table I

Clinical signs in the group of 10 sows

	Sows					Gilts				
	1	2	3	4	5	1	2	3	4	5
Clinically affected	+	+	+	+	+	+	+	+	+	+
Temperature	39.2	39.5	39.2	40.1	40.7	39.4	39.7	40.1	40.6	41.2
Number of days after farrowing when examined	6	1	1	4	3	2	6	2	3	4
Number of piglets born alive	10	8	11	10	8	9	10	10	9	7
Born dead	1	4	0	1	4	0	1	2	9	4
Number alive when examined	7	4	4	6	4	8	6	4	0	4
Milk supply	Good	Poor	Poor	Poor	Poor	Good	Poor	Poor	Dry	Poor
Total number of glands	16	15	14	14	14	16	14	14	14	14
Number of glanda affected	0	5	7	4	5	0	6	5	—	6

appeared to be clinically affected, and also from the cervical and mesenteric lymph nodes, kidney, liver, spleen and lung. Diagnosis of *Klebsiella* infection in 2 other sows (Nos 2 and 4) was obtained by mammary biopsy. Sensitivity tests showed that the *Klebsiellae* isolated were sensitive to neomycin and polymyxin B.

Samples were taken to determine the source of the infection within the herd from fresh supplies of water, food and bedding, from drinking bowls and mangers, from dirty bedding, pen partitions and from the nose, vagina and udder skin of the sows and gilts in the farrowing and fattening houses. *Klebsiellae* were not isolated from food, bedding or water or inanimate objects in the sow houses, but they were isolated from the nose and udder skin of the sows.

Discussion

There are only a few reports of *Klebsiella* mastitis in sows (ADLER, 1951; LANGHAM and STOCKTON, 1953; HELMBOLDT, 1953; JAMKHEDKAR, 1964) and until recently (LAKE and JONES, 1970) it had not been recorded in sows in Great Britain. In this outbreak both acute generalized infection and acute mastitis occurred.

The reason for the sudden appearance of the infection within the herd was not found. Close control of visitors was maintained and access to the pigs was severely limited. The purchase of a boar shortly before the start of the outbreak was viewed with suspicion but no *Klebsiellae* were isolated from preputial or semen samples and there was no relationship between the use of this boar and subsequent disease in sows and gilts.

The possibility of introduction of the disease via the bedding, water or feed was investigated but at the time of sampling they did not contain *Klebsiellae*. The presence of the organism on the skin of the udder and in the nose would suggest that the organism was carried by the sow, and it was not possible to recover *Klebsiellae* from any equipment in the stall house.

No regular pattern was visible in the initial outbreak. The 4 sows that died were third- or fourth-litter sows and not gilts as one might expect. Later in the outbreak both gilts and sows were affected. It was probable that the *Klebsiellae* were transmitted from sow to sow whilst the animals were in the dry sow house. The differences in susceptibility probably due to the degree of contact with the infection would account for the varying clinical picture. It was suggested that resistance developed as the *Klebsiella* initially caused sudden death, then acute mastitis and finally agalactia. Repeated sampling of sows and gilts during May and June failed to reveal the organism.

The early farrowing may have been one of the contributory factors to the high mortality amongst the piglets.

During the period under study from December to March there were 55 farrowings and 592 piglets were born but of these 176 were born dead. Eighty piglets died during the first week of life principally because of agalactia in the sows and subsequent crushing of the weakened piglets. The piglets from the affected sows took 3—5 weeks longer to reach a standard pork weight than their contemporaries from unaffected litters.

The appearance of the disease at or before farrowing, the extremely high body temperature of the sows, possibility of sudden death and lack of response to conventional antibiotic therapy are to be stressed. Diagnosis was difficult without resort to laboratory aids and, in particular, the use of mammary biopsy techniques was to be recommended.

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References

ADGER, H. E.: N. Am. Vet. **32** (1951), 96. HELMBOLDT, C. F., HALE, H. H. and WINN, J. D.: Vet. Med. **48** (1953), 80. JAMKHEDKAR, P. P., VARATA, N. A., VERGURLEKAR, K. G. and SAIGAONIKAR, P. B.: Indian Vet. J. **41** (1964), 385. LAKE, S. G. and JONES, J. E. T.: Vet. Rec. **82** (1970), 484. LANGHAM, F. R. and STOCKTON, J. J.: Mich. St. Coll. Vet. **13** (1953), 112.

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DETECTION OF *MYCOPLASMA MELEAGRIDIS* INFECTION OF TURKEYS IN HUNGARY

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Infectious sinusitis of turkeys caused by mycoplasmas was first described by MARKHAM and WONG (1952). The agent isolated from this disease was designated as strain S_6 (ADLER et al., 1958), later classified as serogroup A (KLECKNER, 1960) which was named as *Mycoplasma gallisepticum* species (EDWARD and KANAREK, 1960).

An airsacculitis caused by mycoplasma other than *M. gallisepticum* was first reported by ADLER et al. (1958). The agent (strain N) was classified as serogroup H (KLECKNER, 1960) and named *M. meleagridis* species (YAMAMOTO et al., 1965).

According to several authors (YAMAMOTO and BIGLAND, 1965; MOHAMED et al., 1965; MOHAMED and BOHL, 1967) *M. meleagridis* infection can be detected in turkey flocks very often, causing airsacculitis and peritonitis, "leg weakness" and embryo mortality (REIS et al., 1970; RHOADES, 1971a, b; ARYA et al., 1971).

Recently the production of turkeys developed intensely in Hungary, but one of the most important veterinary problems is the disease due to air sacculitis and peritonitis. Since this pathological picture was recognized in *M. gallisepticum*-free turkey flocks, it was justified to perform investigations to detect the occurrence of *Mycoplasma* species other than *M. gallisepticum* and to study their biological and pathological properties.

Materials and methods

Two turkey flocks previously found as *M. gallisepticum*-free were examined epizootologically, clinically, pathologically, bacteriologically and serologically.

Mycoplasma examination

Samples from trachea, air sac, peritoneum and oviduct of turkeys with heavy air sacculitis and peritonitis were minced and a suspension of 1 : 10, 1 : 100 and 1 : 1000 dilution was prepared in *Micoplasma* medium B (ERNØ and STIPKOVITS, 1973), VF-medium (BARBER and FABRICANT, 1962) and medium containing chicken meat-infusion (YODER and HOFSTAD, 1962). The cultivation, isolation, cloning, as well as biochemical and serological examination of the strain were performed as described elsewhere (STIPKOVITS, 1973). For serological identification growth-inhibition and metabolic-inhibition tests

were used (STIPKOVITS and VARGA, 1973), using antisera against *M. gallisepticum*, *M. meleagridis*, *M. synoviae*, *M. gallinarum*, *M. anatis*, *M. gateae*, *M. iners*, serogroups: C, D, F, I, J, K, L, Q, N and other mammalian arginine-splitting *Micoplasma* species.

Antibiotic sensitivity

Tylan, Spectam and Gallymycin were diluted in PBS in tenfold dilutions from 10 mg/ml to 0.0001 mg/ml. Filter paper discs 6.0 mm in diameter were soaked with each antibiotic dilution and placed on agar plates inoculated by "running drop" technique with 0.1 ml broth culture (10^4, 10^5 and 10^6 CFU/ml) of various *Mycoplasma* strains, 2 *M. meleagridis* strains (Nos 600 and 618) isolated from the studied turkey flocks, 2 *M. gallisepticum* strains (Nos 613 and 645) and a *M. gallinarum* (No. 614) strain cultivated from other turkey farms. Inhibition zones were measured 4 days after incubation.

Inoculation of turkey embryos

M. meleagridis strain No. 618 was cultivated in medium B without thallium acetate. 0.2 ml of 1 : 10 diluted, 4-day-old *M. meleagridis* broth culture (1.2×10^5 CFU/ml) was inoculated into the allantoic cavity of 9-day-old turkey embryos. As control the same amount of 3 day-old broth culture of *M. gallisepticum* strain No. 613 (3×10^6 CFU/ml) and sterile broth were inoculated into other groups of embryos. Each group consisted of 7 embryos. The time of death was recorded. Re-isolation of the strains and their subsequent identification were performed.

Inoculation of turkeys

0.2 ml of the *M. meleagridis* (No. 618) culture was injected either intratracheally or intrathoracally to two groups of seven turkeys at the age of 3 days. Five turkeys were kept as a control group inoculated with 0.2 ml of sterile broth intratracheally and intrathoracally. The turkeys were observed clinically for 7 weeks. Then the birds were killed and postmortem and bacteriological examinations (including *Mycoplasma* re-isolation) were carried out. Before and after the experiment blood samples were taken for serological examination.

Serological examination

Serum samples collected in both naturally-infected flocks and from experimental birds were checked for the presence of *M. meleagridis* antibodies by growth-inhibition (CLYDE, 1964) and indirect haemagglutination (KROGSGAARD, 1972) and of *M. gallisepticum* antibodies by haemagglutination-inhibition (MÉSZÁROS, 1964) and indirect haemagglutination tests.

Results

Field observation

Significant losses were observed in flock No. 1 in the first and second week (Table I), then deaths decreased in number. After 7 weeks losses increased again. At this time characteristics of *E. coli* infection were found in died turkeys. Deaths in flock No. 2 were of lower rate and prolonged. Pathological picture of the *E. coli* infection was found rarely in diseased birds. No other infectious diseases (Newcastle disease, Arizona infection, cholera, pox, aspergillosis) were observed and no live vaccines were used in the flocks.

No special clinical symptoms were observed in poults in the first week. The weight gain slowed down in 20—30% of the birds from the second week on. Lack of appetite and anaemic mucous membrane were noticed in the diseased birds. Only 2—3% of the turkeys suffered from mild sinusitis and disease of the upper respiratory tract. "Leg weakness" occurred at the age of 8—10 weeks. At the postmortem examination fibrinous peritonitis, abdmoinal air sacculitis and, seldom, salpingitis of variable severity were seen. No synovitis and arthritis were found. Bacteriological examination of the internal organs of young turkeys gave negative results. From the heart, the liver and air sac of older turkeys (7-week-old) *E. coli* was isolated.

From samples of the trachea, and air sacs of turkeys 2 *Mycoplasma* strains (Nos 600 and 618) were isolated. The strains required cholesterol, were sen-

Table I

Death rate in turkey flocks infected by *Mycoplasma meleagridis*

Age, weeks	Death rates in per cent	
	Flock No. 1 (7720 turkeys)	Flock No. 2 (4110 turkesy)
1	2.4	0.5
2	1.8	0.9
3	0.4	0.4
4	0.9	1.2
5	0.4	1.1
6	0.3	0.2
7	1.0	0.3
8	1.0	0.2
9	2.5	0.2
10	3.5	0.2

sitive to sodium polyanethol sulphonate and digitonin, splitted arginine, but did not split glucose, aesculin, arbutin, mannose, galactose, sorbit, cellobiose, xylose and urea, and did not produce "film and spot" and phosphatase. Only the *M. meleagridis* reference serum inhibited the growth and metabolic activity of the strains; no sera prepared against other avian *Mycoplasma* species or serogroups or mammalian arginine-positive *Mycoplasma* gave any positive reaction.

By testing the antibiotic sensitivity of the isolated *M. meleagridis* strains it was found that this species is much more resistant to Tylan (0.01 mg/ml) and Spectam (0.1 mg/ml), and especially to Gallimycin (10 mg/ml) than the *M. gallisepticum* strains (0.01, 0.001 and 0.0001 mg/ml, respectively).

Infection of embryos and turkeys

Results of infection of turkey embryos are demonstrated in Table II, showing a high killing effect of both *M. gallisepticum* and *M. meleagridis* strains. Hyperaemia, oedema, sometimes haemorrhages and fibrinous spots were observed in the dead embryos. The *Mycoplasma* strains re-isolated from the embryos proved to be *M. gallisepticum* and *M. meleagridis*, respectively.

Results of infection of turkeys are summarized in Table III. No death was observed. In the birds killed 7 weeks after intratracheal or intrathoracal infection severe airsacculitis, peritonitis and sometimes salpingitis were detected. No lesions were present in the control birds. *M. meleagridis* was re-isolated from the infected turkeys.

Serological examination

No antibodies against *M. gallisepticum* were detected in serum samples collected from the flocks at various ages and from experimental birds before, and 7 weeks after infection by haemagglutination inhibition and indirect haemagglutination tests. On the other hand, *M. meleagridis* antibodies were

Table II

Results of artifical infection of turkey embryos with *M. meleagridis* and *M. gallisepticum*

Experiment No,	*M. meleagridis*		*M. gallisepticum*		Control	
	Infected/dead embryos	Average survival time, days	Infected/dead embryos	Average survival time, days	Inoculated/ dead embryos	Average survival time
1	7/5	5.6	7/5	8.4	7/0	hatched
2	7/7	4.7	not done		7/0	hatched

Table III

Results of artificial infection of turkeys with *M. meleagridis*

Groups	Number of turkeys	Turkeys with pathological lesions	Serological examination							
			M. meleagridis				*M. gallisepticum*			
			GI*		IHA*		HG*		IHA*	
			pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
Infected intratracheally	7	7	6	1	4	3	0	7	0	7
Infected intrathoracally	7	7	7	0	4	3	0	7	0	7
Uninfected control	5	0	0	5	0	5	0	5	0	5

GI, growth inhibition; IHA, indirect haemagglutination; HG,haemagglutination.

shown in most of the examined field sera by both the growth-inhibition and the indirect haemagglutination tests. Before infection the birds were serologically negative, but 7 weeks after the artificial infection a serological response was confirmed in many birds. The non-infected turkeys remained serologically negative.

Discussion

This is the first report about *M. meleagridis* infection of turkeys in Hungary. From two *M. gallisepticum*-free turkey flocks *M. meleagridis* strains were isolated. The examination of these flocks demonstrated serological positivity of the birds. Indirect haemagglutination and growth-inhibition tests were satisfactory for this purpose, as it was described by MOHAMED and BOHL (1968), and by OGRA and BOHL (1970).

The isolated *M. meleagridis* strains showed a definite pathogenic effect on turkey embryos, as well as on 3-day-old turkey poults, producing fibrinous air sacculitis and peritonitis. These results are in correlation also with published data (REIS et al., 1970; RHOADES, 1971a, b; ARYA et al., 1971).

M. meleagridis infection producing a reduction in weight gain of birds and pathological lesions can be very much influenced by poor hygienic conditions, and by *E. coli* infection resulting in a significant loss of the flocks.

The isolated *M. meleagridis* strains showed a total resistance to Gallimycin (erythromycin) and they were more resistant to Tylan and Spectam too in comparison with *M. gallisepticum*. These results confirmed our general observation (ERNØ and STIPKOVITS, 1973; STIPKOVITS et al., 1974) that the arginine-splitting *Mycoplasma* species are more resistant to erythromycin

than the glucose-positive ones, and the practical observation of ineffectiveness of Gallimycin in flocks infected with *M. meleagridis*.

According to our observation in Hungary the practical importance of *M. meleagridis* infection in turkey flocks is much higher than their *M. gallisepticum* infection. The differences in methods of treatment and control of the *M. meleagridis* and *M. gallisepticum* infections demand their differential diagnosis. From this point of view some alterations in epizootology, clinical symptoms and pathological lesions of these diseases (as the occurrence of disease due to the *M. meleagridis* infection in young turkeys, the lack of symptoms of the upper respiratory tract, predominance of abdominal airsacculitis and peritonitis) are important but they should be completed by serological testing of turkey sera with both *M. meleagridis* and *M. gallisepticum* antigens as well as by isolation of the *Mycoplasma*.

Summary

Mycoplasma meleagridis infection was demonstrated in two *Mycoplasma gallisepticum*-free turkey flocks by isolation of *M. meleagridis* strains from turkeys and detection of antibodies against this species in turkey sera. *M. meleagridis* proved to be more resistant to Gallimycin (erythromycin) than *M. gallisepticum*. Pathogenic role of *M. meleagridis* was proved by artificial infection of turkey embryos and 3-day-old poults. The infected turkeys developed severe airsacculitis and peritonitis and a serological response.

References

Adler, H. E., Fabricant, J., Yamamoto, R. and Berg, J.: Amer. J. Vet. Res. **19** (1958), 440. Arya, P. L., Sautter, J. and Pomeroy, B. S.: Avian Dis. **15** (1971), 163. Barber, T. L. and Fabricant, J.: J. Bact. **83** (1962), 1268. Clyde, W. A.: J. Immunol. **92** (1964), 958. Edward, D. G. and Kanarek, A. D.: Ann. N. Y. Acad. Sci. **79** (1960), 696. Ernø, H. and Stipkovits, L.: Acta vet. scand. **14** (1973), 436. Kleckner, A. L.: Amer. J. Vet. Res. **21** (1960), 274. Krogsgaard, J. A.: Appl. Microbiol. **22** (1972), 756. Markham, F. S. and Wong, S. C.: Poultry Sci. **31** (1952), 902. Mészáros, J.: Magyar Állatorvosok Lapja, 1964, 227. Mohamed, Y. S., Chema, S. and Bohl, E. H.: Avian Dis. **19** (1965), 347. Mohamed, Y. S. and Bohl, E. H.: Avian Dis. **11** (1967), 634. Ogra, M. S. and Bohl, E. H.: Avian Dis. **14** (1970), 364. Reis, R., DaSilva, M. L. and Yamamoto, R.: Avian Dis. **14** (1970), 117. Rhoades, K. R.: Avian Dis. **15** (1971), 762. Idem: Ibidem **15** (1971), 910. Stipkovits, L.: Acta vet. hung. **XXIII** (1973), 315. Stipkovits, L. and Varga, L.: Acta vet. hung. **XXIV** (1974), 139. Yamamoto, R., Bigland, C. H. and Ortmayer, H. B.: J. Bact. **90** (1965), 47. Yamamoto, R. and Bigland, C. H.: Avian Dis. **9** (1965), 108. Yoder, H. W. and Hofstadt, M. S.: Avian. Dis. **6** (1962), 147.

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DATA ON DETERMINATION OF TOXIN F-2 (ZEARALENONE) BY HIGH-PRESSURE LIQUID, GAS AND THIN-LAYER CHROMATOGRAPHY

By

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Mycotoxicoses are caused in animals by feeds contaminated by toxin-producing fungi. Many fungus species have been demonstrated in feedstuffs, and some of these have been found to produce toxin. Although the syndromes associated with mycotoxicoses are generally characteristic, precise diagnosis, identification of the causal agent and elaboration of an appropriate method of detoxification require closer investigations by methods of analytical chemistry.

Fungus species belonging to the genus *Fusarium* have been identified as a main source of mycotoxicoses. Apart from their deleterious effect on animal health, they are also of public health importance, occurring in certain common human foods. In view of this and of the fact that their structure, absorption and metabolism have become known, an improvement in the chemical assays of the toxins is of immediate interest. In the course of related investigations in this Institute, comparative chemical assays of the toxin F-2 (zearalenone) were performed. The methods may be suitable for the demonstration of other toxins as well in feed samples.

Experimental

1. Two methods of toxin extraction were tested in respect of efficiency, purity of the product and economy. The first method (Mirocha et al., 1967) is based on solubility differences between toxin F-2 and contaminating substances, in organic solvents, the second (Mirocha et al., 1974) on a chemical reaction

2. It was examined whether thin-layer chromatography, which is widely employed for the quantitative determination of F-2, can be combined with a photo-densitometric method of evaluation. Different methods of detection (Mirocha et al., 1974; Sarudi, 1974) were checked for sensitivity.

3. In the course of quantitative determinations of F-2 by gas chromatography, the sensitivity of the available instrument for F-2 measurement was tested and the lower limit of measurable quantities was established.

4. Toxin determinations were carried out by high-pressure liquid chromatography, which seems to be a reliable analytical method for the purposes of fusariotoxicosis research.

Materials and methods

Fungus culture. Good-quality maize was sterilized in the autoclave and infected with a *Fusarium culmorum* strain isolated in Hungary, which had previously been shown to cause oedema of the vulva in sows. The fungus was allowed to grow in the maize for one week at room temperature, then the culture was kept for another week at 10—12 °C to promote toxin production, and again at room temperature for one month. The culture was then dried by warm air flow and ground. The toxin was extracted from this meal.

Extraction procedure I. The maize meal was extracted in Soxhlet apparatus for several hours with sixfold excess volume of methylene chloride or chloroform. The extract was distilled, the chloroform-containing residue was transferred into a separatory funnel with acetonitrile and was shaken with an equal amount of petroleum ether. The acetonitrile phase was evaporated, disolved in chloroform and purified by column chromatography.

Extraction procedure II. The maize meal was slightly moistened (10—20%) and extracted with ethyl acetate in Soxhlet apparatus as above. The solvent was removed, the semi-dry residue was reconstituted in chloroform, mixed with 10 ml 0.1 *N* sodium hydroxide, and cautiously shaken several times in a separatory funnel. The combined alkaline phases were adjusted to pH 9.5 with H_3PO_4 and were partitioned again with chloroform. The organic phase was dried with Na_2SO_4 and evaporated.

Column chromatography. Activated silica gel (mesh size: 170—200) (Merck) was filled into a column of 30×1.60 cm. The ratio of dry silica gel to the extract to be purified was 100 : 1. The best resolution was obtained by gradient elution chromatography, with the following programme: chloroform, step-graded acetonous chloroform (2, 5, 10, 30, 50%) and 10% aqueous acetone. The final volume of the last mixture was 100 ml, that of each preceding mixture 50 ml. Fifty fractions, each 8 ml, were collected. Toxin F-2 was eluted in the 12th to 16th fractions (in 10% of the total volume of eluant). The fractions were detected either in UV light or with spot test, after spot application of the eluate to a thin-layer plate previously sprayed with 2% aqueous 4-brom-benzene-diazonium-fluoroborate reagent, which turns yellow in presence of the toxin.

Thin-layer chromatography

Layer: 0.25 mm thick silica gel (Polygram SIL G, Macherey-Nagel Co.).
Developing solvent: methanol : chloroform (5 : 95).
Visualization of spots:

(*a*) in 2% 4-brom-benzene-diazonium-fluoroborate (SARUDI, 1974), yellow colour;

(*b*) in 50% methanol–H_2SO_4 and subsequent exposure at 120 °C for 10 minutes (MIROCHA et al., 1974; MALLINS et al., 1965), dark brown colour;

(*c*) in freshly prepared 1 : 1 mixture of 1% aqueous $K_3Fe(CN)_6$ and 2% aqueous $FeCl_3$ (MIROCHA et al., 1974), vivid blue colour.

A known quantity of standard F-2 toxin was applied as a spot to the chromatography plate and development was carried out in a glass chamber previously saturated with solvent vapours. Quantitative evaluation was made in an Analytrol Beckman type photo-densitometer at 550 nm, using 1 mm slit width.

Plates 20×20 cm in size, coated with Kieselgel G (Merck) in this laboratory, were used for evaluation of extraction experiments and for identification of the chromatographic fractions.

High-pressure liquid chromatography

Apparatus: Pye-Unicam LCM-2 liquid chromatograph.
Detector: moving wire system with flame ionization detector.
Column: 25 cm long by 2.3 mm ID sylilated glass.
Column packing material: Merckosorb SI 60 silica gel (size: 10 μm) (Merck).
Pressure drop: 1260 psi at 0.7 ml/min flow rate.
Eluent: chloroform : methanol (96 : 4).

Standard F-2 toxin was injected into the column in the form of a 5 μg/μl solution in chloroform, while the pressure was reduced to atmospheric. The elution volume of F-2 was 1.96 ml under the given conditions (2.8 min).

Gas chromatography

Apparatus: JEOL type 810 dual channel gas chromatograph.
Detector: FID, with block temperature of 240°C.
Column: sylilated glass, 2 m long, with 2.3 mm inner diameter.
Column packing material: 2% SE-30 or OV-17 stationary phase on Gas-Chrom Q 80—100 mesh solid support. Theoretical plate number 3800.
Carrier: nitrogen, flow rate 20 ml/min at 25 psi.
Injection: 0.5—2.0 μl with Hamilton syringe.

Sylilation: with TRI-SYL (Pierce, USA), 0.1 ml for 0.5 mg material, at room temperature.

The peaks were evaluated with a type Digint 21 electronic digital integrator (CHINOIN, Budapest).

Results

Extraction results differed markedly, depending on the applied procedure. The product obtained with procedure I was less pure than required, because acetonitrile also dissolved substances other than the toxin. The crude extract thus obtained has to be purified further by column chromatography when used for chemical analysis.

Procedure II appeared to be the most suitable for the extraction of resorcyclic acid-lactone-type toxins: the enolates formed in the aqueous phase re-enter the organic phase on acidification. Emulsion formation taking place between the aqueous alkaline phase and chloroform, however, presented a great problem. Various solvents (ether, ethyl acetate) were tested to eliminate the emulsion, but could scarcely attack it, even on exposure for several days. Apart from this difficulty, very little amounts of the toxin were released in the alkaline medium. This difficulty can be surmounted if, at shaking out, caution is exercised to maintain phase interfaces, while the number of alkaline partitions is increased.

Although the extract obtained with procedure II is quantitatively inferior to that obtained with procedure I, its quality is superior, not requiring further purification for use in any kind of chemical analysis.

The *column-chromatography method* is extraordinarily material- and time-consuming. It is nevertheless indispensable whenever larger amounts of extract are desired. Gradient-elution chromatography has made possible the separation of chemically very similar toxins [F-5-3(8'-hydroxy-zearalenon) and F-2] from one another in a highly pure state. The photometric evaluation of this process is shown in Fig. 1, in which the continuous line represents the original toxin mixture (D), and the dotted and broken lines, respectively, the fractions F-5-3 (D_{III}) and F-2 (D_V) obtained on separation.

The main aim of our studies on *thin-layer chromatography* was the comparison of suitable methods for detection of F-2 toxin. Investigators have generally preferred evaluation by UV. Search has been made for other methods which are as efficient as the UV procedure, but simpler and also fit in with the wavelength range of our densitometer. It appears that the azo-dye reaction proposed by SARUDI (1974) and the sulphuric acid treatment whit charring commonly used in lipid analysis are more sensitive than the UV procedure. The results of densitometric measurements are shown in Fig. 2. The contin-

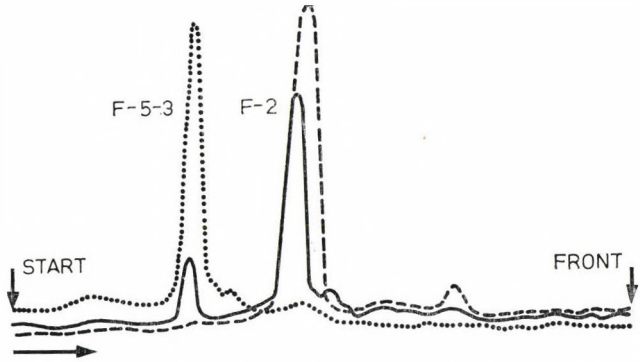

Fig. 1. Photodensitometric evaluation of the original extract and of the toxin fractions separated by column chromatography

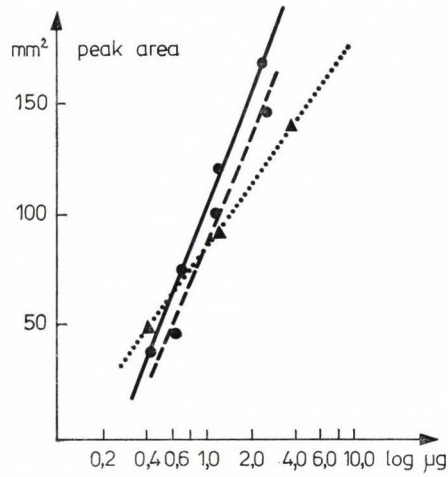

Fig. 2. Evaluation of detection methods by photodensitometry

uous, dotted and broken lines represent the results obtained with the detection methods (*a*), (*b*) and (*c*), respectively; the sensitivity of the former two was 0.3—0.4 μg toxin, whereas of the latter 0.5—0.6 μg toxin which, according to our experience, equal that obtainable by UV development.

The best results were obtained with the combination of methods (*a*) and (*c*). On spraying with reagent (*a*), the resorcylic acid–lactone derivatives showed a yellow colour reaction, and on subsequent application of the sulphuric acid-containing reagent (*b*) all organic compounds present in the system were visualized.

For *gas chromatography*, a volatile trimethyl-sylil derivative (MIROCHA, 1967) was used under the usual conditions of gas chromatography. In extracts obtained by procedure II, the toxin F-2 was determined quantitatively several

times with alpha-D-glucose or lactose used as internal standard. The lowest amount required for analysis was found to be 0.1—0.25 μg. The gas chromatogram of the toxin F-2 (peak No. 2), taken at 4 °C/min under programmed conditions (peak No. 1, alpha-D-glucose), is shown in Fig. 3.

Liquid chromatography was found to be the analytical method of choice for toxin demonstration, because it combines various advantages of the other chromatographic methods. It can be as widely employed as thin-layer and

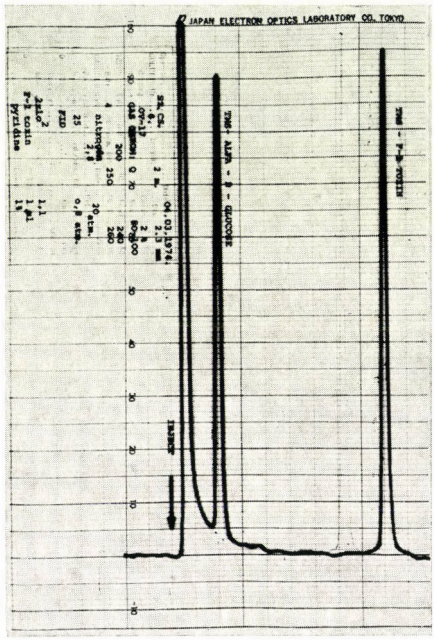

Fig. 3. Gas chromatogram of toxin F-2 (TMS/ether)

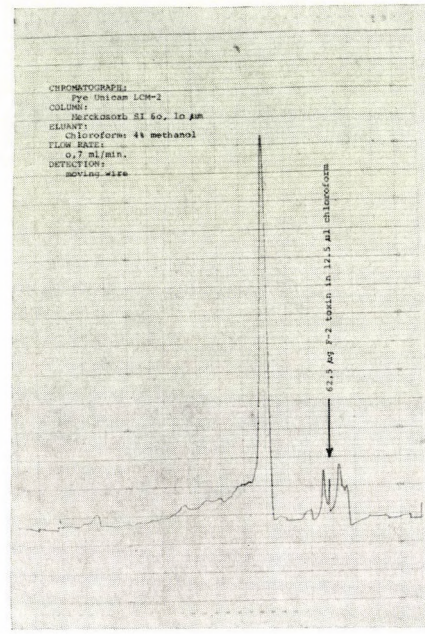

Fig. 4. Liquid chromatogram of toxin F-2

column chromatography, while being as efficient and accurate as gas chromatography. The substances to be analyzed are passed through the column in a soluble state. With the recently developed high-resolution column materials (KIRKLAND, 1971; LOCKE, 1973) the resolution capacity of gas chromatography has been reached. Another important advantage is rapidity: reliable quantitative evaluation needs 100 to 1000 times less time than with column chromatography and 10 to 100 times less than with thin-layer chromatography (KIRKLAND, 1971).

In the present studies 10 μm silica gel (MAJORS, 1972) was used as column material in the separation phase. With chloroform : methanol (96 : 4) used as mobile phase, the chromatogram shown in Fig. 4 was obtained.

The measurements were made in the linear range of the applied detector. The responses obtained with the Pye-Unicam transport detector are plotted against the concentrations of F-2 in Fig. 5.

Toxin determination by liquid chromatography, as described in the foregoing, is only regarded as a preliminary procedure. It is scheduled to increase the sensitivity of the method to the range of 0.1 μg, by using a liquid-chromatographic UV monitor and new chemically bound phases (LEITCH and DE STEFANO, 1973).

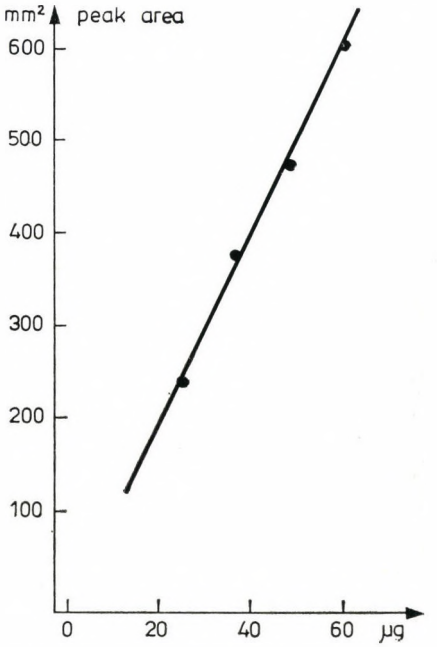

Fig. 5. Detector response of toxin F-2 in liquid chromatography

Conclusions

Alkaline extraction (procedure II) should be preferred to acetonitrile extraction (procedure I) for toxin separation from the crude extract, because the product obtained by the former method is of a higher purity.

Visualization by methods operated in visible light is either equivalent or superior to detection in UV light, if the coloured products formed are evaluated by photo-densitometry.

The liquid chromatography method initiated in these Institutes for toxin determination offers a valuable new procedure in fusariotoxicosis research. Its main advantage over the similar procedures is that the sample requires

no manipulation prior to application, and the chromatographic data can be easily interpreted.

Acknowledgement

The authors are indebted to Professor C. J. MIROCHA (University of Minnesota, USA) for kindly supplying the standard F-2 preparation used in this study.

Summary

The methods of extraction of resorcylic acid–lactone (F-2, zearalenon) toxins were studied with special regard to the end product obtained from the crude extract.

Studies in visualization in thin-layer chromatograms showed that quantitative determination of the fractions can be carried out by evaluation of the colour reactions by photodensitometry.

Quantitative determinations by gas chromatography were examined for sensitivity.

A method elaborated in this Institute for toxin determination by high-pressure-liquid chromatography has the great advantage that dissolved toxin can be used as test material without further manipulation and the chromatographic responses obtained are suitable for quantitative evaluation.

References

KIRKLAND, J. J.: Anal. Chem. **43** (1971), 36A. LEITCH, R. E. and DESTEFANO, J. J.: J. Chromatogr. Sci. **11** (1973), 105. LOCKE, D. C.: J. Chromatogr. Sci. **11** (1973), 120. MAJORS, R. E.: Anal. Chem. **44** (1972), 1722. MALLINS, D. C., WEKELL, J. C. and HOULE, C. R.: J. Lipid Res. **6** (1965), 100. MIROCHA, C. J., CHRISTENSEN, C. M. and NELSON, G. H.: Appl. Microbiol. **15** (1967), 497. MIROCHA, C. J., SCHAUERHAMER, B. and PATHRE, S. V.: J. Assoc. Offic. Anal. Chemists (USA) **57** (1974), 1104. SARUDI, I.: Z. Lebensm. Unters.-Forsch. **154** (1974), 65.

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EXPERIMENTAL INFECTION OF CHICKENS, DUCKLINGS AND GOSLINGS WITH LARVAE OF *AMIDOSTOMUM ANSERIS* (ZEDER, 1800)

By

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There have been scanty and controversial informations available both on the validity and host range of different species of *Amidostomum*, gizzard dwelling nematodes of water birds. Of various species assigned to the genus, 6 and 10 were recognized as being valid by CZAPLINSKI (1962), and RHYZHIKOV and KOBULEJ (1971), respectively. Based on faunistic descriptions, the host list of *Amidostomum anseris* (Zeder, 1800) ranges over 21 species of birds, including also the domestic duck, but not the domestic fowl (SKRJABIN et al., 1952).

The few experimental infections carried out on domestic duck so far yielded inconsistent results. One of the two ducks infected with larvae of *A. anseris* by CRAM (1932) voided eggs in the faeces 40 days later. In order to study the morphology and biology of the nematode in different species of hosts, AKHMEDOVA (1954) infected 6 goslings, 17 ducklings, and 5 chickens. Neither mature nor immature worms were found in the chickens killed at regular intervals from the second postinfection day onwards, whilst adult worms of smaller size and producing less viable eggs, as compared to those in goslings, developed in the ducklings. On the other hand, no worms could be established in any of the 18 ducklings infected at the age of 4, 12, 24 days, 2 or 3 months each with a dose of 2000 or 8000 larvae, nor in the 8 chickens infected at the age of 1, 3, 5 or 8 weeks each with a dose of 2000 larvae, as reported by ENIGK and DEY-HAZRA (1968). According to KOBULEJ and PAPP (1970), chickens may become infected, provided that larvae having completed ecdysis are used for inoculation.

More recently, STRADOWSKI (1972) managed to infect a total of 53 ducklings aged 21 or 42 days, and also several ducks 6 to 24-month-old. While the latter animals exhibited marked resistance to the infection, patent amidostomosis was established in the former ones. There were recorded prolonged pre-patency, smaller and greatly scattered size, reduced fecundity in the worms as well as their rapid elimination from the host.

In addition, death toll of nearly 50% of ducks attributed to infection with *A. anseris* and *A. acutum* in several districts of Romania (OLTEANU et al., 1963) suggests some remarkable pathogenicity.

Some small-scale experiments reported in the present paper were undertaken to amplify the informations on the possible role of domestic fowl and duck in the outbreaks of *A. anseris* infections. These two bird species were chosen in regard to their more or less close contact in the habitat with the most common host, the goose, of this parasite. In view of the higher innate susceptibility of young animals to various infective agents, day-old and several-week-old birds were infected to compare the worm development and pathological alterations in chickens and ducklings with those of goslings.

Material and methods

Day-old Rajna goslings, Peking ducklings and Pilch-Dekalb chickens were obtained from a local hatchery and were kept as described in a previous paper (PHUC and VARGA, 1972). The preparation of infective larvae of *A. anseris*, inoculation, faeces and post-mortem examinations, worm measurements etc., were the same as in the above paper. Some further details of experimental procedures are given under the next heading.

Results

In the first experiment, groups of day-old chickens, ducklings, and goslings were each infected with 500 larvae of *A. anseris*. Between day 5 and 30 of the infection, 1 uninfected control and 5 infected animals from each group were killed at 5-day intervals. In addition, 2 remaining goslings and 4 ducklings were killed on day 67 after infection. Gross lesions in the gizzards of 2 out of 5 infected birds being periodically killed, including also several goslings that died in the course of the experiment, were examined and the worms recovered; while the gizzards of single uninfected and 3 infected animals from every sub-group were opened and fixed in 10% formalin for histological studies (published elsewhere).

None of the gizzards contained gross lesions on day 5 of the infection. Patho-morphological alterations characteristic of amidostomosis were, however, conspicuous in the gizzard of goslings on the 10th day, and minor changes were also noticed in the ducklings at that time, but none in the chickens. On the 15th day, severe haemorrhagic gastritis extending to nearly the whole cuticle, except the most dense round part of horny lining, was shown in the gizzard of goslings. Less confluent lesions, consisting mainly of scattered wavy tracts, were seen also in ducklings (Fig. 1). In one of the two chickens killed on day 15, several haemorrhages of pinhead size were present in the gizzard close to the proventriculus, but no damage could be detected in the other chicken nor in those killed later on.

Lesions were found in all goslings and ducklings killed on days 20, 25 or 30 after infection. Their appearance was consistently more severe in the gizzards of goslings. Although marked lesions were still present in goslings at day 67 of the infection, only traces of changes remained in the ducklings by that time (Fig. 2).

Of 39 goslings a total of 12 animals died of amidostomosis in the course of this experiment, whilst no death due to the equivalent infection occurred in any of the 35 ducklings and chickens, respectively.

Eggs of *A. anseris* first appeared in the droppings of goslings on day 14 of the infection, and following a steep increase, egg counts could be made from

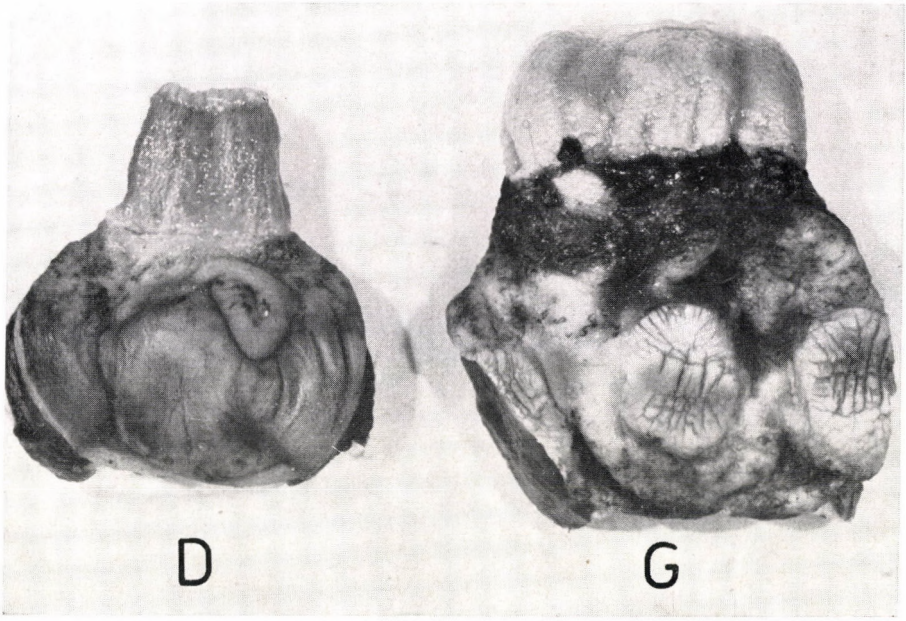

Fig. 1. Lesions in the gizzard of a duckling (D) and a gosling (G) killed 15 days after receiving approx. 500 infective stage larvae of *A. anseris*

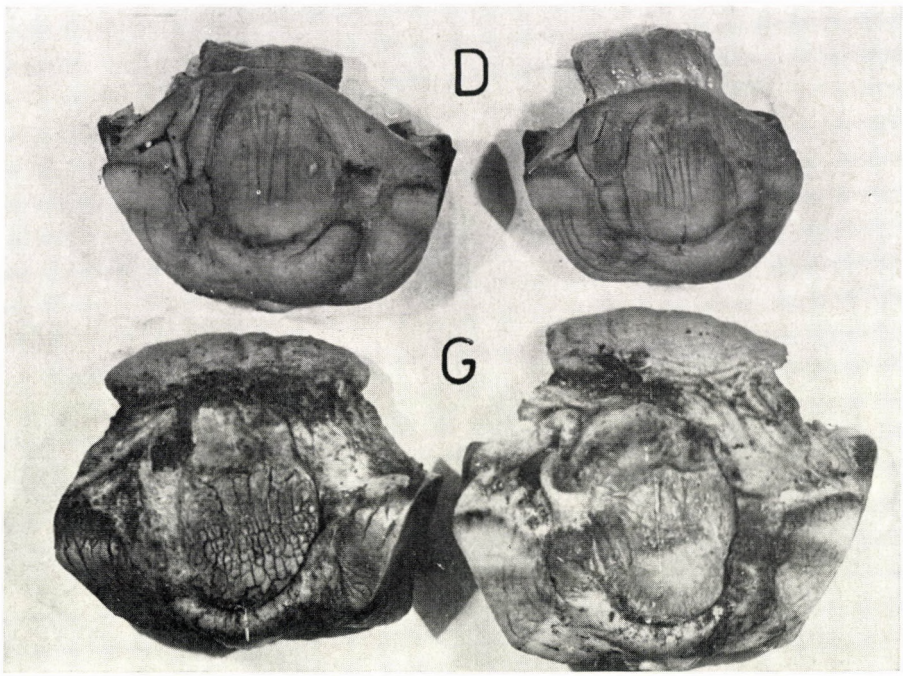

Fig. 2. Lesions in the gizzards of ducklings (D) and goslings (G) 67 days after infection with approx. 500 larvae of *A. anseris*

Table I

Worm recoveries, sex ratio and body length of worms in the chickens (C), ducklings (D) and goslings (G) killed at different time intervals after the infection of birds, each with approx. 500 larvae of *A. anseris*. Measurements were carried out on 30 specimens of worms, if recovered in such number, from each bird

Days after infection	Host	Individual worm counts	Worm sex ratio ♂:♀	Length of worms in mm (mean ± S.D.)	
				♂♂	♀♀
5	C	95, 120	x	1.5±0.3b	
	D	16, 40	x	1.6±0.4a	
	G	47, 49	x	1.8±0.4	
10	C	31, 39	1 : 1.05	3.9±0.8c	4.0±0.6c
	D	56, 233	1 : 1.61	5.7±0.9c	6.7±1.1c
	G	266, 499	1 : 1.14	7.1±0.9	8.3±1.2
15	C	4, 8	1 : 0.42	4.4±1.0	4.7±1.2c
	D	34, 109	1 : 1.03	9.6±1.5	10.6±1.9c
	G	472, 642	1 : 1.06	11.3±1.3	13.6±1.6
20	C	9, 3	0 : 3	—	4.0±0.0c
	D	64, 128	1 : 1.15	11.6±1.7c	14.5±1.9c
	G	205, 431	1 : 1.05	13.8±0.7	17.9±1.7
25	C	0, 0	—	—	—
	D	28, 65	1 : 0.94	12.2±1.7c	16.2±1.9c
	G	410, 427	1 : 1.11	14.0±0.7	19.4±1.5
30	C	0, 0	—	—	—
	D	44, 77	1 : 1.55	11.4±2.2c	13.3±2.6c
	G	187, 330	1 : 0.97	15.3±1.1	22.1±1.6
67	D	0, 3, 10, 14	1 : 2.00	7.3±1.1c	8.6±2.8c
	G	174, 344	1 : 0.85	14.8±1.1	19.5±2.0

Subscript letters indicate differences statistically significant as compared to worms recovered from the goslings: a, $p > 0.05$; b, $p < 0.01$; c, $p < 0.001$ (Student's t-test)

day 18 till the end (i.e. day 67) of the experiment. Eggs in the faeces of ducklings were to be demonstrated by the concentration method between day 15 and 50 and were present in amounts sufficient to make egg counts between day 20

Table II

Percentage distribution of mature female worms of *A. anseris* in ducklings and goslings

Age of worms (days)	Number of female worms examined			
	Ducklings		Goslings	
	Total	Mature (%)	Total	Mature (%)
15	69	7	388	20
20	65	74	200	97
25	32	90	326	100
30	48	39	183	100
67	16	0	160	96

and 40. Egg counts made of pooled faecal samples and recorded every 2 days revealed an average of 300 eggs per 1 g of faeces (E. P. G.), and a peak of 1850 E. P. G. discharged by the goslings, in contrast with an average of 25 and peak of 350 E. P. G. by ducklings. Worm eggs could never be detected in the faeces of chickens nor in any of the uninfected control animals.

Individual worm recoveries, sex ratio and the length of worms at different ages are shown in Table I.

On the grounds of total worm burdens harvested from different hosts between days 5 and 30 of the infection, the following mean ($\pm$ standard deviation) "take" figures were obtained:

chickens: 5.0 ± 8.0
ducklings: 14.9 ± 12.0
goslings: 70.4 ± 37.4.

Female worms harvested from the chickens were all immature. Considerable proportions of the females containing no eggs in the uterus (Fig. 3) were recovered also from the ducklings (Table II).

In the second experiment, cross infection was attempted, using larvae which had been cultured from the faeces either of ducklings or goslings, and stored at 4 °C over 5.5 months prior to their administration to day-old birds. Faeces examination was conducted at 2-day intervals from the 14th day of the infection, and two or three bird in each group were killed on day 42. Tiny lesions in gizzards of ducklings as against the more extensive changes in the goslings were observed in each case and were apparently unrelated to the origin of larvae. The alterations were in line with parasitological findings (Table III).

A group of 5 ducklings at the age of 4 weeks were each administered about 15,000 larvae in the next experiment intended to produce heavily infect-

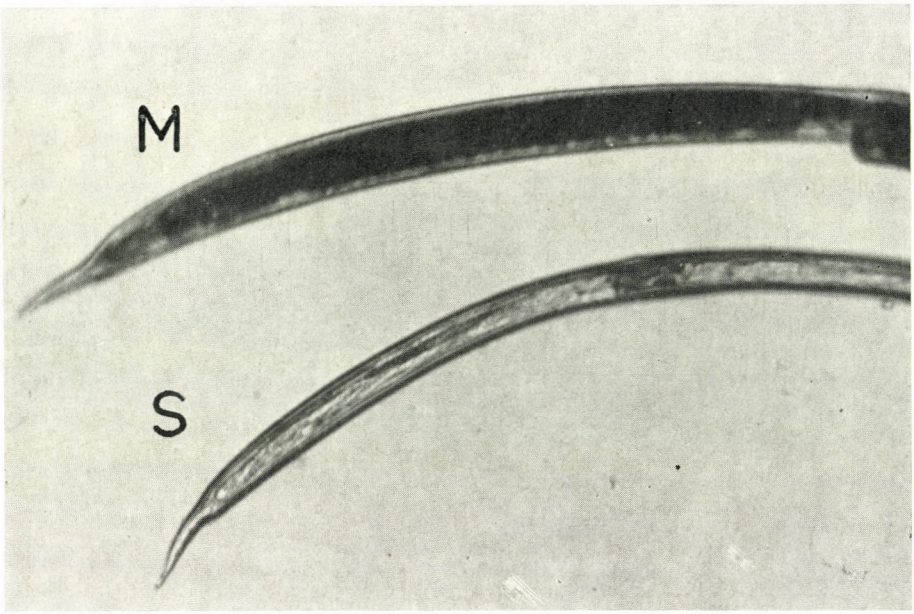

Fig. 3. Hind end of 67-day-old sterile (S) and mature (M) female of *A. anseris* recovered fro m the gizzard of a duckling and a gosling, respectively

Table III

Parasitological findings in the cross-infection experiment where 2 to 3 animals in a group were each receiving approx. 500 larvae of *A. anseris* cultured from the faeces of goslings (G) or ducklings (D)

Host	Origin of larvae	Pre-patency (dyas)	E.P.G. (Mean)	Individual worm counts	Length of worms in mm (mean ± SD)		Mature females (%)
					♀♀	♂♂	
D	G	17	50	2, 5	11.0±1.0	0	0
D	D	16	50	3, 15	11.0±3.9	10.7±2.8	20
G	G	15	125	97, 112, 213	20.2±1.8	14.6±1.0	100
G	D	15	175	490, 503	19.9±0.8	14.3±0.7	100

ed ducklings and large numbers of worm eggs. The latter, however, failed to appear in the faeces even by day 30 of the infection and were, therefore, killed at that time. There were only minute lesions in the gizzards, and the individual worm counts were as follows: 2, 8, 9, 51 and 201 (mean "take": 0.4 ± 0.3). The females measured a length of 7.6 ± 2.0 mm and the males 7.2 ± 1.5 mm (mean ± S. D.). None of the females contained eggs in the uterus.

Table IV

Pre-patency, egg counts, and distribution of mature females among 25-day-old populations of *A. anseris* in ducklings (D) and goslings (G) infected at different ages; figures in parentheses designate numbers of hosts

Species	Age (days) of hosts	No.	No. of larvae in a dose	Pre-patency (days)	E.P.G. (peak)	No. of female worms	
						Total	Mature (%)
D	1	5	400	17	200	286 (3)	35.7
G	1	3	400	14	6500	117 (1)	100
D	7	5	600	22	50	44 (1)	20.4
G	7	2	600	14	2200	142 (1)	100
D	14	6	750	22	50	420 (3)	1.7
G	14	2	750	15	4500	342 (1)	99
D	21	3	1000	32[a]	<50	91 (1)	0[a]
G	21	2	1000	15	—	—	—
D	28	3	1000	36[b]	<50	36 (1)	0[b]

a, 34% of 34 females at day 41 and 45% of 178 females at day 53 of the infection were mature in the remaining 2 ducklings

b, 18% of 11 females at day 36 and 42% of 24 females recovered at day 47 after infection were mature in the remaining 2 animals

In the last experiment, small groups of ducklings and goslings were infected at different age in order to kill them 25 days later and to compare the pre-patency as well as the proportion of mature females. The bulk of animals, unfortunately, died earlier than scheduled, thus comparisons could only be performed on worms recovered from the few survivors (Table IV). At necropsy, severe lesions invariably occurred in the goslings that either died of the infection or were killed, whereas minor changes were only discovered in the ducklings. The cause of death in the groups of ducklings was generally identified as salmonellosis.

Discussion

Larval development not reaching the adult stage of *A. anseris* was established in the chickens which can be regarded as abnormal hosts for this nematode. The larvae grew slowly and were by and large eliminated from the gizzard in 20 days, exhibiting a pattern of abortive infection similar to that

observed in pigeons (JARONY, 1967). This is in harmony with the findings of ENIGK and DEY-HAZRA (1968) as far as the failure to obtain mature worms in chickens is concerned, but is in contrast with the report of AKHMEDOVA (1954) who could not detect larvae either. The reason of discrepancy between the latter and our results may well be in the different ages of hosts, as AKHMEDOVA infected chickens at the age of 1 week. The present experiment on chickens indicates that there is no need of a previous artificial exsheatment of larvae to induce some development in the chickens as advocated by KOBULEJ and PAPP (1970).

It is inferred that *A. anseris* is a virtually harmless parasite for the chickens which do not seem to play any role in the epizootiology of amidostomosis in geese.

Infection of the ducklings, however, resulted in development of mature worms. This observation confirms and extends those reported by CRAM (1932), AKHMEDOVA (1954) and the most comprehensive study of STRADOWSKI (1972). The adaptation of this parasite to the ducklings is apparently dissimilar from its association with the common host, the goose. As shown also in other studies (AKHMEDOVA, 1954; STRADOWSKI, 1972), less and smaller worms with reduced egg-producing capacity and prolonged pre-patent period would develop in the ducklings. The reduced egg production is partly due to the smaller worm burdens established in the ducklings (Table I), and, in particular, to the smaller proportion (Table II) and shorter life span of mature females.

Basically the same features of worm development were observed in the experiment where older than day-old animals were infected. The age of the host is obviously an additional factor resulting in a more efficient inhibition of parasite development (Table IV). In STRADOWSKI's experiment ducks became almost completely resistant to the infection by the age of 0.5 to 2 years.

The extension of the pathological changes in the gizzards is correlated with the actual parasite burdens. While numerous goslings died of the heavy infection in the present experiment, no losses could be ascribed to the equivalent infection of ducklings, a host characterized by low "take" of this parasite. This suggests that the major pathogenic effect resulting in severe outbreaks of amidostomosis in ducks reported by OLTEANU et al. (1963) might rather be attributed to the infection with *A. acutum* than to that of *A. anseris*.

The present cross infection experiment (Table III) is indicative of the possible role of ducklings to transmit the infection to goslings. The success of the latter experiment shows that some of the larvae of *A. anseris* may possess longevity as long as 5.5 months if maintained at 4 °C. KOBULEJ (1956) also reported on survival of the larvae for a minimum of 2 months at 0 °C, and in the study of ENIGK (1969) viability was reserved for 92 days at 2 °C. The infectivity of the larvae kept at 20 to 22 °C greatly diminished within 9 weeks (STRADOWSKI, 1971).

In the light of the alluded reports and present investigations, it is difficult to explain the reason of ENIGK and DEY-HAZRA's (1968) failure to infect ducklings with *A. anseris*. Gross lesions in the gizzards of ducklings infected at the age of 4, 12 or 24 days, and patent infection in the former two age groups, should have been established, as was the case in the corresponding goslings receiving similar oral infections. Their vain attempt to induce percutaneous infection in the ducklings was certainly less surprising.

It is concluded from the present laboratory experiments that domestic duck at the young age can be regarded as a potential host of *A. anseris*. The short-lived infection entails but minor pathological sequelae on the ducklings which may, however, have some limited significance in the epizootiology of amidostomosis by spreading the infection in the much more susceptible goslings.

Summary

Laboratory experiments were carried out to compare the development and pathogenic effec f *Amidostomum anseris* (Zeder, 1800) in chickens and ducklings with those observed in corresponding goslings.

Experimental infection of day-old chickens failed to produce gross lesions in their gizzards. Although no larvae reached the adult stage in this abnormal host, stunted worms could be recovered for 20 days after infection.

In comparison with severe lesions resulting in death of numerous goslings, tiny changes were only produced by the infection in the ducklings. Small proportions of larvae attained the adult stage in ducklings inoculated between the age of one day and four weeks. Inhibition of the worm development in ducklings appeared in reduced "take" and body length of worms, longer pre-patency and short-lived patency of the infection, and also in the presence of immature females in the worm populations. The inhibition greatly increased in aging ducklings.

Larvae of *A. anseris* arising from ducklings and being stored at 4 °C over 5.5 months proved infective both for goslings and ducklings.

It has been suggested that domestic duck susceptible to *A. anseris* at young age suffers little damage of this infection, nonetheless, as a potential host may play some, however not decisive, role in dissemination of the parasite for the highly susceptible common host, the goose.

References

Ахмедова, С. И.: Труды гельминтол. лаб. АН СССР **7** (1954), 375. CRAM, E. B.: J. Parasit. **18** (1932), 48. CZAPLINSKI, B.: Acta parasit. polon. **10** (1962), 125. ENIGK, K. and DEY-HAZRA, A.: Z. Parasitenk. **31** (1968), 266. Эниг, К. и Дей Хазра, А.: Мат. Научн. Конф. Всес. Общества Гельминтол. **2** (1962), 9. Яронь, В.: Helmintologia **8** (1967), 195. KOBULEJ, T.: Acta vet. hung. **VI** (1956), 429. KOBULEJ, T. and PAPP, É.: Personal communication (1970). OLTEANU, G., NEGRU, D. and COMAN, E.: Lucrarile Instit. Cercet. Vet. Bioprep. **2** (1963), 257. PHUC, D. V. and VARGA, I.: Acta vet. hung. **XXII** (1972), 197. RHYZHIKOV, K. M. and KOBULEJ, T.: Comptes-rendus 1er Multicolloque Européen de Parasitologie, Rennes 1 au 4 Septembre 1971, 339. STRADOWSKI, M.: Acta parasit. polon. **19** (1971), 63. IDEM: Ibidem, **20** (1972), 179. Скрябин, К. И., Шихобалова, Н. П., Шульц, Р. С., Попова, Т. И., Боев, С. Н. и Делямуре, С. Л.: Определитель паразитических нематод. Стронгиляты. Москва, 1952.

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GAS CHROMATOGRAPHIC EVIDENCE OF PREGNANEDIOLS IN NON-PREGNANT COW'S URINE

By

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Pregnanediol (5β-pregnan-3α,20α-diol) has been identified by HEITZMAN and THOMAS (1965) in the urine of pregnant cows in the late pregnancy. Several authors suggested that pregnanediol or other epimeric pregnanediols are not present in the non-pregnant cow's urine. WATANABE et al. (1965) failed to identify 3,20-dihydroxy-pregnan derivatives in the urine following administration of progesterone to non-pregnant cows. Recently, by an accurate method of isolation, evidence has been obtained in our laboratory (FEHÉR et al., 1970) for the presence of 5β-pregnan-3α,20α-diol, 5α-pregnan-3α,20α-diol, 5β-pregnan-3α,20β-diol and 5α-pregnan-3β,20β-diol in the urine of non-pregnant cows. The epimers were tentatively identified by thin-layer chromatography (TLC) of hydroxysteroids and acetates, studying the ΔR_M functions (BUSH, 1961) following oxidation or acetylation, and by the analysis of UV absorption spectra. The concentration in urine decreased in the above order of the steroids.

In addition to our previous findings, gas chromatographic evidence is given in the present work on the presence of 5β-pregnan-3α,20α-diol, 5α-pregnan-3α,20α-diol and 5β-pregnan-3α,20β-diol in the urine of non-pregnant cows.

Materials and methods

A Hewlett—Packard Model 402 biomedical gas chromatograph with a flame ionization detector was used. A glass column 6 ft. long by 4 mm I. D. was packed with 3.8% UC-W-98 in Diatoport S, with a mesh-size of 80—100, and operated isothermally at 250 °C. The carrier gas was nitrogen at a flow-rate of 25 or 35 ml/min. The detector was maintained at 270 °C and with an attenuation setting for analysis producing f.s.d. for 3×10^{-10} A. The detector response was recorded on a N—P Moseley Recorder at 0.25 or 0.5 in/min.

A pooled cow's urine specimen obtained at various stages of cycle was hydrolysed with HCl, extracted with ether–ethyl acetate–ethanol (2 : 2 : 1), the extract purified with alkali and water, dried on Na_2SO_4 and evaporated. The ketonic and non-ketonic steroids were separated by Girard treatment (PINCUS and PEARLMAN, 1941), and the non-ketonic fraction subjected to

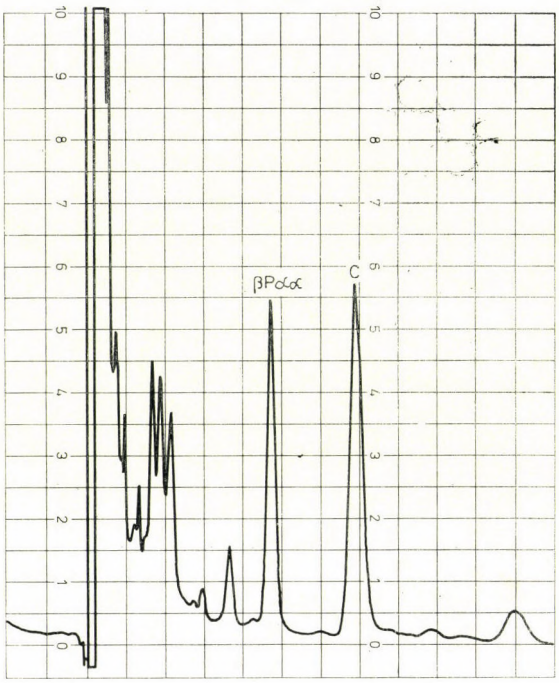

Fig. 1. 5β-pregnan-3α,20α-diol (βPαα) in non-pregnant cow's urine determined as diacetate (Cocholesterol)

digitonin-precipitation (WILSON et al., 1961) to separate 3α and 3β steroids. The 3α-fraction was chromatographed on silicagel G plate in chloroform–acetone (9 : 1). Three fractions, 5β-pregnan-3α,20β-diol + 5α-pregnan-3α,20β-diol, 5β-pregnan-3α,20α-diol and 5α-pregnan-3α,20α-diol, were obtained. The fractions were acetylated (BUSH, 1961) separately, and the acetates rechromatographed in n-hexane–ethyl acetate (8 : 2). The zones were eluted with chloroform, and evaporated. The residue was dissolved in small volume of chloroform, to the solution cholesterol as internal standard was added, and aliquots were subjected to gas chromatography.

Another aliquot of the 5β-pregnan-3α,20β-diol + 5α-pregnan-3α,20β-diol fraction indicated above was oxidized with CrO_3 (BUSH, 1961), and chromatographed on silicagel G with benzene–ethyl acetate (95 : 5). A clear-cut separation of the 5α and 5β dions showed the presence of only 5β-pregnan-3α,20β-diol in the original fraction. Analysis of the 3β-fraction obtained by digitonin treatment revealed no 3β-pregnanediols in cow's urine. For details of the isolation procedure, we refer to our previous communication (FEHÉR et al., 1970).

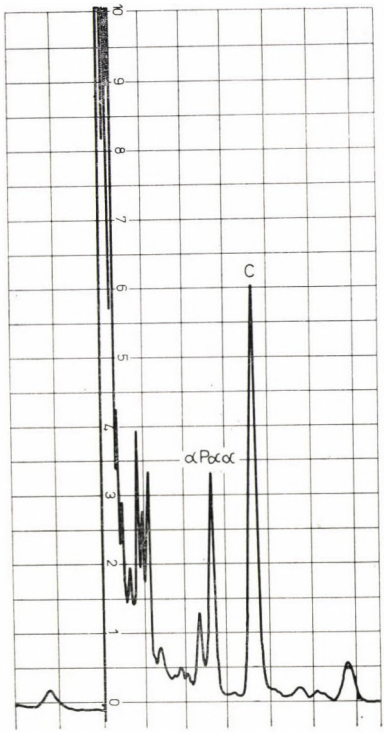

Fig. 2. 5α-pregnan-3α,20α-diol (αPαα) in non-pregnant cow's urine determined as diacetate

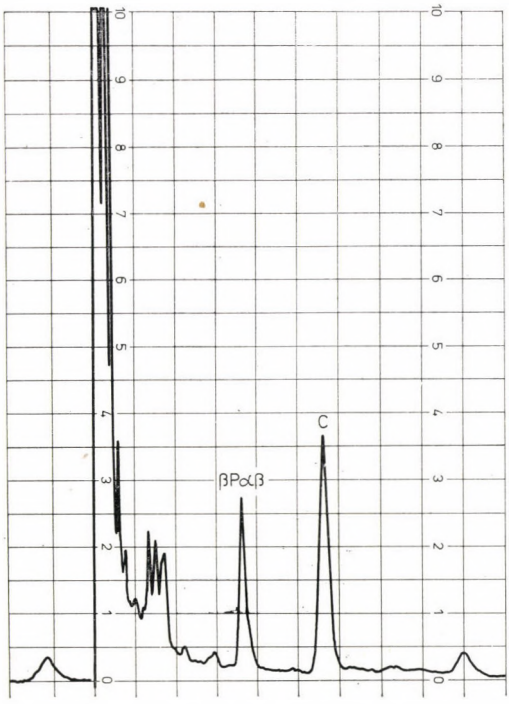

Fig. 3. 5β-pregnan-3α,20β-diol (βPαβ) in non-pregnant cow's urine determined as diacetate

Results and discussion

A study has been made on the gas-chromatographic separation of the eight epimeric pregnanediols as acetates (FEHÉR, 1974). This showed a reliable resolution of the three acetates here isolated. A relative retention time (cholesterol 1.00) of 0.68 for 5β-pregnan-3α,20α-diol-diacetate, 0.73 for 5α-pregnan-3α,20α-diol-diacetate and 0.64 for 5β-pregnan-3α,20β-diol-diacetate was observed. Figures 1, 2 and 3 show the urinary extracts. The analysis is regarded as only qualitative and peak-heights are not proportional to the steroid level in urine.

Several methods have been described for the measurement of progesterone in peripheral blood by gas-liquid chromatography (WYMAN and SOMMERVILLE, 1968; FEHÉR et al., 1975), competitive protein binding (JOHANSSON, 1969; REEVES et al., 1970) or by radioimmunoassay (KUTAS et al., 1972; BRENNER et al., 1973). Recently, we have introduced a simple TLC method for the measurement of pregnanediol in non-pregnant or pregnant cow's

urine (FEHÉR et al., 1970). The present gas-chromatographic approach of pregnanediol assay in cow's urine comfirms the validity of our previous estimations. It is suggested that a numerical increase in individual steroids assayed, and a simultaneous measurement of the hormone in blood and the inactive metabolite in urine will give a complex information on metabolic patterns and correlations in normal and pathological conditions of animal reproduction.

Summary

Evidence has been obtained in a previous study from our laboratory (FEHÉR et al., 1970) for the excretion of 5β-pregnan-3α,20α-diol and other epimeric pregnanediols in non-pregnant cow's urine. The present work supports additional gas-chromatographic evidence for the presence of 5β-pregnan-3α,20α-diol, 5α-pregnan-3α,20α-diol and 5β-pregnan-3α,20β-diol in urine from non-pregnant cows. This gas-chromatographic study confirms the validity of our previous estimations.

References

BRENNER, P. F., GUERRERO, R., CEKAN, Z. and DICZFALUSY, E.: Steroids **22** (1973), 775. BUSH, I. E.: The Chromatography of Steroids. London, 1961. FEHÉR, T.: Magyar Kémikusok Lapja, **29** (1974), 202. FEHÉR, T., BAKSAI, H. E. and HARASZTI, J.: Endokrinologie **56** (1970), 49. FEHÉR, T., FEHÉR, G. K. and BODROGI, L.: J. Chromatogr. **111** (1975), 125. HEITZMAN, J. R. and THOMAS, G. H.: J. Endocrinol. **33** (1965), 455. JOHANSSON, E. B. D.: Acta Endocrinol. **61** (1969), 509. KUTAS, M., CHUNG, A., BARTOS, D. and CASTRO, A.: Steroids **20** (1972), 697. PINCUS, G. and PEARLMAN, W. H.: Endocrinology **29** (1941), 413. REEVES, B. D., DE SOUZA, M. L. A., THOMPSON, E. E. and DICZFALUSY, E.: Acta Endocrinol. **63** (1970), 225. WATANABE, S., DORFMAN, R. I., CRAGLE, R. G. and HAYANO, M.: Steroids, Suppl. **1** (1965), 207. WILSON, H., LIPSETT, M. B. and RIAN, D. W.: J. clin. Endocrinol. **21** (1961), 1304. WYMAN, H. and SOMMERVILLE, I. F.: Steroids **12** (1968), 63.

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STRUCTURAL ABNORMALITIES IN THE MIDDLE PIECE OF THE DRAKE'S SPERMATOZOON TAIL

By

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Abnormal spermatozoon formation has been observed, and discussed in numerous papers. By light microscopy, mainly the abnormalities of the shape had been detected. Only the introduction of microtomy into electron microscopy has allowed to examine the nature and the peculiarities of the spermatozoon structure more thoroughly. Up to now, mostly mammalian spermatozoons have been investigated and numerous abnormalities reported both in shape and structure, occurring either in one part, or in several parts, of the spermatozoon.

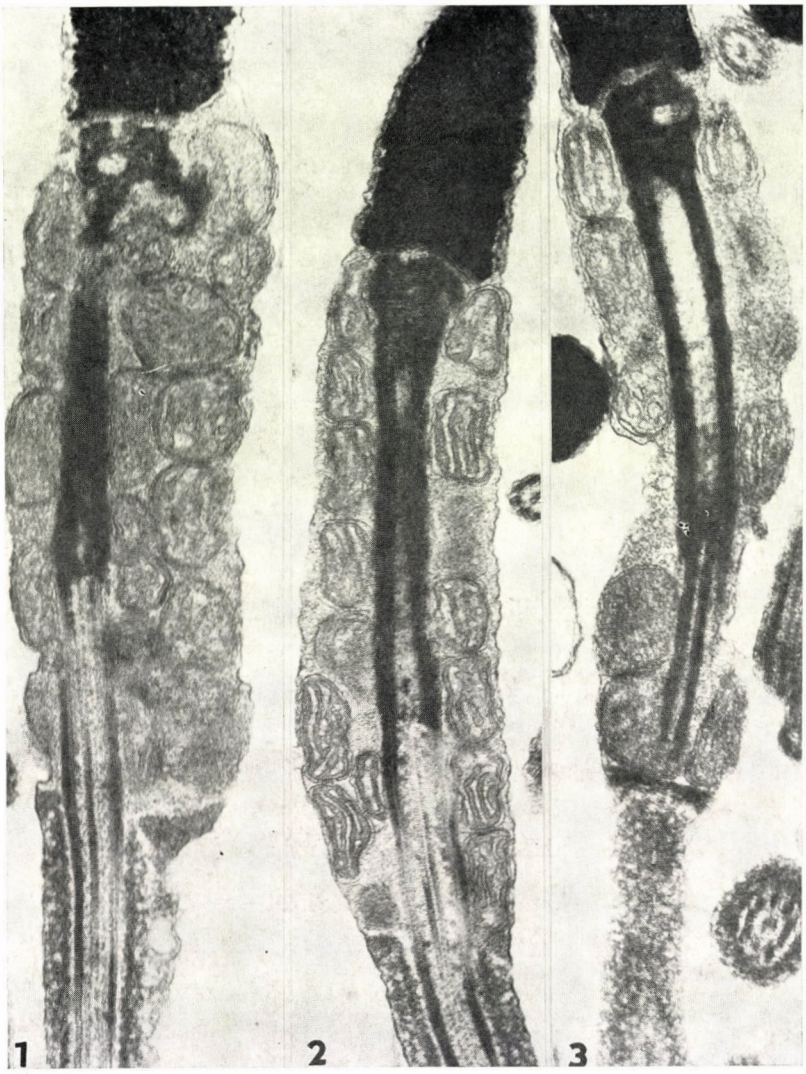
The spermatozoon tail is the place where abnormalities occur most frequently and the middle piece where they are the most dominant. WAKELY and KOSIN (1951), KAMAR and RIZIK (1972), and THURSTON and BIELIER (1972) reported abnormal sperm formation in fowl. In this paper abnormalities observed in the middle piece of drake spermatozoon are reported.

Materials and methods

Sperm was collected by massage from three healthy drakes and discussed the ductus deferens by sqeezing post mortem. Part of the sperm was used for light microscopy and the remaining part was prepared for electron microscopy. The sperm was fixed in 3.5% glutaraldehyde and in 1% OsO_4, both dissolved in 0.1 *M* phosphate buffer of pH 7.2 for 1 hour at 5 °C. After a short rinsing in phosphate buffer, the preparations were dehydrated in ascending concentrations of acetone or ethanol and embedded in Durcupan ACM and Weslopal W. The sections were cut in ultramicrotome TESLA BS 490, stained with uranyl acetate (WATSON, 1958) and lead citrate (REYNOLDS, 1963) and examined in electron microscope TESLA BS 613.

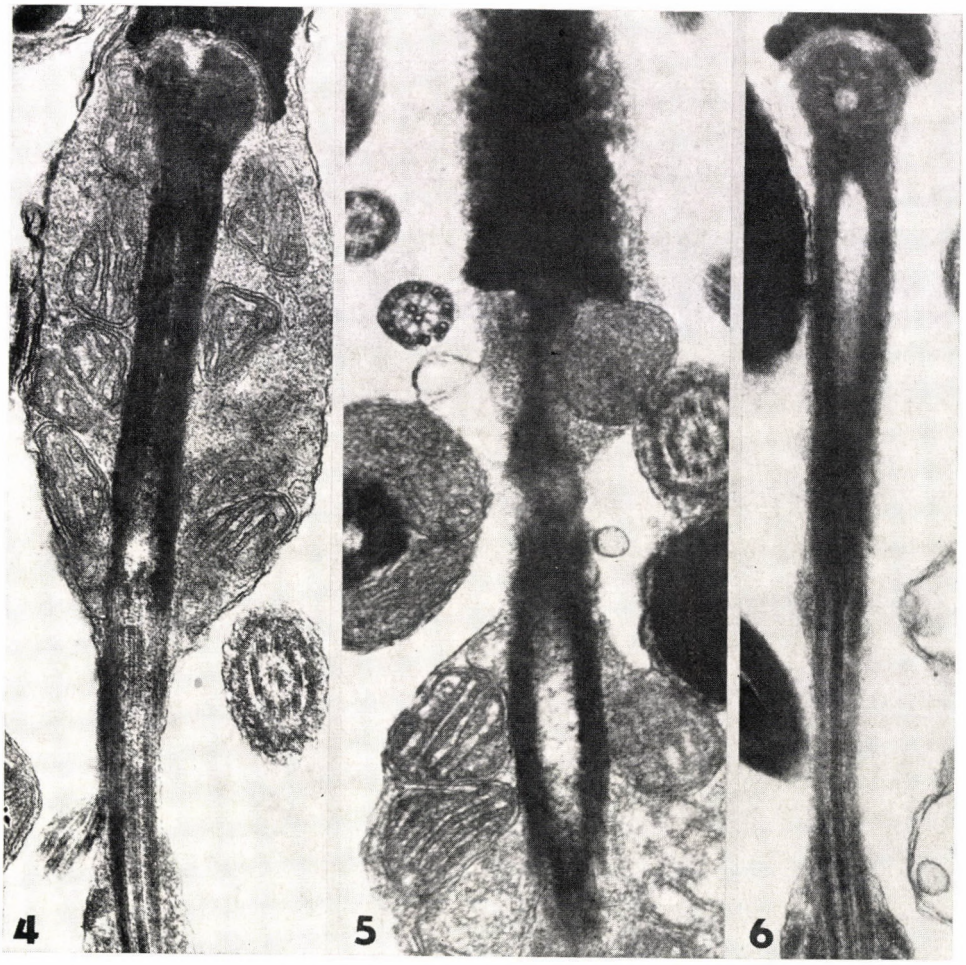
Results

The structural abnormalities of the middle piece of drake spermatozoon have always been located in the mitochondrial sheath. There are changes both in the structure of the mitochondria and in their arrangement. One of the



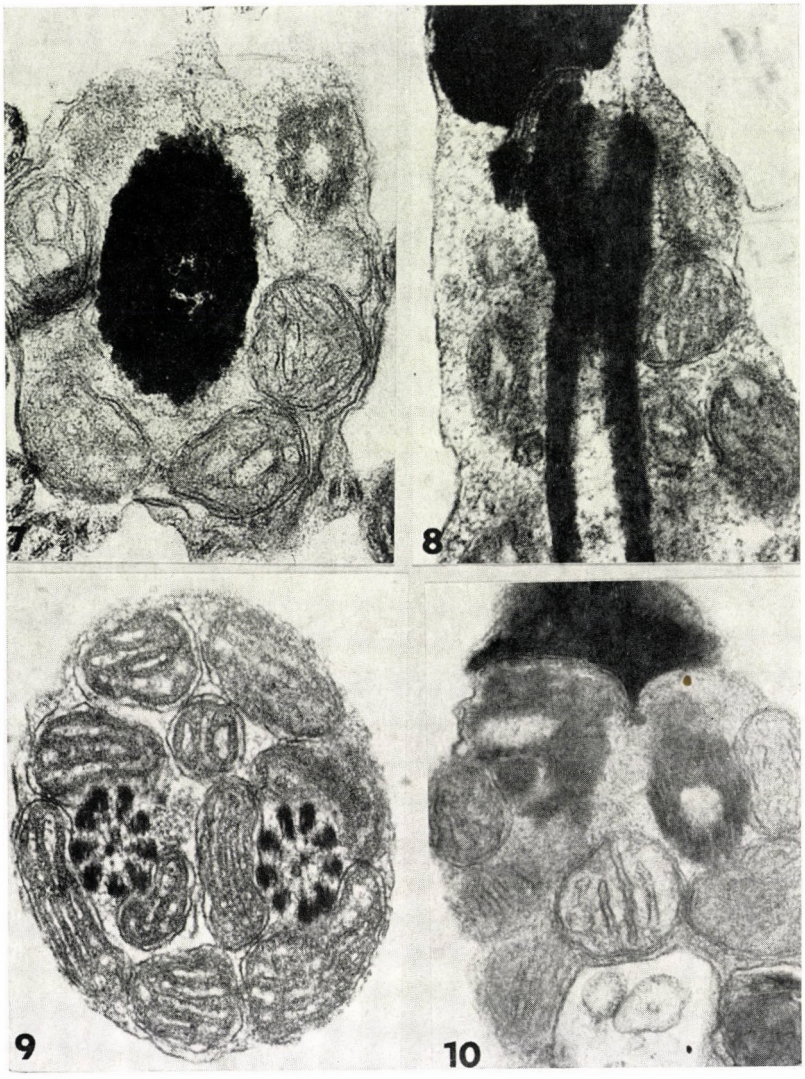
Figs. 1—3. 1, Longitudinal section of middle piece with surplus mitochondria. Note the changes in the articulation between tail and head and in the shape and size of the annulus. ×36,900; *2*, Longitudinal section of middle piece with one surplus mitochondrion. The internal structure of some mitochondria is partially changed. ×36,900. *3*, Longitudinal section of the middle piece; the mitochondria are reduced in number. ×43,050

frequently occurring changes is the irregularity in mitochondrial distribution caused by either the surplus (Figs 1, 2) or the deficient number, or even absence, of mitochondria (Figs 3, 4, 5). The extent of the change depends on the number of the surplus or lacking mitochondria. Both the distribution and the structure of the mitochondria are changed by the presence of accessory mitochondria. Those situated outside the sheath showed changes in shape.



Figs. 4—6. 4, Longitudinal section of tail middle piece. The mitochondrial sheath in the posterior part is missing; in the existing part of the sheath the cytoplasm exceeds the normal cytoplasmic mass. The mitochondria are changed in shape. ×51,600; *5*, Longitudinal section of middle piece. The mitochondrial sheath is localized mainly in the posterior part. The mitochondria are irregular in shape, some of them partly disintegrated. ×36,900; *6*, Longitudinal section of the middle piece with complete aplasia of the mitochondrial sheath. ×41,200

If mitochondria are reduced in number, partial aplasia, if they are absent, total aplasia of the mitochondrial sheath occurs (Fig. 6). The absence of mitochondria may occur in any part of the mitochondrial sheath. Only spermatozoons in which, due to lacking mitochondria, the cytoplasmic membrane was found adjacent to the axial fibril bundle (Figs 3, 4, 5) were considered to show partial aplasia. Empty space in the neck, where the absence of mitochondria is a normal phenomenon in the drake spermatozoons, or that due to insufficient contact between the mitochondria was not considered aplasia.



Figs. 7—10. 7, Transverse section of a sperm head surrounded by mitochondria and distal centriole. ×51,600; *8*, Longitudinal section of the proximal part of tail. Note the abnormality in the articulation between tail and head. ×51,600; *9*, Transverse section of the middle piece of the double tail with surplus mitochondria. ×36,900; *10*, Oblique section of the place of articulation between the double tail and head. ×41,100

Aplasia often occurs when mitochondria accumulate at some place. Such places were found at the anterior and the posterior parts of the middle piece; the mitochondria were changed in structure and, especially, in shape; the changes were conditioned by space limits. The mitochondria were irregular in shape, frequently triangular (Figs 4, 5). The changes, being degenerative, were characteri-

zed by the disappearance of cristae, which were replaced by a structureless matter.

The annulus, which in normal sperms is situated between the mitochondrial sheath of the middle piece and the amorphous sheath of the main piece, in spermatozoons with abnormalities of the middle piece was always present in the same place, even in spermatozoons in which the mitochondrial sheath did not reach the main piece (Figs 4, 6). Its shape, however, was markedly changed, the character of change depending on that in the mitochondrial sheath. In spermatozoons with partial or total aplasia the annulus was less obvious and laterally flattened (Figs 4, 6).

All the above-described changes in the structure of the mitochondrial sheath occurred in spermatozoons in which the tail axis, *i.e.*, the distal centriole and the axial filament complex, further, the place of articulation with the head, were normally developed. Changes were noted sporadically also in the articulation of the tail with the head (Fig. 8). In rare cases, the mitochondrial sheath was found on the spermatozoon head (Fig. 7), and, sometimes, we found that two middle pieces were covered by one cytoplasmic membrane showing all the components and structure of the middle piece (Figs 9, 10).

Discussion

In drakes, as in fowls in general, the mitochondrial sheath in the spermatozoon is arranged in a simpler way than in mammals. It consists of mitochondria situated free around the distal centriole and the proximal part of the axial filament complex (MARETTA, 1974). Consequently, it could be presumed that the occurrence of abnormal spermatozoons in fowls would be less numerous than in mammals, in which the mitochondrial sheath forms a spiral. Yet, our findings show a wide range of abnormalities of the mitochondrial sheath of the spermatozoon from healthy breeding drakes. A small proportion of abnormalities is a current phenomenon and these generally indicate further changes in the spermatozoons, both in mammals and fowls. Changes in the mitochondrial sheath may be due to certain infectious diseases, as observed by CHENOWETH and BURGESS (1972) in bulls, or may appear after exposure to stress, as reported by RATHORS (1969) in rams. Most frequently, the changes in the mitochondrial sheath structure are only a part of more comprehensive changes in the spermatozoon as described in mammalian spermatozoons (KOJIMA et al., 1969; FUJITA et al., 1970; SMITH et al., 1970; PEDERSEN et al., 1971; ROSS et al., 1971). Almost all authors have found changes in the arrangement of the mitochondria in the sheath caused by either deficiency or surplus of mitochondria, just as we have found it in drake spermatozoon. In spermatozoons with surplus mitochondria the changes in the sheath structure are

not as marked as those in the spermatozoons of mammals, where the mitochondrial sheath forms a spiral. Neither did we find changes in the length of the mitochondrial sheath as described by PEDERSEN et al. (1971) and Ross et al. (1971, 1973) in human spermatozoon.

It seems noteworthy that the annular ring was present in every abnormal mitochondrial sheath structure in drake spermatozoon, even in those in which the mitochondrial sheath was missing, contrary to the observation of FUJITA et al. (1970) and CHENOWETH et al. (1970), who failed to find the annular ring in some human spermatozoons.

Several mid-piece abnormalities were described by WAKELY and KOSIN (1951) in turkey spermatozoon; some of them were in accord with our observations, *e.g.*, the absence of the mitochondrial sheath and accumulation of mitochondria at places. However, these abnormalities were found under the light microscope and so we cannot compare the nature of their structure.

Summary

Several structural changes in the mitochondrial sheath of drake spermatozoon are described. The changes are due to disorder in the arrangement of the mitochondria, caused by either lacking or surplus mitochondria. Mitochondrial accumulation is accompanied by changes in their shape. Other, infrequent changes were the presence of the mitochondrial sheath around the spermatozoon head and changes in the articulation of the head and tail.

References

CHENOWETH, P. J. and BURGESS, G. W.: Aust. vet. J. **48** (1972), 37—38. CHENOWETH, P. J., PASCOE, R. R., MC DOUGALL, H. L. and MC COOSKER, P. J.: Brit. vet. J. **126** (1970), 476—481. FUJITA, T., MIYOSHI, M. and TOKUNAGA, J.: Z. Zellforsch. **105** (1970), 483—497. KAMAR, G. A. R. and RIZIK, M. A. A.: J. Reprod. Fert. **29** (1972), 317—325. KOJIMA, Y., ISHIKAWA, T. and MIFUNE, Y.: Jap. J. Vet. Res. **17** (1969), 97—104. MARETTA, M.: Acta vet. hung. **XXV** (1975), 47—52, 53—60. PEDERSEN, H., REBBE, H. and HAMMEN, R.: Fert. Steril. **22** (1971), 156—164. RATHORE, A. K.: Brit. vet. J. **125** (1969), 534—538. REYNOLDS, E. S.: J. Cell Biol. **17** (1963), 208—212. ROSS, A., CHRISTIE, S. and KERR, M. G.: J. Reprod. Fert. **24** (1971), 99—103. ROSS, A., CHRISTIE, S. and EDMONT, P.: J. Reprod. Fert. **32** (1973), 243—251. SMITH, D. M., OURA, C. and ZAMBONI, L.: Nature **227** (1970), 79—80. THURSTON, R. J., and BIELIER, H. V.: Poultry Sci. **51** (1972), 1879. WAKELY, W. J. and KOSIN, I. L.: Am. J. Vet. Res. **12** (1951), 240—245. WATSON, M. L.: J. Biophys. Biochem. Cytol. **4** (1958), 475—478.

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OCCURRENCE OF HONG KONG INFLUENZA A (H3N2) VIRUS INFECTION IN THE BUDAPEST ZOO

By

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In the years following the 1968 Hong Kong pandemic sensitivity to the human influenza virus H3N2 of domestic animals living in close connection with man has been reported to be successfully infected in different percentages (Romváry and Vizy, 1971; Kundin, 1970; Sandow and Wildführ, 1970; Popovici et al., 1972; Harkness et al., 1972), mainly in epidemic periods when farm workers might have been the source of infection. The virus sometimes prevailed in swine stocks for relatively long periods of time (Romváry et al., 1975a). Natural infection of horses (Romváry et al., 1975b), cattle (Izmailov et al., 1973; Fatkhuddinova et al., 1973) and primates (Kalter et al., 1969; Johnsen et al., 1971; Paniker and Nair, 1972) has also been reported. Monkeys (apes) showed severe, occasionally fatal influenza-like illness (Johnsen et al., 1971). Presence of antibodies to the prototype virus (Hong Kong (1/68) in serum samples from chickens and wild birds has been reported from the Soviet Union (Shenderovich and Yefumova, 1969; Zhezmer et al., 1973; Kirianova et al., 1973; Chernetsov et al., 1973; Soldukhin et al., 1973) and in duck sera from the U.S.A. (Slemons et al., 1974). In addition to the demonstration of haemagglutination inhibiting (HI) antibodies, variants of the Hong Kong virus have been isolated from pigs (Kundin, 1970; Popovici et al., 1972; Romváry et al., 1974, 1975a), cattle (Izmailov et al., 1973; Fatkhuddinova et al., 1973), monkeys (Johnsen, et al., 1971), chickens (Zhezmer et al., 1973; Kirianova et al., 1973), duck (Slemons et al., 1974) and bears kept in Zoo (Retescu et al., 1970).

Materials and methods

Virus isolation was attempted from 19 collared doves *(Streptopelia decaocto)* captured on premises of the Budapest Zoo.

Experimental infection. Nine hens were infected intrapharyngeally with influenza strain isolated from collared dove for detection of antibodies in the egg yolk.

Serological tests. HI and virus-neutralization (VN) tests were performed in eight serum samples submitted from the Aviary of the Budapest Zoo and in those from 15 dogs and 11 goats, all kept in the same Zoo. The HI and VN tests were performed as recommended by the WHO in circular No. Z2/180/11/67. If not indicated otherwise, the strain influenza A/H3N2/Hungary/1/73 was used.

The antibodies in the egg yolk were examined as described by Chu and Barhouma (1967). In brief, the albumen was fully removed from the surface

of the yolk sac, to avoid nonspecific HI reaction, a yolk sample sucked off by a syringe was diluted in four volumes of saline and centrifuged for 15 min at 3000 r.p.m. The HI antibodies in the supernatant were titrated as in the serum samples.

Results

Hungary was affected by an influenza epidemic early in 1973. The epidemic was caused by a virus closely related to the human strain influenza A/H3N2/England/42/72. During the epidemic, 19 collared doves captured in the premises of the Budapest Zoo for another purpose were tested for serum HI antibodies. Four doves proved to be positive up to a titre between 1 : 16 and 1 : 32. Virus was isolated from the lungs and bronchi of one collared dove *(Streptopelia decaocto)*. This bird was seronegative when tested with the epidemic strain. The haemagglutinin of the isolate was unrelated to any of the avian influenza virus reference strains and to the NDV; when tested with different variants of the Hong Kong virus, it showed the closest relation to the epidemic strain circulating simultaneously in humans.

At the end of the human epidemic, in March 1973, blood samples were taken from wild birds and mammals kept in the Budapest Zoo. Of the serum samples submitted from the Aviary, those from a *Lampronessa sponsa* proved to be positive up to a dilution of 1 : 16; two *Lampronessa (Aix) galericulata* had a HI titre of 1 : 32 and 1 : 64, respectively. Of 15 dogs four (titre, 1 : 16—1 : 64), of 11 goats two Kameroun goats (1 : 32) proved to be positive. The VN titres agreed well with the HI titres.

Three to 17 months after the end of the epidemic in humans, blood samples were collected from young turkeys and from ducks and layers $1^1/_2$ to 2 years of age. Antibodies to the epidemic strain could not be demonstrated.

Attempts to infect hens with the strain isolated from collared dove

Nine layers were inoculated intrapharyngeally with the strain isolated from collared dove. In the serum samples collected on postinoculation day 13 the hens had HI titres between 1 : 64 and 1 : 128. One week later, even 1 : 256 titres occurred. No titre rise was observed after an intrapharyngeal re-inoculation on the 21st day. In five of the nine re-inoculated hens the serum HI titre had fallen below 1 : 10 by the 36th day following the first inoculation, and in four hens it ranged between 1 : 16 and 1 : 64.

HI antibodies up to 1 : 10—1 : 20 were demonstrated in the yolks of eggs layed 7—9 days after the first inoculation. Subsequently, the yolk titres rose up to 1 : 80—1 : 160 (days 14—16), then tended to decline. From the 21st

day on, the titres did not exceed 1 : 20. After an intramuscular inoculation of the live virus eight days following secondary inoculation, both the HI and VN antibodies in the sera reached a titre of 1 : 64—1 : 512 and then remained at the same level, or slightly increased (1 : 256—1 : 512) over a period of 6 to 7 weeks. In the yolk, the titres ranged between 1 : 20 and 1 : 80 on the 8th day following booster infection. Three weeks later and in the subsequent weeks the yolk titres were equal to the simultaneous serum titres.

Twenty-five cocks were kept for 2—3 months in a room together with dogs experimentally infected and re-infected with the strain Hungary/1/73. At the end of this period three of the cocks had HI and VN antibodies to the same strain up to a titre of 1 : 32—1 : 40.

Discussion

The present results suggest that the wild birds, dogs and goats giving positive serological response to the strain isolated from the simultaneous human epidemic, were susceptible to infection by this variant of the Hong Kong influenza virus. The isolation from a collared dove of a strain of related haemagglutinin is in accordance with this. Supposedly, the animals had been infected from their caretakers. The collared dove is the third avian species after the domestic fowl (ZHEZMER et al., 1973; KIRIANOVA et al., 1973) and duck (SLEMONS et al., 1974) from which a variant of the Hong Kong virus has been isolated. In addition, data have been published on the susceptibility of wild birds — *Clangula hyemalis*, *Gavia arctica* and *Larus argentatus* — living in the arctic areas of the Soviet Union; HI antibody to the Hong Kong virus was demonstrated in 6, 11 and 15%, respectively, of samples collected from these birds. The possibility of droplet infection in birds is suggested by our observation that specific serum antibodies appeared in cocks kept together with dogs artificially infected with a variant of the Hong Kong virus.

The antibodies were demonstrable in the serum of our infected hens for 5—6 weeks; in the yolk sac the titres were lower and the antibodies were demonstrable for a still shorter period of time. The rapid decline of the antibody titre might explain our finding that antibodies to the Hong Kong virus could not be demonstrated in serum samples collected from hens, ducks and turkeys in different areas of Hungary. Intramuscular re-inoculation of the virus was followed by an antibody response demonstrable both in the serum and in the egg yolk; in the latter the antibodies, though they appeared later, reached the same level as in the serum. The high and long lasting yolk antibody level seems to be promising as regards an effective yolk immunity in birds vaccinated against avian influenza.

Summary

Haemagglutination-inhibiting and virus-neutralizing antibodies to the epidemic strain (a variant of the Hong Kong virus) were demonstrated in 23% of serum samples collected in the Budapest Zoo from wild birds, dogs and goats. From the respiratory mucosa of a seronegative collared dove *(Streptopelia decaocto)* a virus strain having a haemagglutinin related to the Hong Kong virus, was isolated.

With the isolate, hens were infected intrapharyngeally. These developed serum antibodies up to a titre between 1 : 16 and 1 : 256, which remained demonstrable for 5—6 weeks. Yolk antibodies appeared later, persisted for a shorter period of time, and reached a lower level than the serum antibodies. An intramuscular re-infection with the same strain was followed by re-appearance of higher-titre antibodies, first in the serum and three weeks later in the yolk. The antibody titres reached approximately the same levels in the yolk as in the serum. The high yolk antibody levels are promising as regards an effective yolk immunity in birds vaccinated against avian influenza.

It is concluded that in connection with human epidemics Hong Kong virus variants may spread from animal to animal; even individuals of different animal species may infect from one another.

References

Chernetsov, Yu. V., Slepushkin, A. N., Sazanov, A. A., Gonsovski, F. K., Obrosova-Serova, N. P., Lvov, D. K., Pysina, T. V. and Zakstelskaya, L. Ya.: Ekologiya Virusov **1** (1973), 46—53. Chu, H. P. and Barhouma, B. R.: In: WHO Circular to Collaborating Laboratories in Study of Animal Influenza (Z2/180/11), 1967. Cretescu, L., Zilisteanu, E., Nisulescu, I., Wagner, G., Cociu, M., Micu, N. and Mastacan, D.: XI. Intern. Symp. Erkrank. Zootiere. Budapest, 1970, 197. Fatkhuddinova, M. F., Kiryanova, A. I., Isachenko, V. A. and Zakstelskaya, L. Ya.: Vopr. Virusol. **1** (1973), 474—484. Harkness, J. W, Schild, G. C., Lamont, P. H. and Brand, C. M.: Bull. WHO **46** (1972), 709. Izmailov, I. A., Atamas, V. A., Knyazeva, N. I., Petrova, M. S. and Kaimakjan, P. V.: Veterinariya, 1973 (5), 69—70. Johnsen, D. O., Wooding, W. L., Tanticharoenyos, P. and Karnjanaprakova, Ch.: J. Inf. Dis. **123** (1971), 365. Kalter, S. S., Heberling, R. L., Vice, T. E., Lief, F. S. and Rodriguez, A. R.: Proc. Soc. Exp. Biol. Med. **132** (1969). 357. Kiryanova, A. I., Fatkhuddinova, M. F., Bakaev, A. E., Smorzhevskaya, L. S., Chumakova, R. F., Isachenko, V. A., Zakstelskaya, L. Ya. and Shaikhanova, Z. T.: Ekologiya virusov **1** (1973), 100—107. Kundin, W. D.: Nature **228** (1970), 857. Paniker, C. K. J. and Nair, C. M. G.: Bull. WHO **47** (1972), 461. Popovici, V., Hiastru, F., Draghici, D., Zilisteanu, E., Matepiuc, M., Cretescu, L. and Niculescu, I.: Acta virologica **16** (1972), 363. Romváry, J. and Vizy, L.: Magyar Állatorvosok Lapja (MÁL), 1971, 49. Romváry, J., Stipkovits, L., Stirling-Mócsy, M. and Farkas, E.: Acta vet. hung. **XXIV** (1974), 335. Romváry, J., Stipkovits, L., Tanyi, J. and Stirling-Mócsy, M.: MÁL, 1975a, 41. Romváry, J., Tanyi, J., Máthé, Zs. and Bartha, A.: MÁL, 1975b, 62. Sandow, B., Wildführ, W.: Mh. Vet.-Med. 1970, 320. Shenderovich, S. Ph., Yefumova, V. A.: Amer. J. Epidemiol. **90** (1969), 400. Slemons, R. D., Johnson, D. C., Osborn, J. S. and Hayes, F.: Avian Dis. **18** (1974), 119. Soloukhin, V. Z., Kasthanova, L. V. and Shumko, V. V.: Ekologiya virusov **1** (1973), 161—164. Zhezmer, V. Yu., Lvov, D. K., Isachenko, V. A., Zakstelskaya, L. Ya.: Vopr. Virusol. **1** (1973). 94—99.

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INFECTION OF DOGS AND CATS WITH THE HONG KONG INFLUENZA A(H3N2) VIRUS DURING AN EPIDEMIC PERIOD IN HUNGARY

By

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In the course of human influenza epidemics, especially in the recent years, infection of domestic pigs, horses, calves, domestic and wild birds has often been reported. Little is known, however, of the reservoir role of dogs and cats. Their potential role was already investigated before the Hong Kong (H3N2) era. Todd et al. (1970) succeeded in re-isolating the influenza A(Singapore)1/57 strain from artificially infected dogs, but the virus did not spread in contact controls. They failed to demonstrate serum antibodies to the Singapore strain in any of 600 blood samples collected in 1964—1966 from dogs.

Paniker and Nail (1970) succeeded in infecting dogs and cats with the prototype strain of the Hong Kong virus (influenza A/H3N2/Hong Kong/1/68) and cage mates of the experimentally infected animals became also infected. The corresponding antibodies were demonstrated in 21.4% of blood samples collected from cats.

Nikitin et al. (1972) re-isolated the Hong Kong virus from the majority of dogs exposed to the virus, and found 5.9% of the dog serum samples collected from different areas of the U.S.A. and England positive. Pysina and Surin (1972) isolated from a sick dog a variant of the H3N2 subtype in the Prymore district of the Soviet Union.

Materials and methods

Virus isolation. Nasopharyngeal fluids from 11 pet dogs were inoculated into the amniotic and allantoic sacs of 9—11-day-old chick embryos and into kidney-cell monolayers prepared from puppies and kittens and colostrum-deprived newborn calves.

Serology. Paired serum samples from the 11 pet dogs and from further 29 dogs were examined for virus-neutralizing (VN) and haemagglutination-inhibiting (HI) antibodies to the strain influenza A/H3N2/Hungary/1/73, which is a virus of human origin closely related to England/42/72 and to a strain isolated by us from a dog during the same epidemic which yielded the Hungary/1/73 strain. The sera examined were treated and the tests were performed as recommended in the circular Z2/180/11/WHO/1967.

Experimental infection. Puppies 3 weeks to 7 months of age, a bitch with her two-week-old puppies, a nursing cat and kits 10-days and 5-weeks of age were inoculated intranasally.

Results

During the 1973 influenza epidemic in Hungary we failed to isolate the virus from nasopharyngeal fluids obtained from 11 dogs 8 months to 5 years of age in puppy and calf kidney-cell monolayers. However, two strains (G112/73 and 108/73), both identical with the Budapest/1/73 strain were isolated from the same dogs in chick embryos. The dogs (1 and 4 years of age) yielding virus had an elevated temperature up to 39.6°C and 40.3 °C, respectively. They were weary and often sneezed. In the families where they lived one or more family members in each were suffering from influenza (clinical diagnosis) at the same time. Blood samples were taken from the 11 dogs at the time of taking nasopharyngeal fluid, and 3—5 weeks later. In the first samples HI antibodies to the Hungary/1/73 specified strain could not be demonstrated, except for one dog who had a 1 : 16 titre. Of the second samples six contained antibodies; the titres ranged between 1 : 64 and 1 : 256. The specificity of the antibodies was confirmed with VN tests. The dogs from which virus was isolated had no antibodies in their first serum samples, but in the second samples, taken 24 and 29 days after the first sampling, respectively, the HI titre was 1 : 64 and 1 : 128. Soon after the human epidemic 15 dogs kept in cage in the Budapest Zoo were examined for serum antibodies. In three cases the HI titre was as high as between 1 : 16 and 1 : 64. In the other samples no antibody could be demonstrated. At the same time blood was taken from 14 dogs kept tied up in the outskirts of a village. These dogs had no contact with either humans (except their feeder), or other animals. All the 14 serum samples proved to be negative.

Experime ntal infection of dogs and cats with influenza A/H3N2/ virus

The two dogs inoculated intranasally with the Hungary(1)73 strain had a temperature elevated up to 40.2—40.4 °C for 1 and 3 days, respectively, beginning on the first and fourth days after inoculation. Their cage-mate (one dog) had a temperature of 40.0 °C on the 4th day. We succeeded in re-isolating the virus from all three dogs over a period of 5—7 days. The HI antibody titres rose to 1 : 64—1 : 128 in the serum of all the three dogs by the 4th week following inoculation. Subsequently, the dogs were re-inoculated with the same virus. Their serum antibody titres reached 1 : 512—1 : 1024 by the 8th day after re-inoculation.

In the following experiment a beagle bitch and three of her five two-week-old youngs were inoculated with the Hungary/1/73 virus. Virus was shed by the dam and each of the 5 puppies over a period of 3 to 7 days. On the 13th postinoculation day the specific antibody titre in the serum of the bitch reached 1 : 256. On the 16th day after the first inoculation the bitch was re-inoculated intranasally. In this case the virus could not be recovered either

from the bitch or from the three inoculated and two uninoculated puppies. Seven weeks after the first inoculation the HI titres of the five puppies ranged between 1 : 32 and 1 : 128. The bitch had a higher titre: 1 : 512. In the whey of the bitch the VN antibody titre was 1 : 20 on the 10th and 49th days after the first inoculation. The three artificially infected puppies showed weariness, anorexia and incoordinated movements and had an elevated temperature in the 5th week after the first inoculation. The incoordinated movements lasted for two weeks, whereafter the puppies were killed. Virus isolation from their organs in monolayer cultures from dog, cat, pig and calf kidney cells was unsuccessful. Three months after the first inoculation the serum antibody titre of the bitch's serum was 1 : 64, that of the serum samples from the two uninoculated puppies was 1 : 16.

With one of the strains re-isolated from one of the pet dogs (G112/73) 3 puppies three weeks of age were infected. These and their uninoculated litter mates had neither elevated temperature nor showed any other symptom of illness. Nevertheless, the virus was re-isolated from the inoculated puppies in the first 4 days, from the uninoculated one even on the 5th day. The HI antibody titres ranged between 1 : 128 and 1 : 256 four weeks after inoculation and was between 1 : 16 and 1 : 128 on day 87. The uninoculated control puppy had a titre of 1 : 16.

In a further experiment three kittens from a 5-week-old litter were inoculated with the Hungary/1/73 strain intranasally; their three litter mates remained uninoculated. Subsequently, all the kittens had a slightly elevated temperature and the virus was successfully re-isolated on the first 5—7 days after inoculation from both the inoculated and the uninoculated kittens. The inoculated ones had serum antibodies up to 1 : 32—1 : 128 on the 34th post-inoculation day, whereas, only one of the uninoculated controls had serum antibodies at the same time (1 : 32). After re-inoculation the serum antibody level rose to 1 : 512. At the same time the contact litter mates were also inoculated intranasally. The HI antibody titres of these reached 1 : 64 to 1 : 256.

Finally, a cat nursing four kittens was inoculated intranasally with the G112/73 strain of dog origin. The dam and the kittens shed the virus up to the 7th and 9th day, respectively. Fourteen days after inoculation the dam had a serum HI titre of 1 : 256, while the titres of the kittens were lower: 1 : 8—1 : 32. The Hungary/1/73 influenza virus strain failed to grow in monolayer cultures prepared from the kidneys of these kittens killed 14 days after the inoculation of their dam.

Discussion

The isolation of the human epidemic strain of influenza A virus from two pet dogs supports the view that pet dogs may become infected with human strains of virus from their masters. The significant increase in the serum HI

antibody titre in further pet dogs as well as the infectedness of dogs in Zoo are consistent with this view. The latter were exposed to infection from feeders and visitors.

Since the artificially infected dogs showed no characteristic clinical symptom, the role of the virus in the clinical picture observed in two dogs yielding virus, needs confirmation. The central nervous system (CNS) signs observed in three infected puppies cannot be explained distinctly with the influenza virus infection, though their uninoculated litter mates did not show similar symptoms.

The dogs of different ages remained symptomless after infection, except for a temperature elevation lasting for 2—3 days. It is of interest that the strain re-isolated from a naturally infected dog, compared to the strain Hungary/1/73 isolated from a human case, proved to be less pathogenic for dogs when administered intranasally. The animals showed no temperature rise, though the virus could be re-isolated from them for 4—5 days.

In the serum of three dogs the antibody titre showed a rise in a period when influenza did not occur in humans. In these cases cats infected with Hungary/1/73 strain kept in the same room might have been the source of infection.

A cat was successfully infected with one of the strains isolated from a naturally infected dog and the virus spread by contact in the litter of the infected cat. The sucklings shed the virus for a longer time than did the infected dogs.

The virus excretion of cats and dogs lasting for several days seems to play a considerable role in the transmission of the virus, especially in crowded cages or rooms, where droplet infection may often occur. This is supported by the observations of NIKITIN et al. (1972), who infected dogs by Hong Kong variant strain, separated them in another room 24 hours after their infection, and exposed susceptible dogs to contact infection. These authors succeeded in recovering the virus only in one of the exposed dogs. Four days later, these dogs infected by contact were transferred into a separated room and kept together with further susceptible dogs but the exposed contact animals did not develop demonstrable infection.

Dogs soon develop local immunity. In our experiments the virus could not be re-isolated from the dogs re-inoculated with the virus 16 days after the first inoculation. The demonstration of HI and VN antibodies in whey 10 and 49 days after inoculation (24 and 63 days after whelping) indicates that antibodies secreted in the milk are available for puppies of convalescent bitches. The fact that a bitch kept in common room with infected dogs and cats became infected, and the demonstration of antibodies in the serum of two cats kept in a wire-cage at a distance of 20—30 cm from dogs infected with the Hong Kong influenza virus, suggest that individuals of these animal species may be infected not only from humans, but also from each other.

Summary

During an epidemic caused by influenza A virus strain closely related to England/42/72, pet dogs became infected by the natural route. The virus was re-isolated from the nasopharyngeal secretions of two sick dogs, and the specific serum antibody level of six pet dogs showed a significant rise. Haemagglutination-inhibiting (HI) and virus-neutralizing (VN) antibodies up to a dilution between 1 : 16 and 1 : 256 were demonstrable in 9 (22.5%) out of 40 postepidemic serum samples collected from dogs kept in different places.

Local immunity of the upper respiratory tract was demonstrable in dogs 16 days after a successful infection with a representative of the human epidemic strain Hungary/1/73. Specific VN antibodies were demonstrable for at least 7 weeks in the whey of a bitch that had been infected intranasally two weeks after whelping.

Dogs and cats were easily infected with the strain Hungary /1/ 73. The infected animals showed some temperature rise and excreted the virus for 3 to 9 days. Even one of the strains isolated from pet dogs was able to spread from an intranasally infected cat in her litter. This strain did not cause temperature rise after intranasal inoculation, but it was successfully re-isolated for 4—5 days from the nasopharynx.

It is suggested that dogs and cats may serve as reservoir for Hong Kong virus and its variants; the virus carried by them may spread in a closed room even in animals of different species.

References

TODD, J. D., LIEF, F. S. and COHEN, D.: Amer. J. Epidem. **92** (1970), 330. PANIKER, C. K. J. and NAIR, C. M. G.: Bull. WHO **43** (1970), 859. NIKITIN, T., COHEN, D., TODD, J. D. and LIEF, F. S.: Bull. WHO **47** (1972), 471. PYSINA, T. V. and SURIN, N. G.: Voprosy virusologyyi **17** (1972), 245.

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SEVERE SINUSITIS IN DUCKLINGS CAUSED BY INFLUENZA A VIRUS

By

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In last two decades avian influenza A virus straines of different antigen groups have been isolated all over the world.

Virus isolation has been successful in every geographical area where ducks were examined (BLASKOVIC et al., 1959; FRAŇO et al., 1958; HIGGINS, 1971; HWANG et al., 1970; MITCHELL et al., 1968; PAUKOVIC et al., 1969; ROBERTS, 1964; ROMVÁRY, 1970; SCHETTLER, 1969, 1970; TANYI, 1972, 1973; TSIMOKH, 1961; VRTIAK and FRAŇO, 1966; WALKER and BANNISTER, 1953). Most of the strains were isolated from ducklings 1—3 weeks of age, some of them from older ducklings. In Hungary, successful isolation from adult ducks has also been reported (TANYI, 1972, 1973). The consequences of the infection have been highly variable. In the absence of additional damaging effects the infection may remain inapparent or sporadic cases without any appreciable loss may occur. However, the losses may grow severe in stocks where housing and/or nutrition are unsatisfactory or the conditions in general are unhygienic.

Materials and methods

Virus isolation

Samples were received from the respiratory tract and other organs of spontaneously died or emergency-slaughtered ducks and from sinus secretions aspirated under sterile conditions from sick ducklings. The samples were homogenized, antibiotics were added, the homogenates were centrifuged and the supernatants were inoculated into the allantoic sacs of 9 to 12-day-old embryonated hen's eggs. The eggs were opened as the embryo had died or 3 to 7 days after inoculation. Three blind passages were carried out until an experiment was closed as negative.

Serological investigations

In the haemagglutination and haemagglutination-inhibition (HI) test chicken and guinea pig erythrocytes were applied. The serum samples were pretreated with heat, trypsin and potassium periodate. Immunodiffusion test was performed as described by BEARD (1970).

Experimental infection

Day-old Leghorn chickens, poults, ducklings, young Leghorn chicken and cocks, furthermore, adult cocks were infected intranasally and into the conjunctival sac with 10^6 $ELDf_{50}$/0.1 ml of 313—V/74 virus isolated from a duck.

Epizootiological observations, symptoms and pathology

In a farm, 2 million duck eggs were incubated in the period from February to October, 1974. Of the ducklings 700,000 were raised in the same farm to be sold as ducks for roast or to be used in the breeding; the others were sold as day-old ducklings or at an age of about three weeks. The egg shell was disinfected with formalin steam before and during incubation. The rate of hatching was 79 $\pm$ 5%; hatching was in some cases biphasic, *i.e.*, about 40% of the ducklings hatched about 16 hrs later than the others. Uni- or bilateral swelling of the infraorbital cavity was first observed late in April, in ducks 10—18 days of age. In the subsequent months, both the severity of the disease and the loss increased.

In the dissemination of infection and in the increasing severity of the disease several factors played a decisive role. First of all, the unexpectedly high rate of hatching caused crowdedness. The cleaning and the disinfection were insufficient because the time interval between the removal of a group of ducklings and the introduction of new ducklings was too short. Furthermore, 15—30% of the ducklings had not reached the required body weight by the end of the third week, therefore, these had to be kept mingled with a younger group for 4—7 days. Finally, because of water supply problems in the farm, the duck-ponds had become plashy.

The outbreak increased in severity up to the end of May, it became milder in June and the morbidity increased again late in July. The number of cases was still considerable in September, when many sick ducklings were found even among the six-week-old.

The first symptoms usually appeared when the ducklings were 10 days old. The affected ducklings often sneezed, serous-mucous secretion was oozing from their nostrils and secretion welled forth from the nostrils on mild compression. Occasionally, feed fragments attached to the oozing secretion, thus rendering the phenomenon most pronounced. By the same time the infraorbital cavity had reached a cherry in size. The coryza, variable in severity, was occasionally accompanied by conjunctivitis. Well-defined sinusitis was observable in 20—40% of the ducklings at 10—18 days of age. The changes were bilateral in 5—10% of the cases. The mildly affected ducklings were vigorous, their appetite was approximately normal; those being severely ill pressed close together, ate very little and were arrested in growth.

From the infraorbital cavity, undulating on palpation, 0.5—2.0 ml whitish-yellowish opalescent, highly viscous, sticky secretion could be aspirated. The secretion sometimes contained fibrinous fragments. The same amount of secretion could be aspirated from the same cavity again after an interval of 24 hrs. In a later stage of illness the infraorbital swelling indurated and secretion could not be aspirated any more.

In the majority of cases the infraorbital inflammation had disappeared by 4—5 days of age, in certain cases, however, it was palpable even later or a hairless scaly skin lesion reminded of the preceding process. The uneven development of the highly affected groups of ducklings remained striking.

In the most critical period, between 10 and 21 days of age, the mortality was by 4—6% higher than usual, the total loss being 4.8 to 22.5%, 10.6% on the average.

At postmortem examination the infraorbital cavity was almost invariably filled with a mucous-serous secretion which sometimes contained fibrinous fragments.

The mucous membrane of the infraorbital cavity was reddened, swollen or dull, with reddish spots. In subacute and chronic cases, the sinus facialis contained a pea- to cherry-sized dry, yellowish detrital caseous substance of laminal structure.

In the air sacs and serous membranes of certain spontaneously died or emergency-slaughtered ducklings fibrinous deposits were observed.

Bacteria could not be demonstrated in most of the mucous secretions; from a few cases ubiquitous bacteria were isolated. From the caseous matter coliform bacteria were isolated, largely in pure cultures.

For therapeutic purposes antibiotics were mixed to the feed, with no considerable success.

Of the farms that had bought of the day-old ducklings, sinusitis outbreaks were demonstrated in two; the housing-hygienic circumstances were objectionable in both. In the other farms, where the conditions were unobjectionable, only sporadic cases occurred. In the latter farms, the mortality did not exceed 4—5% in the critical age of 10—21 days. The relatively low loss can partly be attributed to the fact that the farms buying day-old ducklings received as a rule the best-developed ones. The recovery and the development of the ducklings sold at 3 weeks of age, and still showing clinical symptoms in the different farms also depended on the hygienic conditions in the farm.

Virological examinations

A haemagglutining virus was isolated from the sinus secretion and from the mucous membrane scrapings of each of the ill ducklings tested for virus and from the lungs of more than a half of them. Of 15 samples of caseous sub-

stance only two yielded virus. The inoculated embryos died 48—96 hrs after inoculation. Of the 42 isolates 33 originated from sinus secretions, 7 from lungs and 2 from caseous substances. We attempted to isolate virus from 28 samples of parenchymal organs (liver, kidney, spleen, brain) without any success; the respiratory tracts of the same ducklings did yield virus.

The virus was isolated from one of the ducklings repeatedly, on 11 occasions; the samples yielding virus were taken at 3- to 5-day intervals over 7 weeks. From another duckling, the virus was isolated from both sinuses on three, and from one sinus on two occasions: *i.e.*, the virus could consistently be isolated until no sinus secretion remained to be aspirated. The double agar-gel diffusion test showed that the isolates were type-A influenza virus strains, but they could not be identified with either of the *Hav1* to *Hav6* serotypes of avian influenza A virus.

Experimental infection

The day-old birds inoculated with 0.2 ml allantoic fluid each became weary and lost appetite between the 2nd and the 7th days; they developed poorly, some of them died. In the air sacs of these, and of several birds chosen at random, fine fibrinous inflammation was found. The Leghorn chickens and the young cockerels showed no clinical symptom. The one-year-old cocks were weary and lost appetite between days 2 and 7 and showed circumocular oedematous imbibition.

In the sera obtained between 10 and 12 days after inoculation antibodies to the isolates were demonstrated.

Virus serological investigations

Of 20 ducklings 14 days of age, showing typical symptoms, 16 had antibodies up to 1 : 10—1 : 80 serum dilutions as tested with one of the isolates. Eight day-old ducklings had not such antibodies. In serum samples taken from 5 ducklings weekly up to their age of 9 weeks, the HI titre fluctuated between 1 : 20 and 1 : 80. The antibody level had tended to decline by the 12th week and disappeared by the 15th week. We failed to isolate virus from 10 ducklings 12 weeks of age, separated in a group, but antibodies to the isolates were present up to 1 : 20—1 : 40 in 4 of the same ducklings. In addition, the antibodies in two of the sera contained HI antibodies to the *Hav2* and *Hav6* prototype strains as well. From the same farm *Hav4* strains had been isolated in 1971, but none of the present sera contained antibodies to this virus.

In two adult cocks the serum HI antibody titre to the isolate was 1 : 80. In two young cockerels the titre was 1 : 40 three weeks after inoculation. In

the 4th week the cockerels were reimmunized; their titres rose to 1 : 80 and 1 : 160, respectively, in 7 days. The titres showed a substantial fall by the 12th week.

Discussion

The present observations call attention to certain important aspects of the prevention of avian influenza.

An initially sporadically appearing mild outbreak soon developed into a severe epizootic, owing to crowdedness, to inadequate clearing and disinfection, as well as to mingling of young ducklings with older ones. In this way not only the spread of the outbreak, but also the development of clinical complications was accelerated. Similar clinical picture has been described in turkeys by MOHAMED et al. (1970). The HI antibody level in the sera of convalescent ducklings was lower as compared to the titres observed in other avian species. Similar findings have already been reported by others (ROBERTS, 1964; SCHETTLER, 1969; HIGGINS, 1971). Infectious virus was present in the sinus secretion for a relatively long period of time; from one duckling the virus could be isolated over 7 weeks. The unexpectedly high isolation rate in the present study seems to be attributable to the relatively long-lasting carriership following recovery.

The virus could be isolated from the serous-mucous secretion in almost every case. At the time when the serous-mucous secretion turned into a caseous mass, which can be considered a complication of the influenza infection, the virus could not be isolated any more, except for a few cases.

The majority of the isolates could not be identified with any of the subtypes *Hav1—Hav6*, though they have proved to be influenza A virus strains, as shown by the results of double immunodiffusion tests. Thus, the isolates may belong to subtype *Hav7* or *Hav8*, the antisera to which have not been available for us, or to a hitherto unknown avian subtype. The pathogenicity of the isolates is supported by the great number of clinical cases in ducklings and by the experimental disease in chickens and cocks as well as by the 100% mortality of the inoculated chick embryos.

It is of interest that in a duck stock three subtypes (*Hav2*, *Hav6*, and one at present *unidentified*) of influenza A virus were circulating in 1974 at the same time. Considering earlier experiences in the above-mentioned duck stock within 4 years (1971—1974) four Hav subtypes (in 1971: *Hav4*) of influenza A virus infection has been detected.

Summary

In a duck farm, two million duck eggs were incubated in the period from April to October, 1974. The majority of the ducklings were sold when one-day old, the minority when 3 weeks old. Twenty thousand ducklings were utilized in the breeding. In different groups of ducklings 15—50% were affected by an outbreak characterized by unilateral, occasioally bilateral, sinusitis. The ducklings became free of symptoms within 1—4 weeks, except for complicated cases, when the sinusitis was still recognizable at an age of 8 weeks. In the groups kept in the farm up to adult age, the loss ranged between 4.8 and 22.5% (mean, 10.6%).

Fourty-two haemagglutinating virus strains were isolated from the respiratory tract of the diseased ducklings, most of them from the sinus secretion. In the double agar-gel diffusion test the isolates proved to be influenza A virus strains, but in the haemagglutination-inhibition (HI) test they did not react with antisera to the prototype strains of subtypes *Hav1—Hav6*.

Chickens, day-old ducklings and day-old turkeys experimentally infected with one of the isolates developed fibrinous air sacculitis; adult cocks showed head oedema lasting several days. The artificially infected birds gave a positive immune response, well-demonstrable 10—12 days after infection.

The virus was isolated from all the serous-mucous sinus secretion samples, from several ducklings consistently during a period of 2—7 weeks. However, virus isolation was rarely successful from caseous-fibrinous masses. The HI titres reached relatively low levels in the sera of ducklings after natural infection. From carrier ducklings the virus could be isolated in spite of the presence of homologous antibodies demonstrable up to 1 : 20—1:40 serum dilutions. The serum antibodies had disappeared by the 5th—12th week after recovery.

References

Beard, V. W.: Avian Diseases **14** (1970), 337. Beard, C. W.: Bull. WHO **42** (1970), 779. Blaškovič, D., Rathova, U. and Borecký, L.: Acta Virol. (Prague) **3** (1959), 17. Fraňo, J., Bystrický, U. and Vrtiak, J.: Vet. čas. **7** (1958), 411. Higgins, D. A.: Tropical Animal Health and Production **3** (1971), 232. Hwang, J., Lief, F. S., Miller, C. W. and Mallinson, E. T.: JAVMA **157** (1970), 2106. Mitchel, C. A., Guerin, L. F. and Robillard, J.: Can. J. Comp. Med. **32** (1968), 544. Mohamed, Y. S., Moorhead, P. D. and Bohl, E. H.: Avian Diseases **14** (1970), 410. Paukovič, C., Audi, D. and Aleraj, Z.: Vet. Arh. **39** (1969), 278. Roberts, D. H.: Vet. Rec. **76** (1964), 470. Romváry, J.: Magyar Állatorvosok Lapja (MÁL), 1970, 549. Schettler, C. H.: VIth Congress of the World Veterinary Poultry Association, Beograd, 1969, 13. Schettler, C. H.: Zbl. Vet. Med. Reihe B., Hamburg **17** (1970), 817. Tanyi, J.: MÁL, 1972, 9. Idem: Ibidem, 1972, 13. Idem: Acta Vet. hung. **XXII** (1971), 271. Idem: Thesis, Budapest, 1973. Tsimokh, P. F.: Veterinariya, 1961 (12), 63. Vrtiak, O. J. and Fraňo, J.: Bull. Off. Int. Epiz. **65** (1966), 1231. Walker, R. V. L. and Bannister, G. L.: Can. J. comp. Med. **17** (1953), 248.

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ISOLATION OF MYCOPLASMA OVIPNEUMONIAE FROM SHEEP WITH PNEUMONIA

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The mycoplasma infection of sheep has not been studied in detail so far. *Mycoplasma agalactiae* subsp. *agalactiae*, the aetiological agent of contagious agalactia, was the first of the mycoplasmas isolated from this species (BRIDRE and DONATIEN, 1923). ARISOY et al. (1967) reported on the presence of unidentified mycoplasmas in the eyes and in the nasal cavities of clinically normal animals. Australian authors (ST. GEORGE, 1969; COTTEW, 1971) presented a detailed characterization of mycoplasmas isolated from sheep with pneumonia. CARMICHAEL et al. (1972) also isolated ovine mycoplasma strains in Australia. Some of these isolates were classified as *M. ovipneumoniae* (CARMICHAEL et al., 1972).

In this report isolation of *M. ovipneumoniae* strains from lambs showing chronic respiratory symptoms is described. The isolation of the strains was preceded by an epidemic of adenovirus pneumoenteritis.

Materials and methods

Epidemiological observations. The natural history of an epidemic of pneumoenteritis among fattening lambs was reported previously (BELÁK and PÁLFI, 1974c). In the same farm an adenovirus strain was isolated from acute cases and the aetiological role of the strain was proved. In the majority of the cases, the acute respiratory symptoms became chronic. In this phase of the disease cough and dyspnoea lasted for a long time.

Specimen collection. The lungs of 10 animals with chronic respiratory signs were examined pathologically, parts of 9 lungs were fixed in 10% formalin, embedded in paraffin and the sections were stained with haemalaun—eosin for histopathological studies. Bacteriological and virological investigations were made as described before (BELÁK and PÁLFI, 1974a).

The lungs and the peribronchial lymph nodes were examined for the presence of mycoplasmas. The organs were homogenized and transferred to

mycoplasma medium B (ERNØ and STIPKOVITS, 1973) and Gourlay—Leach's medium (GOURLAY and LEACH, 1970) for cultivation at 37°C. Every second day, the inoculated media were streaked on the same type of solid media. Plates were incubated in candle jar for 3 weeks. The growth of colonies was checked regularly. Agar blocks containing colonies were transferred into Gourlay—Leach's liquid medium and after 5 days of incubation the broth cultures were filtered through Millipore filter (Millipore HAWP O1300,100 ea HA 0.45 μm). Filtrates were streaked on solid medium and if the cultivation was successful, single colonies were picked up for further subcultivation. Each strain was cloned three times.

Biochemical and serological characterization of the isolated mycoplasmas. All strains were examined for criteria of the order *Mycoplasmatales* (International Committee on the Taxonomy of *Mycoplasmatales*, 1972 *viz.*, penicillin resistance, filterability, morphology, absence of bacterial reversion), for classification into family (cholesterol requirement, sensitivity to sodium polyanethol sulphonate and digitonin, growth at 20°C) as well as for species characteristics (ERNØ and STIPKOVITS, 1973). Growth-inhibition tests were performed as described elsewhere (STIPKOVITS and VARGA, 1974), with antisera to 50 mycoplasma species.

Most of the reference mycoplasma strains were obtained from the FAO/WHO Reference Centre for Animal Mycoplasmas (Department of Medical Microbiology, University, of Aarhus, Aarhus, Danemark, Prof. A. E. Freundt), the *M. ovipneumoniae* strains from Mr. G. S. Cottew, CSIRO Division of Animal Health, Parkville, Australia. All antisera used were prepared in our laboratory.

Serology. Sera collected from animals of the examined flock were checked for antibody against *M. ovipneumoniae* by metabolism-inhibition tests with *M. ovipneumoniae* (L 25) reference strains.

Results

Gross pathology. In different areas of the lungs catarrhal, catarrhal-purulent and croupous pneumonia were found, especially in the apical and cardiac lobes, infrequently in other parts, too. Pericarditis and pleuritis were also observed.

Lobar consolidation was frequently seen in the cardiac and apical lobes. The affected areas were reddish or greyish brown and firm. A consistent, muddy, purulent exudate could be expressed from the bronchioles of the cut surface. Enlarged, greyish-white, firm peribronchial lymph nodes were found; these showed an indistinct structure and a rather dry cut surface.

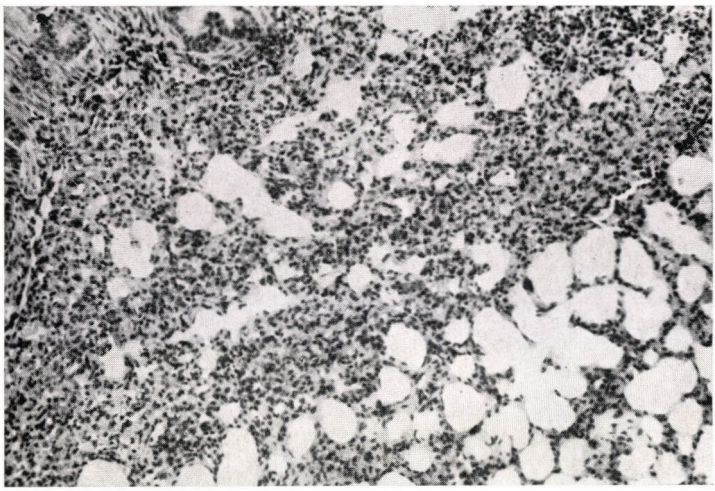

Fig. 1. Peripheral area of interestitial pneumonia, approx. × 110

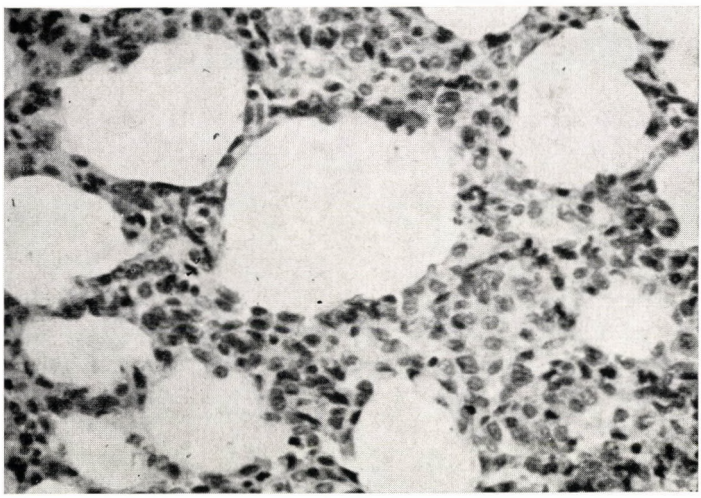

Fig. 2. Alveolar septal thickening, approx. × 275

Microscopic pathology. Alterations were observed in a single lobule or in adjacent lobules.

A marked, intralobular, interstitial pneumonia (Fig. 1) was observed in separated lobules of 3 lungs. There was a marked thickening of alveolar septa (Fig. 2) due to proliferation of histiocytes. Frequently, proliferation of the alveolar epithelial cells was also found. The lumen of a high number of alveoles was filled with desquamated alveolar epithelial cells (Fig. 3). Only a moderate number of neutrophil granulocytes could be observed in the inter-

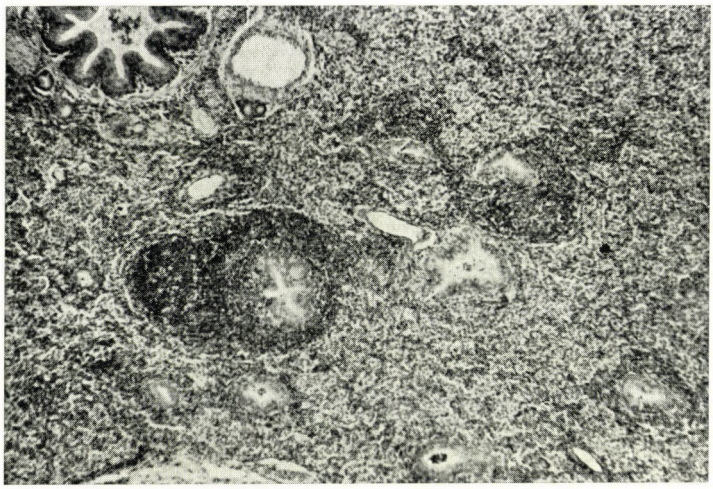

Fig. 3. The alveolar lumen is narrowed due to a marked desquamation and interstitial proliferation, approx. × 45

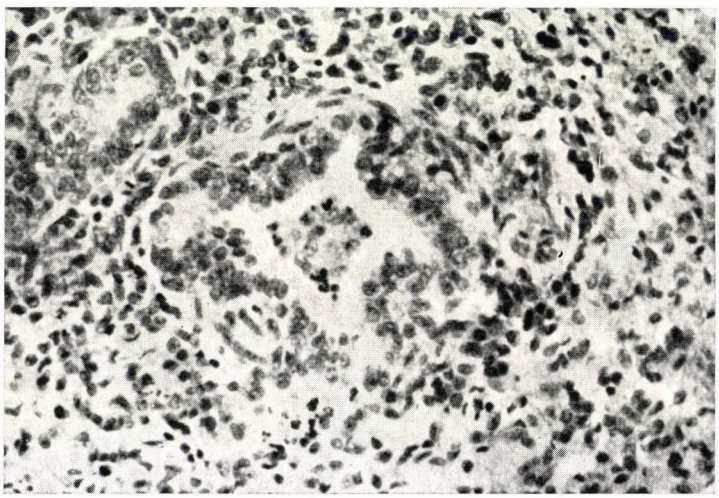

Fig. 4. Epithelial proliferation in small bronchi, approx. × 275

stitium and in a limited number of alveoli and bronchi. Epithelial cell proliferation was present in small bronchi (Fig. 4). Hyperplasia of the peribronchial lymph follicles was also characteristic (Fig. 5).

In further 4 lungs, besides these proliferative changes, a marked neutrophil infiltration was observed in the interstitium and neutrophils and desquamated epithelial cells were frequently present in the alveoli and bronchi. In the peribronchial follicles the hyperplasia was stronger (Fig. 6) than usual with such forms of catarrhal pneumonia.

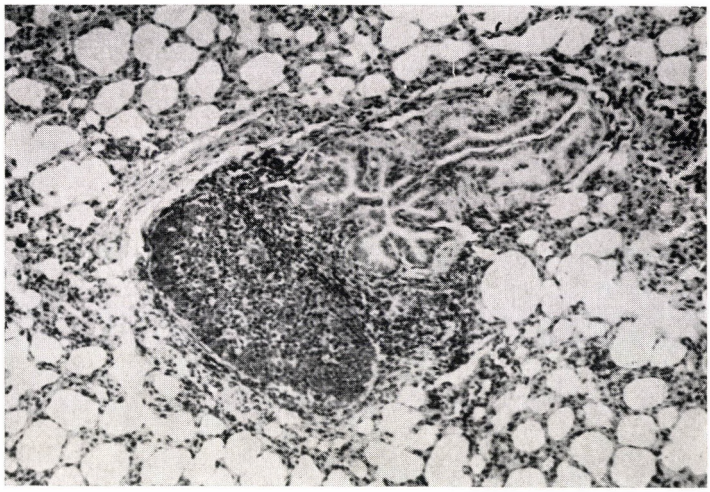

Fig. 5. Hyperplasia of peribronchial lymph follicles in the area of interstitial pneumonia' approx. × 110

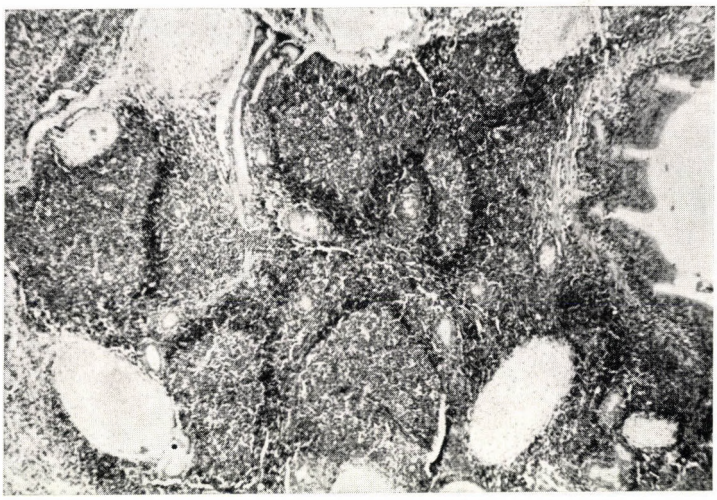

Fig. 6. Severe hyperplastic changes of peribronchial lymph follices, approx. × 45

In the two remaining lungs a severe lobular bronchopneumonia was observed, covering the less dominant proliferative processes.

Bacteriological and virological findings. Pasteurella haemolytica was cultivated from 4 lungs. No viruses could be isolated from the homogenized lungs, even after three successive blind passages in ovine fetal lamb kidney cultures.

Mycoplasma isolation. Of 10 lung samples 7 contained mycoplasmas. All the isolates were penicillin-resistant, had pleomorphous colonies and forms,

they were filterable through the 0.45 μm filter, and did not show reversion. According to cholesterol requirement, digitonin and polyanethol sulphonate sensitivity and inability to grow at room temperature, all the isolates belonged to the family of *Mycoplasmataceae*. All strains grew on Gourlay—Leach's medium.

Two of the 7 strains (Nos 679, 690) grew also on medium B. The remaining 5 strains (Nos 677, 678, 680, 689, 684) splitted glucose, but did not split arginine, urea, aesculin and arbutin and did not form phosphatase and "film an spot". These strains reacted in the growth-inhibition test only with the antiserum *M. ovipneumoniae*. Strains No. 679 and 690 were negative for glucose, arginine, urea, aesculin and arbutin but they produced phosphatase and intensive film and spot. They did not react with any of the sera. Their identification is in progress.

Serology. Althogether 35 sera were examined by the metabolism-inhibition test. The titres varied between 0 and 320.

Discussion

COTTEW (1971) compared mycoplasma strains isolated from diseased sheep with those isolated from the nasal cavities of healthy sheep or goats. CARMICHAEL et al. (1972) suggested that the isolates had been the causative agents of the chronic interstitial pneumonia. SULLIVAN et al. (1973) reported on the natural history of a proliferative interstitial pneumonia and suggested that the disease had been associated with mycoplasmas. Following intravenous or aerosol application of mycoplasma cultures, the latter team was able to produce proliferative pneumonia in experimentally infected lambs.

The mycoplasma strains reported in this article were isolated from the lungs of lambs showing bronchopneumonia of various severity and, also, chronic intralobular interstitial pneumonia. According to their biochemical and serological behaviour, five of the strains proved to be *M. ovipneumoniae*, two further isolates could not be identified yet.

Three months earlier an epidemic of acute pneumoenteritis occurred among the same lambs. In the aetiology of pneumoenteritis the causative role of adenoviruses was proved (BELÁK and PÁLFI, 1974c). After the acute respiratory and enteric disease had ceased, many lambs showed persisting chronic respiratory symptoms. In these cases interstitial proliferative changes in the lungs, desquamation of alveolar epithelial cells, hyperplasia of the peribronchial follicles and an epithelium proliferation in the bronchi were the most important histopathological findings besides the lesions characteristic of catarrhal pneumonia, which was often present. These were similar to those reported by SULLIVAN et al. (1973) in association with natural and experimental mycoplasma infection of sheep.

According to recent studies, several viruses, like reoviruses (BELÁK and PÁLFI, 1974a, b) and adenoviruses (BELÁK and PÁLFI, 1974c) can play a primary role in acute respiratory diseases of lambs kept intensively. Earlier, the aetiological importance of parainfluenza-3 viruses was also reported, firstly in the U. K. (HORE, 1966). These viruses were isolated from sheep showing respiratory symptoms in Hungary, too (BELÁK and PÁLFI, 1974d).

During the acute phase of respiratory disease the isolation of mycoplasmas has not been attempted yet. Further work is required to investigate the pathogenic role of the mycoplasma isolates reported here, either alone or in combined infections with the above viruses. These investigations will show whether a viral background is needed for successful mycoplasma infection.

Summary

Mycoplasma strains were isolated from sheep showing chronic respiratory symptoms. This condition occurred after an epidemic of acute adenovirus pneumoenteritis of lambs. In the lungs interstitial proliferative changes, desquamation of alveolar epithelial cells, proliferation of the bronchial epithelium and hyperplasia of the peribronchial follicles were found histopathologically besides the lesions of catarrhal pneumonia. The mycoplasmas isolated from the homogenized lung tissues were characterized according to their biochemical and serological behaviour. Five strains proved to belong to *Mycoplasma ovipneumoniae*, two further isolates could not be identified yet. The aetiological role of the isolates needs further investigations either alone, or in combination with viruses causing respiratory diseases of sheep.

References

ARISOY, F., ERDAG, O., COTTEW, G. S. and WATSON, W. A.: Türk. vet. Hekim, Dern. Derg. **37** (1967), 11. BELÁK, S. and PÁLFI, V.: Arch. ges. Virusforsch. **44** (1974a), 177. IDEM: Acta vet. hung. **XXIV** (1974b), 241. IDEM: Ibidem **XXIV** (1974c), 327. IDEM: Ibidem **XXIV** (1974d), 281. BRIDRE, J. and DONATIEN, A.: C. R. Acad. Sci. (Paris) **177** (1923) 841. CARMICHAEL, L. E., ST. GEORGE, T. D., SULLIVAN, N. D. and HORSFALL, N.: Cornell Vet. **62** (1972), 654. COTTEW, G. S.: Austr. Vet. J. **47** (1971), 591. ERNØ, H. and STIPKOVITS, L.: Acta Vet. Scand. **14** (1973), 450. GOURLAY, R. N. and LEACH, R. H.: J. Med. Microbiol. **3** (1970), 111. HORE, D. E.: Vet. Rec. **79** (1966), 466. St. GEORGE, T. D.: Austr. Vet. J. **45** (1969), 321. STIPKOVITS, L. and VARGA, L.: Acta vet. hung. **XXIV** (1974), 139. SULLIVAN, N. D., ST. GEORGE, T. D. and HORSFALL, N.: Austr. Vet. J. **49** (1973), 57. Subcommittee on the Taxonomy of *Mycoplasmatales*. Int. J. Syst. Bact. **22** (1972), 184.

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FOOD PREFERENCE AND CONSUMPTION BY MURINE RODENTS

By

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Owing to their intimate association with man and useful animals, rodents of subfamily *Murinae* are incriminated not only in the dissemination of serious zoonoses but also in tremendous economic losses. Hence, several workers have thoroughly studied these pests to elaborate proper scientific control. Food preference and food consumption by different species are points of interest that have not been sufficiently studied, particularly on wild catches in Egypt. The present work is a contribution to this subject.

Materials and methods

Thirteen types of food representing field and stored products as well as prepared animal provender were tested for food preference and consumption. Field collections of every species under study were kept in the laboratory for a few days, then 20 healthy individuals of each species, comprising equal numbers of mature and immature animals of both sexes, were tested.

Each animal was kept in a separate cage provided with drinking water *ad libitum*. Known amounts of three different types of food were offered for 10 days. The average consumption for each type of food per kg body weight was then estimated. Thenceforward other types of food were tested in the same way, and the most preferable types were tested three by three against each other. With this method of screening the most preferable types of tried food, for the corresponding species, stage and sex were determined.

Results and Discussion

As shown in Table I, the food preferred to all other foods was bruised maize for *Rattus norvegicus* and *Acomys cahirinus*, bruised sorghum for *Arvicanthis niloticus*, sound maize for *Rattus r. alexandrinus* and broad beans for *R. r. frugivorus*. Generally speaking, bruised maize and sorghum were pre-

Table I

Food preference in g/kg b.w./day for murine rodents

Group No.	Type of food	*Rattus norvegicus*	*Rattus r. alexandrinus*	*Rattus r. frugivorus*	*Acomys cahirinus*	*Arvicanthis niloticus*
1	Sound maize, sorghum and beans	95.0, 66.4	152.2, 81.5	79.0, 56.5	35.5, 39.8	98.0, 61.5
		35.3	18.2	23.4	65.0	25.5
2	Ears of barley, corn, broad-beans	63.3, 72.1	64.0, 58.3	110.8, 103.6	6.8, 5.6	93.4, 91.0
		93.0	90.8	112.6	15.4	118.6
3	Bruised maize, sorghum and beans	104.9, 103.3	103.8, 119.8	82.2, 100.1	54.7, 54.1	105.1, 108.8
		34.8	14.2	11.7	9.8	37.8
4	Sound wheat, barley and provender	46.4, 55.3	55.0, 67.8	41.8, 48.6	11.8, 19.6	43.0, 51.3
		35.5	26.8	66.8	7.7	23.3
5	Sound maize, broad-beans and	99.7, 85.1	147.0, 81.6	68.3, 125.1		80.6, 113.2
	brouised maize or	119.5				
	Sound sorghum or		121.2			
	Bruised sorghum or			90.2		126.3
	Sound maize, broad-beans and				28.1, 18.3	
	bruised maize				65.9	
6	Sound barley, bruised maize	47.6, 133.2			19.7, 63.0	
	and bruised provender or	11.3			8.1	
	Sound maize, barley and		173.1, 56.5			
	bruised provender or		14.2			
	Sound provender, broad beans			59.0, 124.9		
	and bruised provender or			9.0		
	Bruised sorghum, barley and					131.0, 49.2
	bruised provender					9.8
	F value	35.71	17.57	27.0	91.72	92.8

Table II

Average amount of preferred food consumption according to maturity and sex of murine rats

Rodent species	Preferable food	g/kg b.w./day	Statistical group
R. norvegicus	Bruised maize	143.4, 135.5	(1) Mature males and females
		75.4, 56.3	(2) Immature males and females
			F value = 35.42
			L.S.D. 0.01 = 26.49
			L.S.D. 0.05 = 19.61
R. r. alexandrinus	Sound maize	203.1	(1) Mature males
		175.3	(2) Mature females
		92.9, 107.0	(3) Immature males and females
			F value = 35.42
			L.S.D. 0.01 = 26.49
			L.S.D. 0.05 = 19.61
R. r. frugivorus	Broad beans	160.9, 142.9	(1) Mature males and females
		78.7, 68.9	(2) Immature males and females
			F value = 23.95
			L.S.D. 0.01 = 36.72
			L.S.D. 0.05 = 27.19
Ar. niloticus	Bruised sorghum	161.1, 141.0	(1) Mature males and females
		74.9, 66.4	(2) Immature males and females
			F value = 46.93
			L.S.D. 0.01 = 27.03
			L.S.D. 0.05 = 20.02

ferred to many other foods by all species. The sound form of these grains seemed to be highly palatable for *R. r. alexandrinus* while other species seemed to prefer sound maize. This indicates that stored grains in both sound and bruised forms may be effectively consumed by all species of murine pests.

The grass rat, *Arvicanthis niloticus* and the palm rat, *R. r. frugivorus* showed more preference to ears of barley and to corn and broad beans than other species did. Hence, the importance of these two species in the destruction of unprecessed field crops ought to be considered. The nesting habit of the grass rat in the fields and the commensal nature of the palm rat, which nests mostly on trees outside houses (SETZER, 1963; ARAFA, 1968 and SALIT

et al., 1975d, e), appear to affect their feeding habits. The loss caused by the palm rat may extend, in addition to field crops, to sound provender (Table I).

As regards food consumption (Table II), it appears that the mature stage of murine rats of both sexes required larger amounts of the preferred food than the corresponding immature stage. This is probably due to the need of necessary proteins in grains essential for developing immature rats. Higher protein requirement may be an explanation to the higher consumption by adult males than by females, particularly in the case of *R. r. alexandrinus.*

Results summarized in Tables I and II indicate that *R. r. alexandrinus* consumed the highest amount of food /kg b.w./ day when offered its most preferable diet. It was followed by *Ar. niloticus* and *R. r. frugivorus,* then *R. norvegicus* and, finally, *A. cahirinus.* Table II shows that a mature rat may consume an amount of the preferable food equal to its body weight within less than 10 days, in addition to contamination due to its droppings, urine, hair and smearing. The mature spiny mouse (Table I) needs about 16 days to consume an amount of food equal to its body weight.

Summary

Thirteen types of food representing field-stored products and prepared animal provender were tested for food preference and consumption by murine rodents. Stored grains in both sound and bruised forms are effectively consumed by all species. The importance of the field rat *Arvicanthis niloticus* and the white-bellied rat *R. rattus frugivorus* in the destruction of unprocessed field crops compared with other species is evident. Species of the rodent and its nesting habits play a role in food preference. Food consumption depends on species, maturity and sex. A mature rat may consume an amount of pereferable food equal to its body weight in less than 10 days, while the mouse in about 16 days.

References

ARAFA, M. S.: Studies on ecto and endo parasites infesting rodents in Egypt. Thesis. Cairo, 1968. SALIT, A. M.: Acta Zool. Hung., under publication (1975a, b). SETZER, H. W.: J. Egypt. Pub. Health Assoc. **38** (1963), 51

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BIRTHDAY TRIBUTE TO JÁNOS MÓCSY

János Mócsy, Ordinary Member of the Hungarian Academy of Sciences, completed the 80th year of his creative life on the 30th November, 1975. Although the entire body of Hungarian veterinarians and representatives of veterinarianism from many foreign countries were preparing for the celebration, the Great Old Man of Veterinary Science refused publicity as always, when he could help, and remembered his active years in a little circle of his former pupils and closest coworkers, who assembled to greet him at the University of Veterinary Science in Budapest, between the walls which had witnessed his outstanding teaching and research activities over the long period of four decades. In the warm familial atmosphere of the meeting, Dr. Ferenc Kovács,

professor of Animal Hygiene and Rector of the University, uttered the words of gratitude on behalf of the University and the Hungarian veterinarians for the many outstanding contributions of János Mócsy to world veterinary science in general and Hungarian veterinary science in particular. Now Acta Veterinaria is conveying its birthday greetings to the world-famous scientist, on behalf of its many readers both home and abroad.

Just a list of Professor Mócsy's contributions to veterinary science would demand much more space than is available for this note, not to mention the benefits of his teaching system and personal inspiration. Yet we try to survey at least his greatest achievements on the occasion of this anniversary.

As a scientist, Mócsy has great merits in the development of the so-called "cilinical veterinary medicine". The conception had been initiated by J. Azary at the turn of this century and was evolving as a school of thought in the hands of J. Marek and his group. Mócsy's creative participation in this school resulted in a system of principles, referred to as the "Marek—Mócsy school", in this part of the world. The basic attitude characterizing this school is to push improvement by systematic research, always with an eye to interrelationships. This outlook enabled Mócsy to improve the methods of veterinary clinical examination and to pursue the clinical aspects of veterinary parasitology and toxicology. In Hungary Mócsy pioneered investigations into the trace element supply of domestic animals as well as into the impact of hygienic conditions on animal health. He was aware of the new health hazards threatening the animals in large herds, and he initiated animal-hygienic research in his institute at a time when the establishment of large animal production units had been in its initial stage and most animal scientists had only a vague idea of the risks to be expected. Thus Mócsy is one of the founders of animal hygiene, which has in the meantime evolved as a new, very important branch of veterinary science. It is our pride that Mócsy's school has many followers not only in Hungary, but also abroad. His textbook, which summarizes fundamental knowledge in the fields of veterinary medicine, veterinary clinical diagnostics and animal hygiene, has been translated into nine foreign languages, including three world languages, thus introducing the outlook of the Marek—Mócsy school to all parts of the world.

Mócsy's scientific activities have not been restricted to his chosen field. His interest in science policy has been indefatigable during the 35 years of his membership in the highest scientific body of Hungary. Hungarian science in general, and agricultural sciences in particular, have benefited from his organizer and coordinator activities. He held the post of Secretary in the Department of Agricultural Sciences, Hungarian Academy of Sciences, for a period of 6 years.

As a teacher he distinguished himself not only by profound knowledge of his field and outstanding cultural elaboration, but also by his extraordinary

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РЕЗЮМЕ

ЗНАЧЕНИЕ МЕХАНИЗИРОВАННОГО ВСТРЯХИВАНИЯ ОСНОВНОГО РАЗБАВЛЕНИЯ ОБРАЗЦОВ В ОЦЕНКЕ МИКРОБИОЛОГИЧЕСКОГО КАЧЕСТВА КОРМОВ

Л. ЭТТЕР и И. НИРЕДИ

Обнаружено, что механизированным встряхиванием в течение 5—10 минут образца в разбавителе до микробиологической оценки повышается количество выявимых микробов в нем на 1 экспонент (крайние показатели: 1,7—48 кратные). Состав и соотношение компонентов бактериальной флоры от встряхивания не изменились.

Повышение количества грибков после встряхивания было таким же, но с большим диапазоном колебания (1,3—5000 кратный подъем). Очень большое повышение количества плесневых грибков наступило в образцах с низким количеством плесней. От встряхивания очень часто изменился и состав плесневой флоры. Причина этого, согласно авторам, заключается в необходимости применения очень высоких разбавлений, обеспечивающих определение очень больших количеств плесневых грибков. Данные, полученные после встряхивания, не повышают надежность микробиологической оценки, поэтому авторы не рекомендуют применять встряхивание при микробиологической оценке кормов.

СУДЬБА СПОР ПЛЕСНЕВЫХ ГРИБКОВ В ЖЕЛУДОЧНО-КИШЕЧНОМ ТРАКТЕ МОЛОДЫХ ЦЫПЛЯТ

И. НИРЕДИ, Л. ЭТТЕР, И. ФЕШЮШ и Г. МАЙЕР

Ради решения того вопроса, могут ли попавшие через рот в организм конидии и эндоспоры плесневых грибков вызывать микоз или микотоксикоз, авторы вводили в зоб дневных цыплят споры разных видов плесневых грибков. В эксперименте использовано 7 видов, дружно развивающихся и при температуре живого организма и 3 вида, не развивающихся при такой температуре. В течение 10 дней каждому цыпленку вводили по 500 000-1 000 000 конидий и после 4-хдневного перерыва в течение новых 10 дней по 5—40 миллионов конидий в виде взвеси на физрастворе поваренной соли. Все животные, контрольные включительно, за время наблюдения были здоровыми и развивались безупречно. При вскрытии животных изменений, указывающих на микоз или микотоксикоз, не обнаружено. Некоторые среди использованных в эксперименте видов удалось в течение 3-х, исключительно 10 дней из желудочно-кишечного тракта (главным образом из зоба) реизолировать. Во внутренних органах и крови грибки не обнаружены. На основании своих экспериментов авторы приходят к заключению, согласно которому попавшие в организм через рот конидии не вызывают ни микоз, ни микотоксикоз.

ИЗУЧЕНИЕ ВЗАИМОСВЯЗИ МЕЖДУ ОБНАРУЖИВАЕМОСТЬЮ АНТИГЕНОВ ГРУПП КРОВИ КУР И БОЛЕЗНЬЮ МАРЕКА В ПРИВИТЫХ И НЕПРИВИТЫХ ВАКЦИНОЙ МАРЕКА ПОГОЛОВЬЯХ

М. ПАПП, В. П. ЮХАС и Л. ПАПОЧИ

Статистически проанализированы патологоанатомические данные и таковые групп крови у 1193 кур, вакцинированных против болезни Марека и 1167 непривитых цыплят. Целью анализа явилась проверка резистентности животных к болезни Марека. Обнаружена взаимосвязь между наличием двух антигенов групп крови (B_4 и B_{10}) и резистентностью к болезни Марека. Эта взаимосвязь была статистически достоверной только в отношении антигена B_4, поскольку количество особей B_4 было статистически достаточно высоким. Но благоприятный эффект редкой частоты антигена B_{10} указывает на тот факт, что среди особей B_{10} изменения, характерные для болезни Марека, наблюдались особенно редко. Объяснением указанной взаимосвязи может быть наличие плейотропии или геновой спайки.

ИЗУЧЕНИЕ ФУНКЦИИ ЩИТОВИДНОЙ ЖЕЛЕЗЫ IN VIVO НА БУРЗЭКТОМИЗИРОВАННЫХ ПЕТУШКАХ

ДЬ. ПЕТЕШ и А. ФОДОР

Изучен эффект хирургической бурзэктомии на некоторые параметры функции щитовидной железы бройлерных петушков.

Обнаружено, что доза в 2 μг/100 г живого веса экзогенного трийодотиронина полностью тормозила секрецию щитовидной железы бурзэктомизированных птиц, тогда как доза в 5 μг/100 г живого веса гормона не тормозила функцию контрольных животных, если они содержались на метилмазолевой диете. Сравнением in vitro методов определения темпа выделения тироидного гормона выявлено, что наиболее надежным является так наз. метод прямого выделения Синг-Райнеке. Сравнение дало возможное объяснение правильности данных темпа выделения тироидного гормона, полученных методом Танабе и Комияма (1962).

IN VITRO ЭКСПЕРИМЕНТЫ С ЩИТОВИДНОЙ ЖЕЛЕЗОЙ КУРЫ

А. ФОДОР, ДЬ. ПЕТЕШ и Т. МУРАНИ

В поисках за надежными параметрами биотитрирования тиротропина в in vitro системе с курой как экспериментальным животным обнаружено, что принципы метода Чун и Огура (1960) являются применимыми для этого вида. Активность I^{131}, связанного с протеином, в инкубированных срезах щитовидной железы, заранее обработанных I^{131} птиц, является, согласно авторам, надежным in vitro показателем эффекта тиротропина.

ЭКСПЕРИМЕНТЫ ПО ПРОФИЛАКТИКЕ КОЛИБАЦИЛЛЕЗНОЙ ДИЗЕНТЕРИИ ТЕЛЯТ ПУТЕМ ВАКЦИНАЦИИ

I. Употребление алюминиевой жели в качестве вяжущего вещества

Я. ВАРГА и А. Ф. ФАРИД

После предварительных серологических опытов проводилась экспериментальная вакцинация моно- и тривалентными вакцинами на алюминиевой жели против колибациллезной дизентерии телят.

Патогенные для подсосных телят колибациллезные штаммы представляют собой только 8—10 серологических групп. Большинство штаммов содержит мукоидный К(А) антиген и многие среди них полирезистентны к антибиотикам.

Специфические К антитела против вакцинных штаммов появлялись в крови и молозиве коров, дважды вакцинированных в течение последнего месяца беременности. Телята приобретают иммунитет благодаря наличию антител К в молозиве. Условием надежной вакцинации является точное знание серотипов E. coli, вызывающих заболевание в поголовье телят. При соблюдении гигиенических условий вакцинацией коров по определенной программе можно профилактировать колибациллезную дизентерию подсосных телят.

ИЗУЧЕНИЕ КОЛИБАЦИЛЛЕЗНЫХ ШТАММОВ, ИЗОЛИРОВАННЫХ ИЗ ПАВШИХ ОТ МУКОИДНОГО ЭНТЕРИТА КРОЛИКОВ

Е. ЦИРОК и Ф. ВЕТЕШИ

Серологически изучены 65 колибациллезных штаммов, изолированных из кишечника 6 подсосных крольчат и 52 голов молодняка, павших от колибациллезной энтеротоксемии. Целью изучения явилось выявление того, не формируются ли среди них обычные или менее распространенные энтеропатогенные для человека штаммы.

Среди 65 колибациллезных штаммов 13 (20%) принадлежало к некоторой серогруппе, искаемой авторами, показанной в таблицах 2 и 3. Согласно данным таблицы 4 два штамма среди тринадцати принадлежали в группу E. coli—dyspepsiae (086 и 0125), остальные же 11 в разные патогенные для человека серогруппы.

При изучении чувствительности этих штаммов к антибиотикам бросалось в глаза, что среди изученных штаммов только 35% были чувствительны к тетрациклину.

В испражнениях 17,2% ухаживающего за кроликами персонала обнаружены колибациллезные штаммы, вызвавшие энтеротоксемию у приделенных к ним кроликов.

ЭКСПЕРИМЕНТАЛЬНОЕ ЗАРАЖЕНИЕ ЯГНЯТ ИЗОЛИРОВАННЫМ ИЗ ОВЕЦ ВИРУСОМ, БЛИЗКО РОДСТВЕННЫМ БЫЧЬЕМУ АДЕНОВИРУСУ ТИПА 2. II. ПАТОЛОГОАНАТОМИЧЕСКИЕ И ПАТОЛОГОГИСТОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ

Э. ТУРИ, Ш. БЕЛАК и В. ПАЛФИ

Проводились эксперименты по заражению одним аденовирусным штаммом (Het/3), изолированным из овец, показывающих симптомы заболевания дыхательных органов. Изолят, близкий к бычьему аденовирусу тип 2, вводился в носовую полость и трахею семи ягнят, не получавших молозиво и два ягненка заражались путем контакта.

У заражавшихся животных развились четко очерченные изменения эпителиальных клеток и воспаление слизистой носовой полости, эксудативный бронхиолит и сопровождающая его ателектазия в легких, ограниченная интерстициальная пневмония крупных участков легких, незначительная пролиферация бронхиолярного эпителия. Незначительные поражения стенки сосудов наблюдались в почечных гломерулах, отмечался тубулярный нефроз различной тяжести и местами пролиферация тубулярного эпителия. В мозгу характерных изменений не обнаружено. Характерные внутриядерные включения имелись в эпительных клетках бронхиол, альвеол, слизистой носовой полости и ретикулярных клеток лимфатических узлов. Включениям подобные структуры наблюдались в ядрах некоторых эпителиальных клеток почечных канальцев. Патологоанатомические и патогистологические исследования подтвердили данные клинических исследований, согласно которым вирусный штамм Het/3 патогенен для овец.

ИЗУЧЕНИЕ ДИАГНОСТИЧЕСКОГО ЗНАЧЕНИЯ КЛЕТОЧНЫХ ВКЛЮЧЕНИЙ ПРИ ЧУМЕ СОБАК

М. ДОБОШ-КОВАЧ

При помощи светового микроскопа изучены частота обнаруживаемости, морфология и диагностическое значение включений при чуме собак. Изучены органы 30 павших и 23 убитых на разной фазе болезненного процесса собак. Гистологические препараты фиксировались формалином, заливались в парафин и окрашивались гематоксилин-эозином. В раз-

ных органах всех 53-х животных с неодинаковой плотностью обнаружены включения. Включения регистрировались в мозгу (в цитоплазме, часто и в ядре нервных и разных глиозных клеток) и эпителиальных и железистых клетках разных органов (главным образом в цитоплазме, реже в ядре).

Согласно данным гистологических исследований в уротелии, выстилающем мочевой пузырь, в 73%-ах случаев имелись включения; если наряду с мочевым пузырьем изучались и легкие (эпителий бронхов и альвеолей), то включения имелись в 95%-ах животных; а если кроме этих органов изучению подвергались и желудок, третье веко и головной мозг (мозжечек и Аммоновы рога), то включения обнаруживались во всех трупах. На основании наблюдений рекомендуется при диагнозе чумы собак готовить гистологические срезы из упомянутых 6 мест. В случае мацерации мочевого пузыря целесообразно изучение эпителиальных клеток почечной лоханки.

Проведены и ориентировочные электронномикроскопические исследования, на основании чего дается описание ультраструктуры цитоплазматических включений в эпителиальных клетках мочевого пузыря. Для них характерно, что они состоят из сплетений нуклеокапсидов промера в 18 наномикронов.

ЭКСПЕРИМЕНТЫ ПО ПРОФИЛАКТИКЕ КОЛИБАЦИЛЛЕЗНОГО ЭНТЕРИТА ПОРОСЯТ ОТЪЕМЫШЕЙ ПУТЕМ ВАКЦИНАЦИИ

I. Употребление алюминиевой жели в качестве вяжущего вещества

Я. ВАРГА и А. Ф. ФАРИД

После предварительных бактериологических и серологических исследований проведена профилактическая вакцинация против колибациллезного поноса поросят-отъемышей вакциной, содержащей колибациллезные штаммы 041 : К/А, 0138 : К81, 88ab, 0141 :К85 и 0147 : К89, 88ac, адсорбированные к алюминиевой жели.

От двухкратной вакцинации до отъема наступило образование специфических в отношении вакцинных штаммов О и К антител. Но антитела формировались только к антигенам К88 и в образцах сыворотки не обнаруживались антитела ни К(В), ни К(А). В экспериментальных и контрольных группах гибель равнялась 3,6 и 6,7%-ам, соответственно.

Поскольку вакцина стимулирует образование прочного иммунитета только к антигену К88, она защищает только от штаммов, содержащих этот компонент. Таким образом ее не следует применять в настоящей форме в условиях практики.

ОБЛУЧЕНИЕМ ОБУСЛОВЛЕННЫЕ ИЗМЕНЕНИЯ ФАБРИЦИЕВОЙ СУМКИ И ЗОБНОЙ ЖЕЛЕЗЫ ВО ВРЕМЯ ОНТОГЕНЕЗА ЦЫПЛЯТ

Р. ДЖУРГЕА, С. МАНЧУЛЕА и И. ИЙЕШ

Рентгеновское облучение вызывает возрасту соответствующие модификации Фабрициевой сумки и зобной железы. При облучении в возрасте 3 дней нет признаков реакции инволюции, тогда как облучение в 3-хнедельном возрасте уже после первых нескольких часов сопровождается признаком инволюции. От применяемой авторами дозы (100 R) наступили обратимые изменения как в фабрициевой сумке, так и зобной железе. Изменения в зобной железе — в силу ее большей чувствительности к облучению — были более выраженными, чем в фабрициевой сумке.

НАБЛЮДЕНИЯ ПРИ ВСПЫШКЕ МАСТИТОВ СРЕДИ СВИНОМАТОК, ВЫЗВАННЫХ КЛЕБСИЕЛЛАМИ

С. Х. ДОН

Описывается течение и клиника вспышки маститов свиноматок после опороса, вызванной клебсиеллами. Анализируется экономический эффект эндемии.

ОБНАРУЖЕНИЕ MYCOPLASMA MELEAGRIDIS В ИНДЕЙКАХ ВЕНГРИИ

Л. ШТИПКОВИЧ, А. А. ЭЛ-ЭБЕДИ и Л. ВАРГА

Изолировались штаммы Mycoplasma meleagridis в двух индеечных поголовьях, благополучных по Mycoplasma gallisepticum; в сыворотке животных отмечены антитела против возбудителя. M. meleagridis оказалась менее чувствительной к галлимицину (эритромицин), чем M. gallisepticum. Патогенный характер M. meleagridis доказан путем заражения индюшечного эмбриона и 3-хдневных индюшат. У зараженных животных развилось воспаление воздухоносных мешков и перитонит; в их сыворотке появились антитела против возбудителя.

ОПРЕДЕЛЕНИЕ ТОКСИНА F-2 (ЗЕАРАЛЕНОНА) ПУТЕМ ЖИДКОСТНОЙ ВЫСОКОГО ДАВЛЕНИЯ, ГАЗОВОЙ И ТОНКОСЛОЙНОЙ ХРОМАТОГРАФИИ

Ф. КОВАЧ, Ч. САТМАРИ и М. ПАЛЮШИК

Изучались способы экстракции токсина резорбцил-ацид-лактона (F-2, зеараленона) с особым вниманием на конечный продукт сырого экстракта.

Эксперименты по тонкослойной хроматографии показали, что количественное определение фракций возможно на основании оценки цветных реакций при помощи фотоденситометрии.

Количественное определение путем газовой хроматографии осуществлялось ради оценки чувствительности этого метода.

Метод выявления токсина путем жидкостной хроматографии при высоком давлении, разработанный на кафедре зоогигиены Университета ветеринарных наук обладает тем большим преимуществом, что растворенный токсин можно использовать без предварительной манипуляции изучаемого материала и хроматографические показатели удобны для количественной оценки.

ЭКСПЕРИМЕНТАЛЬНОЕ ИНВАЗИРОВАНИЕ ЦЫПЛЯТ, УТЯТ И ГУСЯТ ИНВАЗИОННЫМИ ЛИЧИНКАМИ AMIDOSTOMUM ANSERIS (ZEDER, 1800)

Д. В. ФУК и И. ВАРГА

В лабораторных экспериментах проведено сравнительное изучение развития и патологического эффекта Amidostomum anseris (Zeder, 1800) в цыплятах, утятах и гусятах.

Искусственной инвазией дневных цыплят не удалось вызвать видимых патологических изменений в их мышечном желудке. В этом ненормальном хозяине личинки не дорастали до половой зрелости, но отсталые в развитии нематоды обнаруживались вплоть до 20-ого дня инвазии.

В сравнении с тяжелыми постинвазионными изменениями в гусятах, обусловившими гибель многих животных, в утятах имелись только незначительные патоанатомические изменения. В утятах, инвазированных в возрасте 1—18 дней, выжила до половой зрелости только незначительная часть инвазированных личинок. Торможение развития нематоды в утятах проявилось в слабости инвазии и в меньших размерах нематоды, в задержке развития до половой зрелости, в краткой выживаемости половозрелых нематод и в наличии в половозрелых популяциях незрелых самок. С возрастом утят торможение развития нематоды значительно усилилось.

Личинки A. anseris от утят, хранившиеся 5,5 месяцев при температуре 4 °C, оказались инвазионными как для гусят, так и для утят.

На основании своих экспериментов авторы приходят к заключению, согласно которому утка в молодом возрасте успешно инвазируется инвазионными личинками A. anseris, но инвазия не сопровождается тяжелыми патологоанатомическими изменениями. Этот вид хозяина может играть некоторую роль в распространении инвазии среди сильно восприимчивых хозяев — гусей.

ОБНАРУЖЕНИЕ ПРЕГНАНДИОЛОВ В МОЧЕ ХОЛОСТЫХ КОРОВ ПУТЕМ ГАЗОВОЙ ХРОМАТОГРАФИИ

П. ФЕХЕР, Ф. ОРОС, Л. БОДРОГИ и Я. ХАРАСТИ

В предыдущей работе доложено (Fehér и сотр., 1970) о выделении мочей холостых коров 5 β-прегнан-3α, 20α-диола и других эпимерных прегнандиолов. Данные настоящей работы, полученные при помощи газовой хроматографии, являются новым подтверждением выделения мочей холостых коров 5β-прегнан-3α, 20α-диола, 5α-прегнан-3α, 20α-диола и 5β-прегнан-3α, 20β-диола.

СТРУКТУРАЛЬНЫЕ УРОДСТВА СРЕДНЕЙ ЧАСТИ ХВОСТИКА СПЕРМИЯ СЕЛЕЗНЯ

М. МАРЕТТА

Описываются разные структуральные изменения митохондриальной оболочки спермия. Причиной изменений является расстройство в расположении митохондриев, вызванное либо наличием их избытка или недостатка. Избыток митохондриев сопровождается изменением их формы. Менее частыми уродствами явились наличие митохондриальной оболочки вокруг головки спермия и изменений сустава головки и хвостика.

ОБНАРУЖЕНИЕ ЗАРАЖЕНИЯ ВИРУСОМ ХОНГ КОНГ (ИНФЛЮЭНЦА A/H_3N_2) В БУДАПЕШТСКОМ ЗООПАРКЕ

Й. РОМВАРИ и Я. ТАНИ

Гемагглютинацию тормозящие и вирус нейтрализирующие антитела к эпидемическому вирусу (вариант вируса Хонг Конг) обнаружены в 23%-ах образцов крови, полученных от диких птиц, собак и коз зоопарка в Будапеште. Из слизистой дыхательных путей серологически отрицательной перепели (Streptopelia decaocto) изолирован вирусный штамм, содержащий гемагглютинин, близкий вирусу Хонг Конг.

Этим вирусом интрафарингеально заражены куры. В них образовались антитела до титров 1 : 16—1 : 256, выявимость которых продолжалась 5—6 недель. Антитела в желтке появились позже, их выявимость была короче и их титр был ниже титра антител сыворотки. После внутримышечной реинфекции тем же штаммом последовало повторное появление антител, но уже в более высоком титре, сначала в сыворотке, а после — в желтке. Титры антител в желтке поднялись примерно до того же предела, как и в сыворотке. Высокий уровень титра антител в желтке сулит эффективным желточным иммунитетом птиц, привитых против инфлюэнцы.

Авторы приходят к заключению, согласно которому варианты вируса Хонг Конг при эпидемии инфлюэнцы среди людей могут переходить с животного на животное. Не исключена даже возможность заражения друг друга особей разных видов животных.

ЗАРАЖЕНИЕ СОБАК И КОШЕК ВИРУСОМ ИНФЛЮЭНЦЫ ХОНГ КОНГ A(H_3N_2) ВО ВРЕМЯ ОДНОЙ ЭПИДЕМИИ В ВЕНГРИИ

Й. РОМВАРИ, Й. РОЖА и Э. ФАРКАШ

Во время одной эпидемии, вызванной штаммом вируса инфлюэнцы А, близким вирусу England/42/72, щенки заразились естественным путем. Вирус реизолирован из выделений назо-фарингеальной слизистой двух больных собак; в крови же 6 щенков наблюдалось статистически достоверное повышение специфических антител. Гемагглютинацию тормозящие (ГТ) и вирус нейтрализирующие (ВН) антитела до титров 1 : 16—

1 : 526 наблюдались в 9 от 40 образцов сыворотки (22,5%), полученных после эпидемии из разных мест страны.

Местный иммунитет высшего участка дыхательных путей 16 дней после удачной инфекции собак репрезентативным штаммом эпидемии Hungary/1/73 доказан. Специфические ВН антитела выявлялись в течение не меньше 7 недель в молочной сыворотке суки, зараженной две недели после щенения.

Собаки и кошки легко заражались штаммом Hungary/1/73. У животных наблюдался некоторый подъем температуры и выделение вируса в течение 3—9 дней. Один штамм, изолированный из щенков, после заражения в нос кошачей самки, распространился в ее помете. Этот штамм не вызывал повышения температуры после заражения в носовую полость, но зато реизолировался из назофаринкса в течение 4—5 дней.

Авторы приходят к заключению, согласно которому собаки и кошки могут служить резервуаром для вируса Хонг Конг и его вариантов. В закрытом помещении носимый ими вирус может распростанится даже на животных другого вида.

ТЯЖЕЛЫЙ СИНУСИТ У УТЯТ, ВЫЗВАННЫЙ ВИРУСОМ ИНФЛЮЭНЦЫ А

Я. ТАНИ, Й. РОМВАРИ, Д. ДЕРЖИ и И. ШАРИ

На одной утиной ферме с апреля по октябрь 1974 г. проводилась инкубация 2 миллионов утиных яиц. Большинство утят продано в 1-дневном возрасте, меньшая часть — в 3-недельном возрасте. Двадцать тысяч утят утилизировано для племенной цели. В разных группах утят 15—50% животных был поражен одно- или двухсторонним синуситом. В течение 1—4 недель за исключением комплицированных случаев утята стали бессимптомными. В случае компликации синусит наблюдался до возраста 8 недель. В группах, содержавшихся на ферме до половозрелого возраста, гибель животных равнялась 4,8—22,5%-ам (в среднем 10,8%).

43 гемагглютинирующих вирусных штамма изолировано из дыхательных путей больных уток: большинство из выделений синуса. При изучении изолята в диффузии на двойном агарном желе патогенное начало оказалось вирусным штаммом А инфлюэнцы, но в реакции торможения гемагглютинации он не реагировал с антисыворотками с прототипными штаммами подтипов Hav 1—Hav 6.

Цыплята, 1-дневные утята и индюшата успешно заражены одним среди изолятов. У них образовалось фибринозное воспаление воздухоносных мешков; у взрослых петухов возникла эдема головы, продолжающаяся несколько дней. У искусственно зараженных птиц выработалась иммунная реакция, хорошо демонстрируемая после 10—12 дней.

Вирус изолировался из всех образцов серозно-мукозных выделений синусов, во многих случаях беспрерывно в течение 2—7 недель. Зато редко удавалось изолировать вирус из казеозно-фибринозных масс выделений. В сыворотках утят после естественного заражения титры торможения гемагглютинации достигали невысокого уровня. Из утят-носителей вирус удавалось изолировать, если гомологические антитела демонстрировались при разбавлении 1 : 20—1 : 40. Антитела из сыворотки исчезали к 5-й — 12-й неделе.

ИЗОЛИРОВАНИЕ MYCOPLASMA OVIPNEUMONIAE ИЗ ОВЕЦ С ПНЕВМОНИЕЙ

Л. ШТИПКОВИЧ, Ш. БЕЛАК, В. ПАЛФИ и Э. ТУРИ

Микоплазматические штаммы изолировались из овец, показывающих клинику хронического заболевания респираторных органов, наступившего после эпизоотии аденовирусного пневмоэнтерита среди ягнят. В легких кроме признаков катаральной пневмонии наблюдались пролиферативные изменения, десквамация альвеолярных эпителиальных клеток, пролиферация эпителия бронхиол и гиперплазия перибронхиальных фолликул. Изолированные из гомогенизированной легочной ткани микоплазматические штаммы определялись на основании их биохимических и серологических свойств. Пять штаммов оказались принадлежащими к Mycoplasma ovipneumoniae; принадлежность двух изолятов не удалось определить. Этиологическая роль изолятов требует дальнейшего изучения как их одних, так и в комбинации с вирусами, вызывающими заболеваемость респираторных органов.

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pedagogical abilities and informal contact with his pupils. His personal qualities, love to work, keen sense of duty, orderliness, and fascinating informality never failed to impress his pupils and coworkers as an example to be followed. His teaching was not only a source of profound professional knowledge, but also an education of character. The value of a school is always judged by the performance of the pupils. The activities of two generations of Hungarian veterinarians have attested to the far-reaching influence of János MÓCSY, the teacher.

Educational policy was also his great concern. For many years he held the post of Dean, first of the Veterinary Faculty, later of the independent University of Veterinary Science.

The outstanding merits of János MÓCSY won him also official recognition. The Hungarian Government awarded him several high orders, including the Golden Medal of Labour, the Kossuth Prize, the First-Degree National Prize and, on his 80th birthday, he was awarded the Order of the Banner of the Hungarian People's Republic, Second Degree. As a professional acknowledgement he holds the title of Honorary Doctor of the University of Veterinary Science in Budapest and of the Humboldt University in Berlin, the Hutyra Memorial Medal, and he was the first awardee of the Marek Memorial Medal, founded at the University of Veterinary Science in Budapest. Holder of so many awards of honour, he still may find greatest satisfaction in witnessing how the seeds of his teaching and work flourish into bloom in the professional activities of his pupils and continue to develop in the hands of a next generation of veterinarians.

János MÓCSY retired from his university chair as long as 15 years ago, but veterinary scientists and practitioners still profit from the treasury of his knowledge, because he is always ready for help and advice. As a birthday greeting, we wish that he may stay with us in good health for many years to come as our spiritual mentor in many professional problems awaiting resolution.

Acta Veterinaria Academiae Scientiarum Hungaricae, Tomus 25 (4), pp. 283—289 (1975)

USE OF D-CYCLOSERINE BITARTARATE FOR SELECTIVE ISOLATION OF CLOSTRIDIA

By

J. TAKÁCS and Emőke Z. IMREH

Central Laboratory, Veterinary Meat Control Service, Budapest

(Received May 22, 1973)

Inhibitory substances have been increasingly preferred in diagnostic and food control laboratories as components enhancing the selectivity of the media used for isolation of pathogenic and food-poisoning clostridia. However, the majority of the inhibitors fail to properly inhibit the propagation of the facultatively anaerobic bacteria when used in a low concentration, and inhibit the clostridia to be isolated when used at higher concentrations.

FÜZI and CSUKÁS (1968, 1969) employed blood agar plates containing 800 μg/ml D-cycloserine bitartarate to facilitate the isolation of *Clostridium perfringens* and other pathogenic clostridia. HARMON et al. (1971) used 400 μg/ml D-cycloserine in their TSC agar medium above all for *C. perfringens* isolation.

D-cycloserine bitartarate (Chinoin) was added to several media commonly used in Hungary for food microbiological studies, in order to assess their value for the isolation of *C. botulinum* and *C. perfringens* strains by comparative evaluation.

The composition of the media used in the above experiments is precisely described below, for reasons of experimental reproduction, whereas the test organisms are specified only in the Tables.

Materials and Methods

Preparation of media. The following broth was used as basal broth:

Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$)	2 g
NaCl	2 g
Glucose	1 g
Witte peptone	2 g
Richter peptone	2 g
Yeast extract (prepared according to EÖRSI, 1947)	5 ml
Meat extract	10 g
Distilled water	1000 ml
Autoclaved at 121 °C for 20 min	
Final pH 7.6—7.7	

Preparation of modified Holman's Cooked Meat Medium (CCM). Minced, boiled and subsequently dried horse meat was distributed in test tubes, 2 g $Na_2HPO_4 \cdot 12H_2O$, 2 g NaCl, 5 g yeast extract, 1 g glucose, 4 g peptone, 10 g meat extract and 1 g starch were added to 1000 ml distilled water, boiled thoroughly. Final pH 7.8—8.0. Filtered through filter paper. Fifteen ml amounts of the filtrate were distributed in the test tubes containing the dried horse meat. Autoclaved for 20 min at 121 °C. Stored at +4 °C until used.

Preparation of sulphite agar with egg-yolk-emulsion. One g glucose, 5 g peptone, 10 g meat extract, 20 ml yeast extract (prepared according to EÖRSI, 1947) and 20 g agar-agar were added to 1000 ml distilled water. The mixture was boiled. Final pH 7.2. Autoclaved for 20 min at 121 °C. To 446 ml medium cooled to 50 °C 6 ml each of 5% ferrum citricum oxydatum and 5% sodium sulphite solution (both G5-filtered) were added under sterile conditions. Finally 0,20 g D-cycloserine, dissolved in 10 ml sterile brotte at 50 °C temperature was added to the medium and 32 ml egg-yolk emulsion. The latter was prepared from two egg-yolks and 25 ml saline under sterile conditions. Twenty-five ml amounts of the finished medium were distributed in Petri dishes already containing the test material, and were allowed to solidify.

Preparation of blood agar with gelatine added. Fifteen g agar-agar, 5 g gelatine and 1 g glucose were added to 1000 ml basal broth. Final pH 7.6—7.7. Autoclaved for 20 min at 121 °C. The ingredients were dissolved in a water bath, and the medium was cooled to 45 °C; 20 ml sterile defibrinated blood was added to 400 ml medium.

Preparation of inocula. Spore suspension. Each test strain was inoculated into 150 ml Holman's CMM placed in an Erlenmeyer flask, and incubated for 20 days at 32 °C. The incubated cultures were filtered through sterile gauze and care was taken to squash the meat fragments retained on the gauze. The filtrate was centrifuged at 6000 r.p.m. for 30 min and the supernatant was decanted. The sedimented spores were washed in five changes of distilled water. Each lot was stored at +4 °C in rubber-stoppered vials, each containing 50 ml distilled water and glass beads.

The density of the spore suspensions was adjusted to that of an oversaturated barium chloride solution and the spore counts were determined by dilution technique, using CMM medium.

The CMM cultures were incubated for 7 to 35 days at 32 °C, subsequently exposed to 20 min heat shock by inoculation into media kept at 80 °C, and were finally chilled in cold tap water.

D-cycloserine, 800 or 600 μg/ml, was added to blood agar medium, and 600 μg/ml was added to modified sulphite agar medium containing egg-yolk emulsion. Media without D-cycloserine served as controls. Inoculation was made either directly from 48-hr CMM broth culture by streak technique, or dilutions of broth culture or of purified spore suspensions in 0.1% peptone-

containing saline were similarly applied. Three plates were inoculated by streak technique from each dilution, using a 0.1 ml inoculum per plate. On the media serial dilutions of 24-hr CMM cultures of *C. perfringens* were titrated.

The inoculated solid media were incubated for 48 hours at 37 °C, under anaerobic conditions according to KOCH (1934). The mean germ counts were assessed for each dilution from the colony counts on three replica plates.

Results

It was shown in preliminary experiments that 800 μg/ml D-cycloserine bitartarate did not influence the growth of most test strains (*C. novy* B 9691, *C. tetanomorphum* 288, *C. aerofoetidum* 505, *C. botulinum* A 2812, *C. bifermentans* 506 and *C. sporogenes* 533) on blood agar plates. However, in accordance with the findings of FÜZI and CSUKÁS (1969), the strain *C. histolyticum* 7123 failed to grow in the presence of D-cycloserine bitartarate, and the strain *C. botulinum* B 3807, although did grow, but lost its haemolytic activity. Unlike FÜZI and CSUKÁS (1968) we found that 800 μg/ml D-cycloserine had a marked inhibitory effect on the outgrowth of *C. perfringens* strains on blood agar plates. This contradiction is probably due to methodical reasons, *viz.*, FÜZI and CSUKÁS pursued the selective effect of the inhibitor by employing its different concentrations, whereas we used different dilutions of the cultures for determining the degree of growth inhibition.

The quantitative determination of inhibition by D-cycloserine was carried out with broth cultures of various clostridium strains diluted up to 10^{-12}. Table I shows that the presence of 800 μg/ml D-cycloserine in the medium markedly inhibited the growth of all the three *C. perfringens* strains tested.

We obtained both with 600 and 800 μg/ml of D-cycloserine a powerful inhibitory effect on the outgrowth of aerobic or facultatively anaerobic bacteria on blood agar plates. Among the facultatively anaerobic strains tested (see Table II) only *Erysipelothrix insidiosa*, *Serratia marcescens* and *Aerobacter cloacae* were able to grow, each at a depressed rate.

On sulphite agar prepared with egg-yolk emulsion and elevated agar content (20 g/litre), addition of 600 μg/ml D-cycloserine did not seem to interfere with the outgrowth of any of the *Clostridium* strains tested (Table III): whether undiluted broth, diluted broth, or spore suspension were used as inoculum. Colony shape, sulphite reduction (black colonies), a well as lipase and lecithinase activities characteristic of the species were exactly the same as on the control media prepared without inhibitor. Counts obtained with corresponding dilutions in experimental and control series also were in the same order of magnitude. Since the type A, B and C strains of *C. botulinum* were still sparsely growing after 48 hrs, the results were read at 72 hrs of incubation.

Table I

Growth of various clostridium strains on blood agar plates, containing 800 μg/ml D-cycloserine on common blood agar and in CMM

Strains	Colony counts after 48-hr incubation under anaerobic conditions in		
	CMM	common blood agar (control)	blood agar containing 800 μg/ml D-cycloserine
C. sporogenes 533	10^8	$4.4 \cdot 10^7$	$4.2 \cdot 10^7$
C. botulinum A 2012	10^7	$2.1 \cdot 10^7$	$1.9 \cdot 10^7$
C. bifermentans 506	10^9	$3.5 \cdot 10^7$	$1.2 \cdot 10^7$
*C. perfringens** 10240	10^8	$1.75 \cdot 10^6$	no outgrowth
*C. perfringens** 8235	10^8	$2.4 \cdot 10^6$	no outgrowth
*C. perfringens** 1808 (authors isolate)	10^{12}	$3.5 \cdot 10^8$	$2.1 \cdot 10^3$

* The dilutions of *C. perfringens* cultures were prepared from CMM broth, incubated for 24 hrs.

Table II

Growth of facultative anaerobic microorganisms on blood agar prepared with 600 and 800 μg/ml D-cycloserine bitartarate and on common blood agar, anaerobic incubation for 48 hrs at 37 °C

Strains	Common blood agar medium (control)		Blood agar medium containing 600 or 800 μg/ml D-cycloserine	
	growth	haemolysis	growth	haemolysis
B. cereus 100003 ATCC	+	+	—	—
B. megaterium 747	+	+	—	—
B. subtilis 100008 ATCC	+	+	—	—
Str. faecium J5 Sarajevo 70 C	+	—	—	—
04 *Str. durans* OKI 80 174	+	—	—	—
L4 *Str. zymogenes* NCTC 8 176	+	+	—	—
02 *Str. liquefaciens* OKI 80 172	+	—	—	—
01 *Str. faecalis* OKI 80 171	+	—	—	—
Erysipelothrix insidiosa (authors' isolate)	+	+	+	(+)
Listeria monocytogenes (authors' isolate)	+	+	—	—
Corynebacterium pyogenes (authors' isolate)	+	+	—	—
E. coli 33001	+	—	—	—
S. paratyphi B	+	—	—	—
Proteus mirabilis 110	+	—	—	—
Serratia marcescens	+	—	+	—
Aerobacter cloacae (authors' isolate)	+	—	+	—

Facultatively anaerobic strains were also tested for growth ability in the above medium (Table IV): out of these, exclusively *Serratia marcescens* showed an undisturbed growth, but failed its pigment-producing ability. A depressed growth was noted with *Streptococcus faecalis*, *Str. zymogenes* and *Corynebacte-*

Table III

Growth of *C. botulinum*, *C. sporogenes* and *C. perfringens* strain on sulphite agar plate containing egg-yolk emulsion with and without 600 μg/ml D-cycloserine

Strains	Colony counts after 48-hr anaerobic incubation		
	in CMM	on sulphite agar containing egg-yolk emulsion	on sulphite agar containing egg-yolk emulsion + 600 μg/ml D-cycloserine
C. botulinum A 7272	10^7	$2.61 \cdot 10^6$	$1.5 \cdot 10^6$
C. botulinum B 3807	10^{12}	$8.3 \cdot 10^9$	$7.3 \cdot 10^9$
C. botulinum C 3732	10^{12}	$3.0 \cdot 10^9$	$1.5 \cdot 10^9$
C. botulinum D Canada 53	10^{10}	$8.5 \cdot 10^8$	$7.9 \cdot 10^8$
C. botulinum E 8550	10^6	$1.7 \cdot 10^5$	$1.2 \cdot 10^5$
C. botulinum F 1 TP	10^{12}	$7.7 \cdot 10^9$	$2.5 \cdot 10^9$
C. sporogenes 3679	10^{12}	$3.7 \cdot 10^9$	$3.1 \cdot 10^9$
C. perfringens 1008 (authors' isolate)	10^{12}	$6.6 \cdot 10^7$	$2.6 \cdot 10^7$
C. perfringens 8335	10^{12}	$14.3 \cdot 10^6$	$7.0 \cdot 10^6$

Table IV

Growth of facultative anaerobic bacteria on sulphite agar containing egg-yolk emulsion with or without D-cycloserine

Strains	Sulphite agar containing egg-yolk emulsion	Sulphite agar containing egg-yolk emulsion + 600 μg/ml D-cycloserine
B. cereus 100003 ATCC	+	—
B. megatherium 747	+	—
B. subtilis 100008 ATCC	+	—
Str. faecium J5 Sarajevo 70 C	+	—
04 *Str. durans* OKI 80 174	+	—
L4 *Str. zymogenes* 8176 NCTC	+	(+)
02 *Str. liquefaciens* OKI 80 172	+	—
01 *Str. faecalis* OKI 80 171	+	(+)
Staphylococcus aureus (authors' isolate)	+	—
Erysipelothrix insidiosa (authors' isolate)	+	—
Listeria monocytogenes (authors' isolate)	+	—
Corynebacterium pyogenes (authors' isolate)	+	(+)
E. coli 33001	+	—
S. paratyphi B 5	+	—
Proteus mirabilis 110	+swarming	—
Serratia marcescens 1	+, pigment: +	+, pigment: —
Aerobacter cloacae (authors' isolate)	+	—

(+), depressed growth.

Table V

Growth of *C. botulinum* A, *C. sporogenes*, *C. bifermentans* spores and *C. perfringens* on common blood agar and blood agar containing 600 μg/ml D-cycloserine

Strains	CMM	Common blood agar	Blood agar containing 600 μg/ml D-cycloserine
C. botulinum A 7272	10^7	$3 \cdot 10^{91}$	$3 \cdot 10^{91}$
C. sporogenes 3679	10^{12}	$9.5 \cdot 10^{11}$	$7.9 \cdot 10^{10}$
C. bifermentans 506	10^{12}	$2.1 \cdot 10^{10}$	$1.2 \cdot 10^{8}$
C. perfringens 1808	10^{12}	$1.1 \cdot 10^{7}$	$5.2 \cdot 10^{6}$

rium pyogenes. Thus, the growth potential of the facultatively anaerobic strains was essentially the same in the above medium as in blood agar plates containing 800 μg/ml D-cycloserine.

With 600 μg/ml D-cycloserine added to blood agar plates the plating efficiency of diluted spore suspensions was reduced by 1—2 orders of magnitude compared with the control series (Table V), and the growth of certain *C. perfringens* strains was inhibited to a still greater degree. Thus in those cases in which the isolation of *C. perfringens* strains is pursued, the D-cycloserine content of the selective medium should be decreased to 400 μg/ml, and incubation should be made at 46 ± 0.5 °C.

Conclusions

Blood agar containing 800 μg/ml D-cycloserine bitartarate proved to be a suitable medium for the selective isolation of most test organisms. The applied concentration of D-cycloserine, however, had a considerable inhibitory effect on the growth of *C. perfringens* strains and completely depressed that of *C. histolyticum*.

At 600 μg/ml concentration in sulphite agar containing egg-yolk emulsion, D-cycloserine bitartarate did not interfere with the lecithinase, lipase, and sulphite-reduction activities of various clostridium species. All but a few strains maintained full growth ability in this medium, while the growth of aerobic spore formers was inhibited.

All *C. perfringens* strains tested grew readily at 400 μg/ml concentration of D-cycloserine bitartarate in the medium, if the incubation temperature was maintained at 46 ± 0.5 °C. Thus, this is the concentration of choice when the isolation of *C. perfringens* is pursued.

At 600 μg/ml concentration of D-cycloserine in blood agar plates, the efficiency of plating of spore suspensions was reduced by 1—2 orders of magnitude compared to the controls. The selectivity of the blood agar containing

600 μg/ml D-cycloserin enevertheless roughly corresponded to that of the plates containing 800 μg/ml of the inhibitor. Both blood agar and sulphite agar plates prepared with 600 μg/ml inhibitor content and egg-yolk emulsion proved to be suitable for routine selective isolation of all *Clostridium* species except certain *C. perfringens* strains. However, for the isolation of the latter strains 400 μg/ml D-cycloserine concentration should be used and the cultures should be incubated at 46 ± 0.5 °C.

Sulphite agar with egg-yolk emulsion also enables the reading of the lecithinase and lipase activities, and on blood agar media containing 400—600 μg/ml D-cycloserine, apart from colony morphology, haemolytic activity comes into display. On this basic *C. botulinum* can easily be differentiated from other clostridia. The sulphite agar medium containing egg-yolk emulsion and 400 μg/ml D-cycloserine and 20 g/l agar is suitable also for the pour-plate technique. The plates should be incubated at 46 ± 0.5 °C. Thus, the plate count of viable *C. perfringens* cells can be determined.

Summary

D-cycloserine bitartarate was tested for growth inhibition in blood agar and in sulphite agar medium containing egg-yolk emulsion, with special regard to the selective isolation of various *Clostridium* species. At 800 μg/ml concentration, D-cycloserine depressed the growth of *C. perfringens* strains and blocked that of *C. histolyticum*. At 600 μg/ml D-cycloserine concentration in sulphite agar containing egg-yolk emulsion and 20 g/l agar, all *Clostridium* species except *C. perfringens* grew readily and displayed undisturbed lecithinase, lipase and sulphite-reduction activities characteristic of the species. *C. perfringens* strains grew only when the concentration of the inhibitor was reduced to 400 μg/ml and the incubation temperature was maintained at 46 ± 0.5 °C, while the outgrowth of the accompanying flora was depressed by the inhibitor.

C. botulinum and *C. perfringens* can be readily detected if blood agar plates with 600 μg/ml D-cycloserine and sulphite agar plates containing egg-yolk emulsion and 600 or 400 μg/ml (*C. perfringens*) D-cycloserine are used simultaneously. Colony morphology, character of haemolysis is to be seen on blood agar, lecithinase and lipase activity on sulphite agar containing egg-yolk.

References

EÖRSI, A.: Népegészségügy **30** (1947), 1172. FÜZI, M. and CSUKÁS, Zs.: Kísérletes Orvostudomány **20** (1968), 123. IDEM: Ibidem **21** (1969), 331. STANLEY, M. H., DONALD, A. H. and JAMES, T. P.: Applied Microbiol. **4** (1971), 688. KOCH, F. E.: Zbl. Bakt. Abt. I. Orig. **132** (1934), 358. TAKÁCS, J. and NARAYAN, K. G.: Magyar Állatorvosok Lapja (MÁL), 1965, 211. TAKÁCS, J.: Arch. Lebensmittelhyg. **22** (1971) 101. IDEM: MÁL, 1972, 275.

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OCCURRENCE OF CONTAMINATING VIRUSES IN VARIOUS SWINE FEVER VIRUS STRAINS

II. STUDIES ON GILLESPIE'S CYTOPATHIC TYPE-A STRAIN AND ON OTHER STRAINS

By

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The cytopathic changes caused by contaminating adenoviruses in tissue cultures inoculated with swine fever (SF) virus were described in a previous publication (BODON, 1966). SF virus strains causing changes not characteristic of adenoviruses or any known viral agent are dealt with in this paper.

GILLESPIE et al. (1960) found one cytopathic strain when propagated six SF virus strains in primary renal epithelial cell cultures obtained from 5—16 weeks old piglets. The growth medium consisted of 50% bovine amniotic fluid, 46% Earle's solution and 4% lamb serum. This medium was later replaced by another, consisting of 86% Parker 199, 4% lamb serum, 10% of a 5% lactalbumin hydrolysate solution and antibiotics. The growth medium is generally believed to have a decisive influence on the development of the cytopathic effect (CPE). GILLESPIE and his coworkers did not describe the CPE in a detail, but they reported that the SF virus isolate maintained in tissue culture produced all characteristic symptoms of swine fever in susceptible pigs and the isolate was neutralizable by specific SF antiserum.

BASS and RAY (1963) studied GILLESPIE's cytopathic isolate (type-A SF virus strain) by acridine orange fluorescence in embryonic pig kidney epithelial cell culture. Three to five days after inoculation a focal CPE appeared: the cells became rounded, the cytoplasm was shrinking and some cells underwent necrosis. Cells in the centres of the foci even detached from the tube wall. At the marginal parts cell degeneration was seen.

CHUNG TAO LEE (1962) examined the CPE caused by GILLESPIE's strain in embryonic pig kidney cell culture by electron microscope. He described the changes as follows: the cells became swollen and rounded, mitochondrial degeneration and cytoplasmic vacuolation developed, the nucleoli enlarged, and osmiophilic electron-dense aggregations appeared in the nucleus. The vacuoles began to appear on the second day after inoculation, the other cytoplasmic changes on the tnird day, whereas the nuclear changes appeared only on the fifth day, or later. CHUNG TAO LEE regarded the spherical electron-dense aggregations seen in the nucleus as SF virions or SF virion precursors.

The present author (BODON, 1965) studied the cytopathic strain PAV-1 by the acridine orange fluorescence technique in embryonic pig kidney epithelial cell culture. He found (BODON, 1967) that three to five days after inoculation the confluent monolayer became discontinuous, part of the cells detached, cytoplasmic vacuoles appeared, the cellular RNA level rose, the nuclei became shrunken and assumed an eccentric position. Taking into consideration the type of changes, and the results of immunofluorescence and agar gel diffusion precipitation tests, the CPE was clearly due to virus diarrhoea (VD) virus. Since, however, the PAV-1 strain was not directly supplied from GILLESPIE's laboratory, its contamination with VD virus may well have taken place during maintenance in tissue culture.

BACHMANN et al. (1967) disagreed with our conclusion (BODON, 1965), because they did obtain the characteristic CPE described by GILLESPIE et al. (1960) in the 28th, 135th and 205th tissue culture passages of the strain PAV-1. By electron microscopy they saw particles 14—16 nm in diameter, but these could be scarcely differentiated from the cell organelles.

They failed to demonstrate either adeno- and/or VD viruses or mycoplasmas and, accordingly, concluded that the CPE was due to the SF virus.

HRABÁK and DUBANSKY (1970) infected susceptible pigs with the 29th passage of the SF virus strain PAV-1 in primary pig kidney cell culture. Although the animals did not develop the disease, they showed hyperthermia and leucopenia 3—7 days after infection. The pigs killed in this period exhibited a haemorrhagic diathesis. Unlike the pigs infected with virulent SF virus, those treated with the tissue culture passage did not show microscopic lesions in the central nervous system. The strain PAV-1 proved to be highly immunogenic, but did not produce CPE in primary pig kidney cell culture (HRABÁK, 1971).

Materials and Methods

SF virus strains

1. PAV-1 — Gillespie's type A cytopathic SF virus strain maintained in freeze-dried samples.

2. VSP — SF-virus-containing defibrinated blood, obtained from Bulgaria.

3. AVSP — tissue-culture-adapted attenuated SF virus strain, obtained from Bulgaria in freeze-dried condition.

4. MVSP — tissue-culture-adapted SF virus strain, obtained from Bulgaria.

5. VPP — SF-virus-containing defibrinated blood, obtained from Romania.

6. RVVS — SF virus-containing serum, obtained from Romania.

The strains listed under 2—6 were not characterized as cytopathic, but their examination for possible contamination with other viruses was regarded as desirable.

Tissue culture

The PK-15 permanent pig kidney epithelial cell line (CHUNG TAO LEE, 1962) was used throughout. It was maintained in 200 ml flasks, in Parker M 199 solution (90%), containing 10% colostrum-deprived calf serum and antibiotics (streptomycin and kanamycin). After the outgrowth of the confluent monolayer, subpassage was done as follows: the growth medium was removed and 7 ml 0.25% trypsin solution was pipetted into the flask, which was then returned to the incubator (37 °C). After 30 to 45 min the flasks were checked for detachment of the monolayer. If the cells tended to detach, 7 ml growth medium was added to the cell suspension, the contents of the flask were thoroughly shaken, stirred several times by pipetting. In this way suspended single cells were obtained. Of the suspension 7 ml were transferred to another flask as passage material, while the rest was distributed to Leighton tubes in 1 ml amounts, after addition of 25 ml medium to adjust the cell density.

The outgrowth of the confluent monolayer took 2—4 days in the flask and 2 days in the tubes.

Subsequently, the medium was exchanged for a serum-free Parker 199 solution and the cultures were infected with SF virus. The incubation period was 6—7 days, while each culture was checked daily for CPE. The medium was not exchanged from infection to final reading. Appropriate control tubes, similarly treated except for virus infection, were set up with each series. After 6 or 7 days the coverslip cultures were removed from the Leighton tubes and were stained with acridine orange (BODON, 1967).

All six SF virus strains studied were carried through 20 passages in the PK-15 cell cultures.

Results

In the non-infected control tubes no CPE appeared in the first 9 passages, but from the 10th passage on two types of change were seen, (a) focal rounding of a few cells and (b) rounding of many cells over the entire area of the monolayer (Table I.).

Roughly similar changes appeared in the cultures infected with any of the 6 virus strains tested; differences were found only in the time of outset, and in the persistence or disappearance of the CPE.

To properly interpret the focal and diffuse types of cell rounding, we scrutinized the characteristics of CPE due to all known virus groups carefully. However, the CPE produced by the six strains was not identical with any known type of virus-induced cell change, and it also appeared in the cultures not infected with SF virus.

Discussion

Out of the examined strains the PAV-1 isolated was the most intriguing, because it has been often used as an SF virus strain (COGGINS and BAKER, 1964; GILLESPIE et al., 1971; COGGINS and SHEFFY, 1961). The original lyophilized sample was labeled with the titre $10^{-1.8}$ $TCID_{50}$/0.1 ml, but the remaining strains were submitted without such information and all we knew was that they had been used either for vaccine preparation or laboratory tests.

Furthermore, most authors previously engaged in experiments with the PAV-1 cytopathic SF virus strains had used primary kidney cell cultures. We attempted the use of a cell line and have ab ovo taken into consideration that permanent cell cultures are less susceptible to virus infection than primary ones. Twenty transfers were therefore made to allow enough time for adaptation. In spite of this the risk had been taken that the PK-15 line might have remained resistant to SF virus even after as many as 20 passages. This

Table I

Cell changes in PK-15 permanent pig kidney cell cultures inoculated and not

Strains	Passage No.									
	1	2	3	4	5	6	7	8	9	10
VPP	0	0	0	0	0	0	0	+	±	+
VSP	0	0	0	0	0	0	0	0	±	+
AVSP	0	0	±	0	0	0	0	0	0	+
MVSP	0	0	±	0	0	0	0	0	0	+
RVVS	0	±	0	0	0	0	0	0	0	+
PAV-1	0	0	0	0	0	0	0	+	±	+
Control	0	0	0	0	0	0	0	0	0	0

Symbols used: ± focal rounding of a few cells; + diffuse rounding of many single cells

had, however, little support because other authors had already used this cell line for SF virus studies (PIRTLE and KNYAZEFF, 1968; AYNAUD, 1968; DANNER and BACHMANN, 1970).

None of the strains showed a characteristic CPE in the course of passaging. Accordingly, contaminating cytopathic viruses were not associated with the strains and if SF virus was present in the test materials, it was clearly not cytopathic. Infection experiments on susceptible pigs were, however, omitted because our studies were centered on CPE. The low titre of the PAV-1 strain could initially account for the absence of CPE, but 20 transfers are generally sufficient for effecting a notable titre rise in the later stage of passaging.

The changes seen in both virus-infected and control cultures were indicative of ageing of the cells rather than of a specific, virus-induced CPE. Such changes are sometimes seen in aged cultures of both primary and permanent cells. Epithelial cells are considerably more sensitive than fibroblasts, owing to their high oxygen requirement. Furthermore the epithelial tissue produces a proteolytic enzyme. The more intensely the epithelium is growing, the stronger the lytic action of the enzyme, and PK-15 cells are noted for fast growth. Discontinuities — fenestrae — often appear in the confluent monolayer, which gradually become larger and finally merge with one another. Proteolytic enzyme production by the epithelial cells can be depressed by addition of inhibitors, e.g., with the sera of certain animal species (horse, calf). However, high inhibitor concentrations in the applied serum suppress cell division. When the nutrient medium has been exhausted, the cells become rounded, and finally disintegrated.

In such cases, control cultures not treated with viral agent greatly help evaluation, especially in the case of viral agents requiring a long — 6 to 10-

inoculated with swine fever virus strains

Passage No.									
11	12	13	14	15	16	17	18	19	20
+	0	+	0	+	+	+	±	0	+
+	+	+	±	±	±	0	±	0	+
+	+	+	0	+	+	+	±	0	+
+	±	+	0	0	0	+	±	0	+
+	±	+	0	0	0	+	±	0	+
+	±	+	0	±	0	+	±	0	+
±	±	±	±	+	+	0	0	0	+

over the entire monolayer; Control: Non-infected PK-15 cultures.

day — culturing time. Spontaneous cell changes have sometimes been found in control cultures of the PK-15 line, even under appropriate conditions of maintenance.

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Summary

The swine fever (SF) virus strain PAV-1, characterized in the United States as cytopathic, as well as five other SF virus strains failed to produce CPE in our hands. The five strains had been submitted for examination from Bulgaria and Romania. The changes appearing in PK-15 permanent pig kidney epithelial cell cultures infected with the strains were not characteristic of any known group of viruses and similar changes also appeared in control cultures not infected with virus. This and the type of changes suggest that these were due to ageing or spontaneous degeneration of the cells rather than to SF virus.

References

AYNAUD, J. M.: Rech. Vét. **1** (1968), 25. IDEM: C. R. Acad. Sci. Paris **226** (1968), 535. AYNAUD, J. M., GALICHER, C., LOMBARD, J., BIBARD, C. and MIERZEJEWSKA, M.: Ann. Rech. Vét. **3** (1972), 209. BACHMANN, P. A., SHEFFY, B. E. and SIEGL, G.: Arch. ges. Virusf. **22** (1967), 467. BASS, E. P. and RAY, T. D.: JAVMA **142** (1963), 112. BODON, L.: Acta vet. hung. **XV** (1965), 471. IDEM: Ibidem **XVI** (1966), 321. IDEM: Magyar Állatorvosok Lapja (MÁL), 1967, 466. CARBREY, E. A., STEWART, W. C., KRESSE, J. I. and LEE, L. R.: 69th Ann. Proc. US Livestock San. Ass. October 1965, p. 487. CHUNG TAO LEE, R.: Cornell Vet. **52** (1962), 39. COGGINS, L. and BAKER, J.: Am. J. Vet. Res. **25** (1964), 408. COGGINS, L. and SHEFFY, B. E.: 65th Ann.

Proc. US Livestock San. Ass. October 1961. DANNER, K. and BACHMANN, P. A.: Zbl. Vet. Med. **17B** (1970), 353. GILLESPIE, J. H., SHEFFY, B. E. and BAKER, J. A.: Proc. Soc. Exp. Biol. Med. **105** (1960), 679. GILLESPIE, J. H., SHEFFY, B. E., COGGINS, L., MADIN, S. H. and BAKER, J.: 65th Ann. Proc. US Livestock San. Ass. October 1961. HRABÁK, R. and DUBANSKY, V.: Acta Vet. (Brno) **39** (1970), 457. HRABÁK, R.: Personal communication, 1971. MENGELING, W. L. and DRAKE, L.: Am. J. Vet. Res. **30** (1969), 1817. MENGELING, W. L.: Arch. ges. Virusf. **23**, (1968), 27. MENGELING, W. L., PIRTLE, E. C. and PACKER, R. A.: Am. J. Vet. Res. **31** (1970), 2019. PIRTLE, E. C. and KNYAZEFF A. J.: Am. J. Vet. Res. **29** (1968), 1177. PIRTLE, E. C.: Am. J. Vet. Res. **30** (1969), 209. IDEM: Ibidem **30** (1969), 1913.PIRTLE, E. C. and MENGELING, W. L.: Am. J. Vet. Res. **31** (1970), 1795.

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THE SPAYING OF PIGS

(OVARO-HYSTERECTOMY OR OVARIOTOMY)

By

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(Received March 18, 1974)

The spaying of gilts by hormone overdoses was recommended in the 'fifties by BAJEZ (1953, 1954) and, in Hungary, by TANGL (1953) and CSEH and PAÁL (1953). Since, however, practical observations (TORDAY and DRATSAY, 1954) failed to substantiate the initial promising results, hormonal sterilization of the sow was prohibited by an order (No. 81.875/963) of the Ministry of Agriculture and Food for public health reasons. Thus the original practice of surgical spaying by oophorectomy was resumed.

Removal of the ovaries through an incision in the flank, the so-called Chinese procedure first performed in Shanghai, had been recommended for sow sterilization already by the end of the past century (HOFFMANN, 1890). In Hungary, LÁSZLÓ (1925) dealt with the practical aspects of the operation in a greater detail, and subsequently HETZEL (1935) advocated its advantages so that its application was very widely spread in the country during the 'thirties and 'forties. The main difficulty met with by the practitioners was the exploration of the right ovary in the sow recumbent on her right side. In view of this, PARISIS (1949) proposed that exploration should be done along the ligamentum teres uteri (Fig. 4).

This and other disadvantages of the above procedure, *viz.*, weight loss during fasting for 48 hrs (TANGL, 1953), depression of resistance (LÁSZLÓ, 1950) and the difficulty of restraining animals weighing 50—60 kg on the spaying table tilted at the angle of 60°, initiated efforts for the elaboration of a more simple procedure, which makes possible the use of both the right and left hand for the operation. The related studies were partly based on JOSHUA's (1965) good results obtained on spaying bitches by ovaro-hysterectomy at 10 weeks of age, *i.e.*, before reaching sexual maturity.

Experimental

Surgical removal of the ovaries and uterine cornua (ovaro-hysterectomy)-spaying before puberty

Ten weeks old gilts, weighing 25—30 kg, were spayed on the operating table. The animals were laid on one side, and a two-inch incision was traditionally made cranial to the second and third posterior teats, *viz.* in the centre of the abdominal wall. Fasting for 12 hours is sufficient in the case of this operation, because only one ovary and one uterine tube, *viz.*, those nearby

the incision have to be exteriorized (Fig. 1). The same applies to the non-fasted pig operated upon in the same position. At 10 weeks of age the ovary is usually undeveloped, the size of a lentil seed, but sometimes it may already be of normal size. The sexually immature gilt can be laid on either side for the surgery, because, as mentioned above, it is not necessary to exteriorize the contralateral ovary by manual exploration of the abdominal cavity. One uterine horn is exposed after the other, to find the minute ovaries, as recommended by

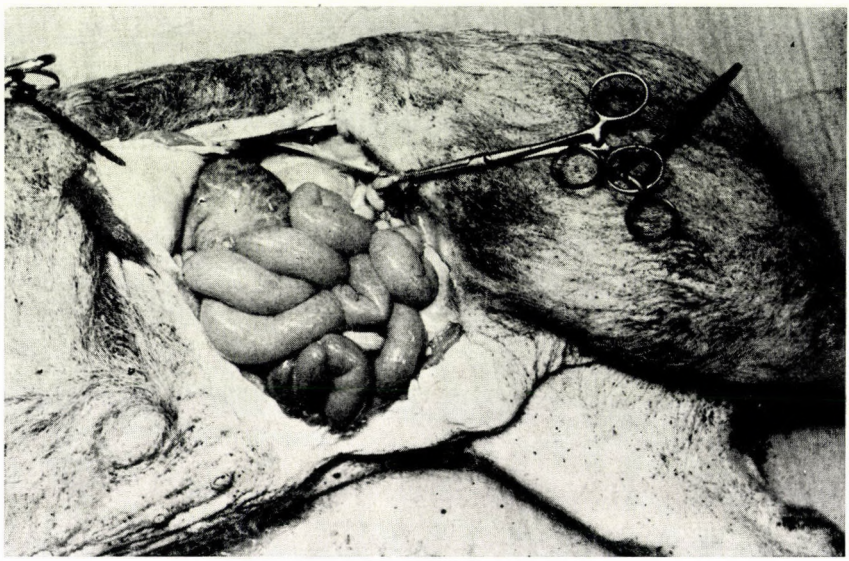

Fig. 1. The reserve space in the abdominal cavity is filled by intestines. The left — upper — ovary is caught in tweezers; the ovarial suspensory ligament is taut. (The sow lies on her right side.)

HETZEL (1942). This procedure is still resorted to when the ovary is not easily found at the side on which the animal lies. Being out of function at 10 weeks of age, and consequently not hyperaemic, the ovaries can be simply crushed with an emasculator. Another approach is to reach into the abdominal cavity with one finger, and to exteriorize both cornua along with the ovaries by lifting the body of the uterus at the bifurcation of the horns. Subsequently, the cornua are cut off at their origin, and the ovarian ligaments are crushed.

Ovariectomy on the operating table — spaying after puberty

Good results have been obtained for more than 10 years by performing ovariectomy in growing gilts, weighing 30—35 kg, with the animal laid on its right side on the operating table. Most gilts coming under this weight category are 3—4 months old, but fastly growing breeds may attain the weight required

for the operation by 10 weeks of age. Little physical exercise is necessary to restrain such animals and to lift them on the operating table. In slowly-growing breeds the ovaries are practically of full size at 3—4 months of age, thus their removal is by the traditional surgical approach (HETZEL, 1942), *viz.*, exploration for both ovaries is made along the cranial margin of the ovarial suspensory ligaments.

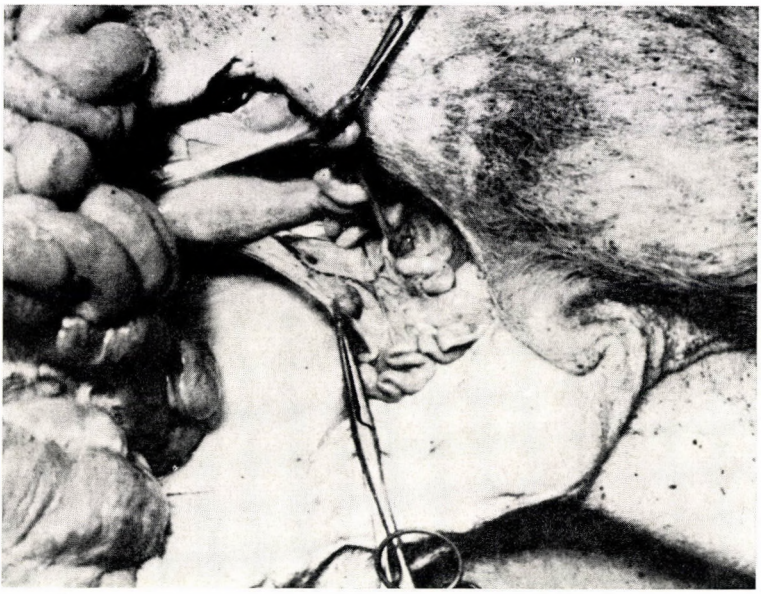

Fig. 2. The reserve space in the abdominal cavity is empty. Both ovaries are caught in tweezers. The free margins of the ovarial suspensory ligament are extending almost parallel with the rectum. If the middle- and forefinger are introduced into the abdominal cavity in cranio-caudal direction beneath or above the rectum, the ligament, and at its end the ovary, is caught between the fingers. (The sow lies on her right side.)

Preparation for the operation

Serial laparotomy studies have shown that the operation can be safely performed 24 hrs after the last feeding. Thus 24-hr fasting is sufficient, but this should be complete. Even the straw bedding, especially fresh straw, should be removed, to prevent its ingestion, which often results in intestinal gas accumulation. This enhances the peristaltics and causes the small intestines to bulge into the posterior third of the abdominal cavity, which normally serves as a reserve space allowed for the distension of the visceral hollow organs (intestines, uterus, urinary bladder). The reserve space is empty in Fig. 2, but it is filled out by bloated small intestines in Fig. 1. The latter condition interferes with the surgical procedure. Although the fasting gilts should be deprived of any food, they have to be provided with drinking water, especially in the hot summer season.

Laying the pig on the table

Adult sows should lie preferably on their right side, because the pig is the only species having the caecum at left. The duodenocaecal and renolienal ligaments fuse with one another in adult age.

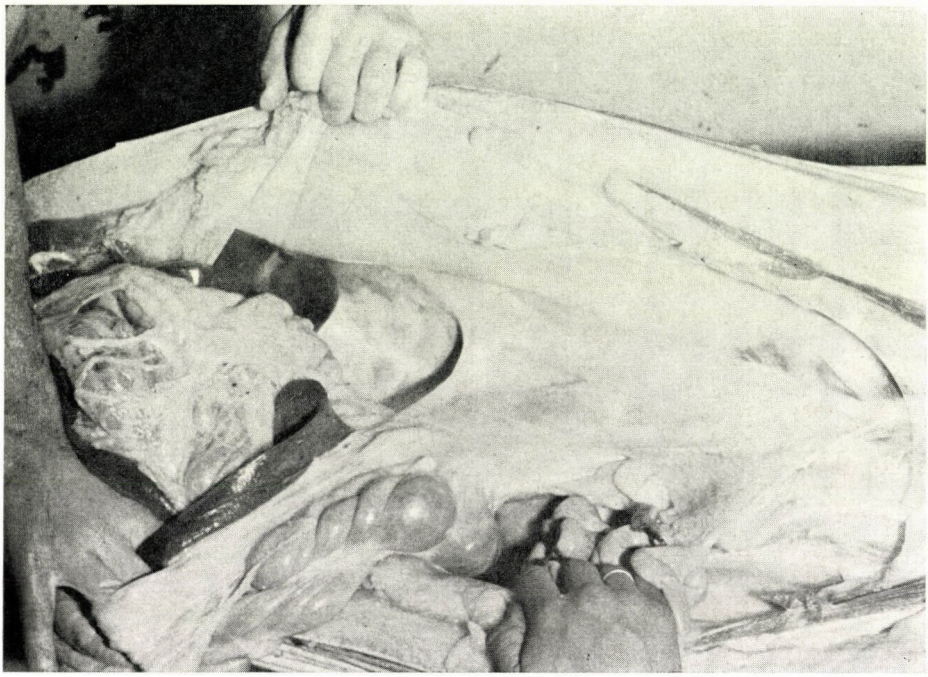

Fig. 3. The lig. reno-caecale et lienale, passing from the left kidney to the caecum and the colon is exposed in a slaughtered pig. It is because of the position of this ligament that the sow has to lie on its right side for surgical spaying

The fused ligament has its origin at the duodenum, from where it passes to the left kidney and, in a returning arch, to the caecum and colon. One branch of the ligament is extended to the spleen. Thus the position of the ligament resembles that of a leaf in book; the upper part corresponds the duodenocaecal ligament, the lower part to the renocaecal ligament, which sends one branch to the spleen (Fig. 3). If the sow lies on her right side, the caecum and colon, supported by the left kidney, are descending on the right abdominal wall owing to their weight, compressing the motile small intestines against it, so that the abdominal reserve space is left empty for easier exploration by the finger. If the sow lies on its left side, the anatomical conditions are less advantageous for the intervention, because the caecum and the colon descend on the left abdominal wall and the small intestines, thus released from compression, fill the empty reserve space, and interfere with exploration for the ovaries.

The position of the intestines depends not so much on the horizontal or tilted position of the spaying table — a bench of tortures — as on laying the appropriately fasted sow on her right side.

In the latter position, exploration for the upper — left — ovary is easily done along the cranial margin of the ovarial suspensory ligament (Fig. 2). Exploration for the lower — right — ovary along the ligamentum teres uteri

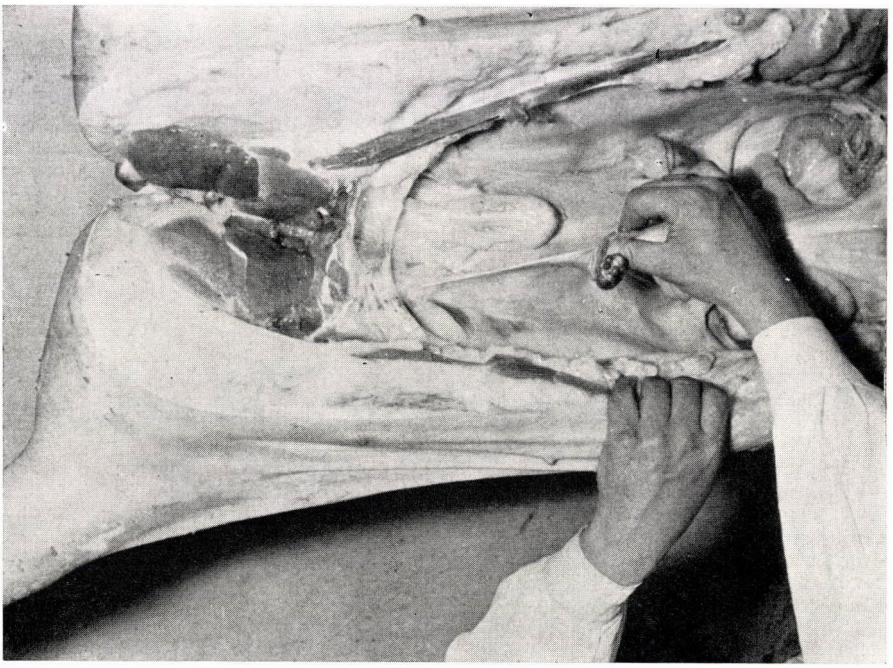

Fig. 4. Free margin of the left lig. teres uteri, exposed in slaughtered sow

(Fig. 4) is not practicable with this procedure if the animal lies on a horizontal table, because under such conditions the said ligament is not found in the segmental plane of the second and third posterior teats. Accordingly, exploration for the lower — right — ovary should be made between the free cranial margins of the ovarial suspensory ligaments, at the caudal end of the reno-colonal ligament (Fig. 3), by reaching into the lower part of the abdominal cavity beneath the colon. This is performed according to the technique recommended by PARISIS (1949): with the middle- and forefinger of the left hand introduced craniocaudally into the "rachopsoas" sulcus, the free margin of the ovarian suspensory ligament, and at its end the ovary is caught between the two fingers (Fig. 2).

Spaying on horizontal or tilted operating table is difficult with adult sows ruled out from breeding and destined for fattening. These animals are

so heavy that their lifting on a table requires the assistance of several attendants. Thus, such animals should be preferably operated upon on the ground, *viz.* on a clean lawn. HETZEL (1942) himself had reported about spaying sows on the lawn. According to CSEH (1955), Caesarean section may also be employed for spaying, but this has been little practised. Ovariotomy in the lactating sow for prolongation of the lactation period has not been fashionable in Hungary.

Acknowledgement

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Summary

Since in Hungary hormonal spaying of gilts and sows has been prohibited by legal order for more than a decade, spaying by surgical method has been practised again ever since. Most pigs kept by smallholders in the country are fattened for slaughter for the family's own use, and the owners are anxious to have such animals neutered by the veterinarian.

Certain difficulties involved in the so-called Chinese method of spaying, which had been very widely spread in the country during the 'thirties and 'forties, stimulad efforts for the modification of the traditional operating technique.

The present paper is the report of a modified procedure, recommended for spaying of gilts before puberty, at 10 weeks of age, when the body weight of the animals is about 20—30 kg. The operation, involving the removal of both ovaries and uterine cornua (ovaro-hysterectomy) can be easily performed whether the animal lies on its right or left side, because exteriorization of the lower-seated ovary is not necessary, this being explored either by exposure of the uterine horns segment by segment, or by lifting and exposing both cornua and ovaries simultaneously, with one finger pushed beneath the body of the uterus at the bifurcation.

Three to four months old gilts and sexually mature sows are spayed by ovariotomy on the operating table, with the animal lying on its right side. The forefinger and middle finger are introduced into the abdominal cavity in cranio-cauddal direction, along the rectum. Thus, the free margin of the ovarial suspensory ligament is caught between the fingers, at the end of which the ovary is attaching on either side.

References

BAJEZ, E.: Wiener tierärztl. Mschr., 1953, 226; 1954, 295, 597. CSEH, S.: Szülészeti Műtéttan. Budapest, 1955. CSEH, S. and PAÁL, S.: Magyar Állatorvosok Lapja (MÁL), Továbbképzés 12, 1953. HETZEL, H.: A sertések miskárolása. Budapest, 1935, 1942. HOFFMANN, L.: Mh. prakt. Tierheilkunde **1** (1890), 249. JOSHUA, O.: Vet. Rec. **23** (1965), 642. LÁSZLÓ, S.: Az ovariotomia gyakorlati kivitele sertésen. Thesis. Budapest, 1925. IDEM: MÁL, 1950, 185. PARISIS, E.: Agrártudomány **11** (1949), 399. TAMÁS, L. and FELLNER, F.: Állatorvosi Sebészeti Műtéttan. Budapest, 1969. TANGL, H.: Állattenyésztés **3** (1952), 283; **4** (1953), 259. TORDAY, F. and DRATSAY, GY.: MÁL, 1954, 280.

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DEMONSTRATION OF *SALMONELLAE* IN SAUSAGES OF HIGH FAT CONTENT

By

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We have shown (TAKÁCS and NAGY, 1969) that of all slaughtered animals pigs are the most frequent reservoirs of *Salmonellae*. Swine pick up *Salmonellae* from the feed or environment, but insects, rodents, birds and even man can serve as mediators of infection. Pigs carrying small populations of the pathogen in the gut usually show no symptoms.

Meat inspection, however careful, is not in itself sufficient for the detection of all symptomless carriers. If the standards of abattoir sanitation are low, *Salmonellae* easily become disseminated with the faeces, accounting for sometimes 100% contamination of meat surfaces.

Meat products processed without heat treatment (TAKÁCS, 1964; TAKÁCS and NAGY, 1969) easily become contaminated with *Salmonellae*, especially in the summer season, when more than 50% of them may become contaminated, although the greater part of the contaminating bacteria perish during drying and ripening. Diminution of water activity and low pH (SCHEIBNER, 1969) in the ripened product either kill, or stop multiplication of *Salmonellae*, while the cells embedded in fat are preserved in an inactive, but still infective state (VAN SCHOTHORST and VAN LEUSDEN, 1972).

Examination for *Salmonellae* of each batch of finished Hungarian-type sausage products, cured by salting, cold smoke and drying, has been compulsory in Hungary since 1972, but it had been a general practice in the nationalized meat industry for about 10 years previously. Comparative studies were conducted in this laboratory to evolve a rapid, and efficient, *Salmonella* test for the purpose of compulsory routine examinations. This was necessary because sausages prepared without heat treatment contain 45—47% fat, 3.5—5.0% salt and 28—30% water in the finished state, and *Salmonellae* may still be present in them after ripening. Organisms trapped in fat may remain unnoticed at microbiological examination, accounting for false negative results in infected batches. Comparative examinations performed to prevent such misjudgements are reported in this paper.

A. Experiments with various enrichment methods

Material

Four pairs of sausages were chosen at random from each batch, and one half of each pair was used for the preparation of a pooled sample. From specimens kept in water of 45—48 °C for a few minutes the casing was removed with a sterile lancet. Care was taken not to touch the filling.

The filling was minced in a sterile mincing machine; a separate mincer and other tools, all sterilized, were used for each batch sample. The minced filling was thoroughly mixed with a sterile glass rod and samples were measured for *Salmonella* tests.

Method

Each sample was examined by three different methods, *viz.*

A/1: a procedure conforming to the prescriptions of the National Formulary XII;

A/2: a procedure conforming to the standard prescriptions issued by the International Standard Organization (ISO);

A/3: a procedure elaborated in this laboratory.

Details of the test procedures and positive test results are shown in Table I.

Results

Thirty samples were parallelly examined by each procedure A. Twenty-six samples were found to be negative, four contained *Salmonellae.*

Conclusions

The lactose-containing pre-enrichment medium (A/1) was not practicable in examining of sausages prepared without heat treatment because the concurrent microflora shifted the pH to 4.0—4.5.

The buffered peptone-water pre-enrichment medium (A/2) was favourable when tetrathionate was used for enrichment.

The tetrathionate-containing enrichment media (A/1—a, A/2—a, b, A/3—a, b) proved to be considerably more efficient than the media containing selenite (A/1—b, A/2—c). This accords well with the results of our earlier studies on meat and meat products (Takács, 1964).

The 1% polysorbate solution (A/1), being a good fat solvent proved to be advantageous, but the polysorbate concentration proposed under A/2 was

insufficient because it even failed to satisfactorily dissolve the fat component of 25 mg test material on transfer to the tetrathionate enrichment medium.

Incubation temperatures of 42—43 °C were more advantageous than 35 or 37 °C, in respect of both rate of isolation and fat dissolution. In spite of this 37 °C incubation was not omitted in the further experiments.

The procedures that proved to be practicable in these experiments were thereafter regularly employed in routine *Salmonella* examinations to decide which of them is the most efficient in the detection of *Salmonellae*, with special regard to their serogroups.

The test procedures were also compared for sensitivity of detecting *Salmonellae* in samples artificially inoculated with minimal doses of the pathogen.

B. Selection of the test procedures most sensitive in routine examination

Material

On a total, 136 samples were taken from batches submitted for routine examination; out of these, 31 proved to contain *Salmonellae*.

Schedules of experiments

Three schedules are shown in Table II.

Schedule B/1 was used for testing the influence of the temperature of incubation.

Schedule B/2 served for the examination of the joint influence of incubation temperature and fat solvent.

Schedule B/3 was employed to determine the joint influence of varying of pre-enrichment, incubation temperature and fat solvent.

The results are included in Table II.

Conclusions

Table II shows that the highest rate of positivity (31 positive samples) was reached with method B/2—b, *i.e.* when 25 g homogenized test material was placed in 225 ml Bierbrauer's tetrathionate enrichment medium containing 1% Tween 80, and the mixture was incubated for 24 $\pm$ 2 hrs at 42—43 °C. Procedure B/2—a was the same as procedure B/2—b except that the incubation temperature was 37 °C. By this procedure only 16 samples proved to be positive. Procedure B/2—b proved to be superior also in respect of the number of detectable *Salmonella* O serogroups.

Table 1

Results obtained with procedures A/1, A/2 and A/3

Method of examination		A/1		A/2			A/3	
		a	b	a	b	c	a	b
Pre-enrichment	Amount of samples	25 g		25 g			5 g	5 g
	Lactose broth + 1% Tween 80	225 ml						
	Buffered aqueous peptone				100 ml			
	Correction of pH by N NaOH	pH: 7.0						
	Pre-enriched material incubated at 35 °C	24 hrs						
	37 °C				6—24 hrs			
Enrichment	Amount of pre-enriched sample	0.1 ml	0.1 ml	25 ml	25 ml	25 ml		
	Enrichment medium containing tetrathionate	10 ml					45 ml	45 ml
	Tetrathionate medium + 0.66% Tergitol 7			225 ml	225 ml			
	Enrichment medium containing selenite		10 ml			225 ml		
	Enriched material incubated at 35 °C	24 hrs	24 hrs					
	37°C			3 days		3 days	24 hrs	
	42—43 °C				24 hrs			24 hrs

Transfer	to modified XL agar	+	+					
	to modified brillant green–phenol red agar	+	+	+	+	+	+	+
	to *Salmonella-Shigella* agar	+	+					
	to crystal violet-neutral red-bile agar			+	+	+		
	to modified Drigalski agar						+	+
	Plates incubated at 35 °C	24 hrs	24 hrs					
	37 °C			24 hrs	24 hrs	24 hrs	24 hrs	24 hrs
Test results		A/1		A/2			A/3	
		a	b	a	b	c	a	b
with 4 known positive samples		samples found positive						
		—	—	4	4	—	2	4

Table II

Results obtained with procedures B/1, B/2 and B/3

Method of examination			B/1		B/2		B/3	
			a	b	a	b	a	b
Amount of test material			25 g		25 g		25 g	
Pre-enrichment	Buffered aqueous peptone						100 ml	
	Pre-enriched material incubated at 37 °C						24 hrs	
Enrichment	Amount of pre-enriched material						25 ml	25 ml
	Bierbrauer's tetrathionate enrichment medium		225 ml	225 ml				
	Bierbrauer's tetrathionate enrichment medium + 1% Tween 80				225 ml	225 ml	225 ml	225 ml
	Enriched material incubated at 27 °C		24 hrs		24 hrs		3 days	
	42—43 °C			24 hrs		24 hrs		24 hrs
Transfer	to modified brillant green–phenol red agar		+	+	+	+	+	+
	to modified Drigalski agar		+	+	+	+	+	+
	Plates incubated at 37 °C		24 hrs	24 hrs	24 hrs	24 hrs	24 hrs	24 hrs
Test results			No. of samples found positive					
with 31 known positive samples			9	14	16	31	17	18
O-serogroup distribution of the *Salmonella* isolates		B	6	8	9	17	10	10
		C_1			2	4	2	2
		E	3	4	4	6	3	4
		L		1		3	1	1
		Mixed: B + E		1	1	1	1	1

C. Sensitivity tests

The *Salmonella* species occurring in Hungary most frequently were used for artificial ioculation of sausage fillings. The strains belonged to the O serogroups B, C_1, C_2, D and E. In general only one strain was present in each inoculum, but mixed infections were also carried out with B + E and C_1 + C_2 strains.

Preparation of the inoculum

Each test strain was inoculated into the liquid basal medium elaborated by one of us (TAKÁCS, 1964) and was incubated for 24 ±2 h at 37 °C. Three parallel dilution series, in the range of 10^{-5} to 10^{-15}, were prepared from each culture to obtain the dilution in which 1—9 *Salmonella* organisms were present per ml.

In every case, 1 ml inoculum was added to a 25 g sample of sausage filling that had been proved negative for *Salmonella*; the dilutions containing 1—9, 10—99, 100—999, 1000—9999 and 10 000—99 999 per ml were tested.

The sensitivity tests were carried out according to schedules B/1, B/2 and B/3.

Results

The experimental results are summarized in Table III.

Conclusions

Based on the data in Table III the ranges of sensitivity for the different methods as determined with different O serogroups were as follows. (The sensitivity values are expressed in the lowest *Salmonella* counts resulting in bacterial growth.)

Method	Range of detectability	
	Salmonella	count per 1/g
B/1 a	4 —	3,999.9
b	0.04—	39.9
B/2 a	0.04—	39.9
b	0.04—	3.9
B/3 a	0.04—	399.9
b	0.04—	399.9

Table III

The lowest *Salmonella* counts

Method of examination		*Salmonella* counts used for inoculation														
		in 25 g	1–9							10–99						
		in 1 g	0.04–0.36							0.4–3.9						
			B	C_1	C_2	D	E	B + E	$C_1 + C_2$	B	C_1	C_2	D	E	B + E	$C_1 + C_2$
	a															
B/1	b					+					+			+		
	a					+					+	+		+	+	+
B/2	b		+	+	+	+	+	+								+
	a			+		+				+				+	+	
B/3	b			+		+				+				+	+	

+, the crosses indicate the lowest input of *Salmonella* counts demonstrable with the applied

It is clear that method B/2—b, *viz.*, incubation in 1% Tween 80 containing Bierbrauer's tetrathionate enrichment medium at 43 °C, was of the highest efficiency: it made possible the detection of B, C_1, C_2, D and E *Salmonella* O groups even when the *Salmonella* count per 25 g test material was as low as 1—9.

Discussion

In contrast to other authors (MORRIS and DUNN, 1970), we found that *Salmonella* detection in meat products of high fat contents requires not only a relatively high (42—43 °C) incubation temperature, but also the presence of a surface-active fat solvent. Otherwise certain O serogroups cannot be detected unless 100—999 or more germs are present in 25 g test material.

Incubation at 42—43 °C enhanced the lipid-solvent action of Tween 80 compared with the 37 °C incubation temperature.

Pre-enrichment with lactose or buffered aqueous peptone was of low efficiency.

Enrichment media based on selenite proved to be unsuitable for the purpose at 35, 37, 42 and 43 °C incubation temperatures alike.

It is recommended that the B/2—b procedure, clearly the most efficient of all methods tested, should be performed as follows: 25 g homogenized sample should be added to 225 ml Bierbrauer's enrichment medium containing

demonstrable by different methods

of sausage fillings

100 – 999							1000 – 9999							10 000 – 99 999						
4 – 39.9							40 – 399.9							400 – 3 999.9						
B	C_1	C_2	D	E	B + E	$C_1 + C_2$	B	C_1	C_2	D	E	B + E	$C_1 + C_2$	B	C_1	C_2	D	E	B + E	$C_1 + C_2$
	+		+				+				+	+				+				+
+		+			+	+														
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method in the given volume of sample.

1% Tween 80 and incubated for 24 ±2 hrs at 42—43 °C. Subsequent transfers should be made to modified brillant green–phenol red agar (TAKÁCS, 1964) and modified Drigalski agar (TAKÁCS, 1964), and the selective and differentiating media should be incubated for 24 ±2 hrs at 37 °C. This procedure is sensitive enough to detect 1—9 viable *Salmonella* cells present in 25 g test material. Another great advantage of the procedure is its rapidity: evidence of the absence of *Salmonellae* can be obtained in 2×24 hrs and the differentiation of suspect or *Salmonella*-infected batches is possible in 3×24 hrs. A pre-enrichment would prolong the procedure by 24 hrs and the results would yet be less precise; thus pre-enrichment is not recommended for the testing of sausage-type finished meat products prepared without heat treatment.

Summary

Three procedures were compared in the detection of *Salmonellae* in sausages of high fat contents preserved by cold smoke, salting and drying, without heat treatment. The efficiencies of enrichment media at 37 and 43 °C incubation temperatures were also compared. Incubation for 24±2 hrs at 42—43 °C of 25 g homogenized sausage filling in 225 ml Bierbrauer's tetrathionate enrichment medium containing 1% Tween 80 proved to be the method of choice. No satisfactory results were obtained with the use of lactose solution or buffered aqueous peptone as enrichment medium. Enrichment in Tween 80-containing Bierbrauer's medium has an additional advantage, *viz.*, batches free from *Salmonellae* can be identified within 2×24±2 hrs and suspect and *Salmonella*-infected batches can be differentiated within 3×24±2 hrs. Thus, this procedure is practicable for routine use.

References

EDEL, W. and KAMPELMACHER, E. H.: Bull. World Health Organization **39** (1968), 487. MORRIS, K. G. and DUNN, C. G.: Appl. Microbiol. **20** (1970), 192. SCHEIBNER, G.: Mh. Vet.-Med., 1969, 388. VAN SCHOTHORST, M. and VAN LEUSDEN, F. M.: Zbl. Bakt. Hyg. I. Abt. Orig. A **221** (1972), 19. TAKÁCS, J.: Az Enterobacteriaceae családba tartozó baktériumok elkülönítése a laboratóriumi húsvizsgálatban, különös tekintettel a salmonellákra. Thesis. Budapest, 1964. TAKÁCS, J. and B. NAGY, GY.: Zbl. Bakt. Hyg. I. Ref. **218** (1969), 305. ISO/TC 34/SC 6WG 2/143: Meat and Meat Products. The detection of *Salmonellae*. 1969, National Formulary XIII. General Tests.

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EFFECT OF AGE, NUTRITION AND GONADOTROPHIC HORMONE THERAPY ON SERUM ALKALINE PHOSPHATASE ACTIVITY IN EWE LAMBS

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Literary data indicate wide variations in the activity of serum alkaline phosphatase (AP) among and within the different species of domestic animals, suggesting that serum AP is subjected to the influence of several factors. One of the most important factors affecting AP activity was postulated to be the growth rate. Growth rate is influenced by age, nutrition, genetic and hormonal balance, *etc.* The present investigation was conducted to study the effect of age, nutrition and gonadotrophic hormone therapy on the AP activity in the serum of ewe lambs from after weaning till puberty.

The serum AP activity in sheep and cattle decreases progressively with age (ALLCRAFT and FOLLEY, 1941; MYLREA and HEALY, 1968; TOMBELSON et al., 1973). KUNKEL et al. (1953) reported similar observation on bovine serum, however, when these authors subdivided the animals into three age groups (8—12, 17—26 months and 3—4 years), they failed to found any correlation between age and AP activity. JACKSON (1952), TUBA and MADSEN (1952) in rats, CHROOKSHANK et al. (1952) in ewes and cows and BO CRABO et al. (1973) stated that the AP activity could reflect the nutritional status. LI et al. (1947) reported that the serum AP was affected by the administration of pituitary hormones.

Materials and Methods

Sixty merino ewe lambs (average age 15 weeks; 23.8 kg body weight) were randomized in three nutritional groups, viz., Gi, Gii and Giii, 20 animals in each group. The animals in the different groups were matched for body weight and age. Gi was fed on 1.0 kg alfalfa hay plus 0.3 kg lamb grain mixture, Gii on 1.3 kg alfalfa hay, and Giii on 1.0 kg meadow hay plus 0.3 kg crushed maize day/animal. The respective rations were offered twice daily to lambs at 12-hr intervals in group feeding. Clean water was available ad libitum and the animals were kept in well-lightened, good ventilated, closed pens all over the experimental period.

Blood samples were regularly collected at 4-week intervals from 15 lambs (5 per group), by jugular vein puncture at 10 a.m. Other 14 animals were subjected to blood sampling at 41 weeks of age. The serum AP activity was determined according the method of BODANSKY (1932, 1933).

Half of the animals were injected with choriogonadotrophic hormone (HCG, Choriogonin, G. Richter, Budapest) with 100, 150, and 250 I.U each at 32, 36 and 40 weeks of age, respectively. The other half served as control. The statistical analysis was performed according to SNEDECOR and COCHRAN (1967).

Results and Discussion

Effect of age

Mean ± standard error, range, standard deviation and coefficient of variation for AP activity as affected by the age of lambs are presented in Table I and Fig. 1. The enzyme activity showed no significant change within each of periods from 19 to 31 and from 34 to 41 weeks of age. Therefore, the values within each period were combined. The overall averages were 6.68 and 12.48 B.U./100 ml serum, respectively. In general, the values were within the ranges reported by ALLCRAFT and FOLLEY (1941), FORD (1958), LEAVER (1968) and EL-ABDIN and HAMSA (1972) for the ovine serum.

The progressive decline with advancing age, reported by ALLCRAFT and FOLLEY (1941), ROUSEL and STALLCUP (1966), MYLREA and HEALY (1968) and TUMBELSON et al. (1973), was not proved in the present study. However, a very slight decline in the AP activity was observed between 19 and 31 weeks and between 34 and 41 weeks. On the other hand, significant sudden rises were noticed at 34 weeks as well as at 45 weeks. These sudden rises in the enzyme activity seem to be due to changes in the reproductive activity of sex organs and endocrine glands, rather than in advancing in age. The first rise may be attributed to the starting of ovarian function, though no sign of heat was observed at that time. The ovarian function was proved by testing the chlorine

Table I

Serum AP activity in merino ewe lambs at various ages

Date of sampling	Age, weeks	No.	Enzyme activity, B.U/100 ml,			
			Mean ± S.E	Range	S.D	C.V, %
11 May	19	15	6.69±0.66	1.9—11.2	2.57	37
8 June	23	15	6.62±0.60	1.5— 9.8	2.35	35
5 July	27	15	7.25±1.03	1.5—18.9	4.01	55
3 August	31	15	6.17±0.54	2.7— 8.5	2.09	33
31 August	35	15	13.14±1.57	4.8—27.7	6.09	45
28 September	39	15	13.17±1.24	7.8—22.5	4.81	36
12 October	41	29	12.20±0.90	3.6—22.6	4.86	39
9 November	45	15	17.62±1.63	9.7—41.0	6.13	34

content in the cervical mucus by a semiquantitative method developed by BAKSAI (unpublished data). The relationship between the ovarian activity and the chlorine content in cervical mucus was reported by VAN DER WESTHUYSEN and VAN NIEKERK (1969), TURNBULL et al. (1967) in ewes and by VEZNIK et al. (1964) and EL-NAGGAR et al. (1970) in cows. The second rise observed at 45 weeks was coincident with the time of conception in 71% of the

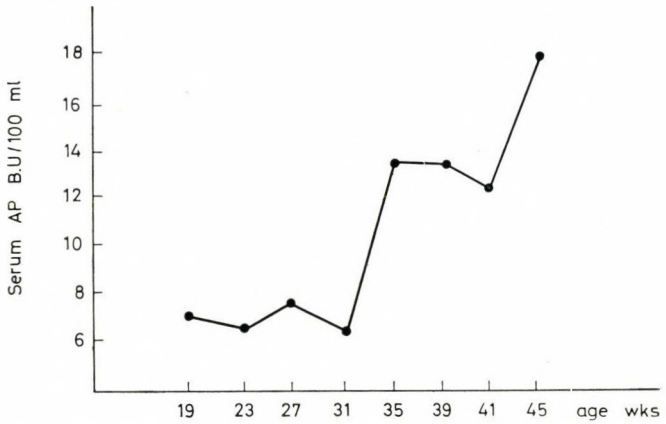

Fig. 1. Serum AP activity as a function of age in merino ewe lambs

lambs. When the data were combined on the basis of incidence of conception, the enzyme activity was 19.45 *vs.* 13.20 B.U/100 ml serum for conceived and nonconceived animals, respectively.

Effect of HCG

Data concerning the effect of HCG treatment on the AP activity are shown in Table II and Fig. 2. It appears that the enzyme activity did not significantly vary before the hormone injection. The hormone administration increased the activity in the serum of treated animals compared with the untreated: 15.02, 14.73 and 14.40 *vs.* 11.27, 11.61 and 10.01, respectively. The enzyme activity at 45 weeks (on 9th Nov.) became again similar for the two groups (17.83 B.U/100 ml for treated and 17.53 B.U/100 ml for untreated lambs). This may also explain the rises in the enzyme activity previously reported on the basis of ovarian function and incidence of conception.

The results concerning the hormone effect are consistent with those demonstrated for leucocyte AP activity by POLISHUK and DIAMANT (1973), who stated that the enzyme activity reached peak values on and about the day of the luteinizing hormone surge in human subject. They suggested that the enzyme is under hormone control.

Table II

Effect of HCG treatment on serum AP activity in merino ewe lambs

Date of sampling	Treatment	No.	Enzyme activity, B.U/100 ml			
			Mean ± S.E	Range	S.D	C.V, %
3 Aug.	Before	7	6.33±0.78	3.9— 8.8	2.07	31
		8	5.81±1.54	2.7— 9.7	4.35	74
31 Aug.	Treated	7	15.02±1.66	8.6=20.5	4.40	29
	Untreated	8	11.27±2.46	4.8—27.7	6.96	58
28 Sept.	Treated	7	14.73±1.20	9.8—19.7	3.18	21
	Untreated	8	11.61±1.95	7.8—22.5	5.46	46
12 Oct.	Treated	15	14.40±1.37	7.9—22.6	5.32	36
	Untreated	14	10.01±0.81	3.6 15.8	2.92	29
9 Nov.	Treated	6	17.83±2.60	9.7—28.3	6.37	35
	Untreated	8	17.53±3.81	10.0—41.0	10.78	61

Treatment: HCG (Choriogonin, G. Richter, Budapest (i.m. injected, doses 100, 150 and 250 I.U on 17 Aug., 7 Sept., and 5 Oct., respectively.

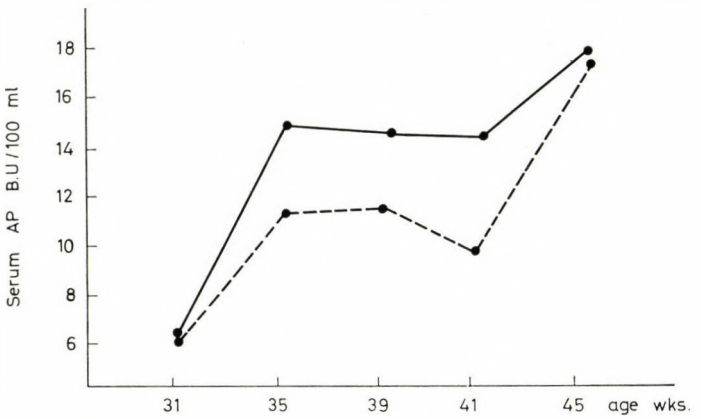

Fig. 2. Effect of HCG treatment on serum AP in ewe lambs ●———● treated; ● — — — ● untreated

Effect of nutrition

Table III and Fig. 3 indicate the effect of nutrition on the serum AP activity in ewe lambs. The enzyme activity in serum of lambs of Gi was only slightly higher than that for lambs of either Gii or Giii. The corresponding activity did not vary between Gii and Giii except on 9th Nov., about the conception time. This may reflect the conception rate rather than the nutritional status (conception rate for Gii was 40% *vs.* 60% for Giii). The difference in the enzyme activity due to nutrition is consistent with the data reported by TUBA and MADSEN (1952), JACKSON (1952), CHROOKSHANK et al. (1952) and

Table III

Effect of nutrition on serum AP in merino ewe lambs

Nutritional group	Av. body wt, kg	No.	Enzyme activity, B.U/100 ml			
			Mean ± S.E	Range	S.D	C.V, %
Gi	32.20	5	7.56±0.60	6.0— 9.4	1.35	17
Gii	29.30	5	4.36±0.86	1.9— 7.4	2.17	48
Giii	29.80	5	7.84±1.18	4.9—11.2	2.67	34
Gi	34.60	5	7.84±0.57	6.2— 9.8	1.28	16
Gii	31.00	5	7.06±0.72	4.5— 8.5	1.61	22
Giii	31.50	5	4.96±1.35	1.5— 9.0	3.02	60
Gi	39.80	5	10.90±2.66	3.7—18.0	6.29	57
Gii	34.60	5	5.34±1.31	1.5— 9.0	3.10	57
Giii	34.00	5	5.92±0.80	2.6— 7.5	1.90	32
Gi	41.60	5	7.08±0.85	5.4— 7.9	2.01	28
Gii	37.60	5	5.82±0.94	2.7— 8.5	2.22	38
Giii	38.20	5	5.62±0.96	3.9— 8.0	2.29	40
Gi	44.80	5	15.26±3.20	9.2—27.7	7.57	49
Gii	39.40	5	12.87±2.87	4.8—20.5	6.78	52
Giii	40.00	5	11.94±1.37	8.5—15.4	3.24	27
Gi	47.20	5	13.62±2.35	8.9—22.4	5.65	41
Gii	41.40	5	13.14±2.36	8.9—19.7	5.57	42
Giii	43.40	5	12.76±1.81	7.8—18.4	4.29	33
Gi	40.90	9	12.92±1.88	3.6—22.4	5.94	14
Gii	41.60	10	14.08±1.79	8.9—22.6	5.12	12
Giii	40.50	10	9.69±0.57	6.3—11.9	1.82	18
Gi	47.80	5	21.98±5.58	12.7—41.0	12.48	56
Gii	43.25	4	13.95±2.41	10.0—21.0	4.83	21
Giii	43.20	5	16.32±9.56	9.7—26.5	7.97	48

Gi, 1.0 kg alf. hay + 0.3 grain mixture; Gii, 1.3 kg alf. hay; Giii, 1.0 kg meadow hay + 0.3 kg crushed maize.

Bo CRABO et al. (1973). Such influence did not appear for Gii or Giii, since the rate of gain was about the same, however, it was considerably higher for Gi (Table III and Fig. 4). This is in agreement with data reported by FLETCHER et al. (1956) and BOGART et al. (1963), who found positive correlation between rate of gain and enzyme activity. On the other hand, LI et al. (1947) indicated that the enzyme activity was influenced by the pituitary somatotrophic hormone. This is also inconsistent with the present study.

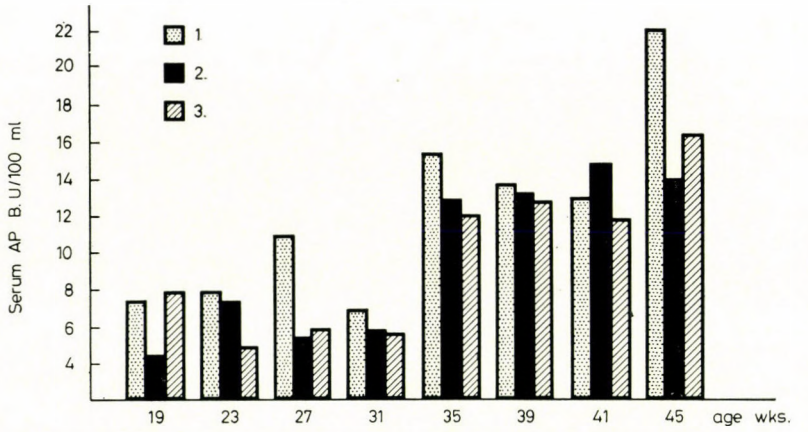

Fig. 3. Effect of nutrition on serum AP activity in merino ewe lambs. 1, Gi; 2, Gii; 3, Giii

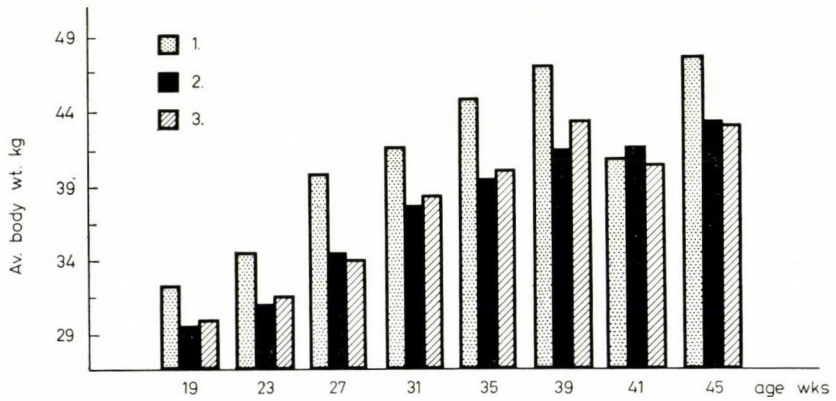

Fig. 4. Average body weight of ewe lambs in different nutritional groups. 1, Gi; 2, Gii; 3, Giii

Summary

The serum alkaline phosphatase was determined in ewe lambs at 4-week intervals. Average of enzyme activity at 19—31, 35—41 and 45 weeks was 6.68, 12.48 and 17.62 B.U/100 ml serum, respectively. The rise in activity at 35 weeks was coincident with starting of ovarian activity which was proved by fluctuation in cervicomuous chlorine content, although sign of heat did not appear at that time. The second rise was at conception time (71% of the animals conceived). Treatment with HCG increased the enzymeactivity. Lambs fed on alfalfa hay plus grain mixture had better gain and showed higher activity than those fed on either alfalfa hay only or meadow hay and crushed maize. The effect of age was not proved in the present study.

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References

ALLCRAFT, W. M. and FOLLEY, S. J.: Biochem. J. **35** (1941), 254. BO CRABO, N., BJÖRKLUND, E. and SIMONSSON, A.: Acta Vet. Scand. **14** (1973), 93. BODANSKY, A.: J. Biol. Chem. **99** (1932); **101** (1933), 93. BOGART, R., AMPY, F. R., ANGLEEMEER, A. F. and JOHNSTON, W. R.: J. Anim. Sci. **22** (1963), 993. CHROOKSHANK, H. R., CHALLIHAM, M. R. and GALAM, M. R.: J. Anim. Sci. **11** (1952), 560. EL-ABDIN, Y. Z. and HAMSA, S. M.: J. Egypt. Vet. Med. Assoc. **32** (1972), 247. EL-NAGGAR, M. A., BAKSAI-HORVÁTH, É. and KATONA, F.: Fortpfl. Haust. **7** (1970), 123. FLETCHER, J. L., SHRODE, R. R. and KUNKEL, H. O.: J. Anim. Sci. **15** (1956), 1119. FORD, E. J. H.: J. Anat. **52** (1958), 447. JACKSON, S. H.: J. Biol. Chem. **198** (1952), 553. KUNKEL, H. O., STOKES, D. K. Jr., ANTHONY, W. B. and FURTELL, M. F.: J. Anim. Sci. **12** (1953), 765. LEAVER, D. D.: Res. Vet. Sci. **9** (1968), 265. LI, C. H., KALMAN, C. and EVANS, H. M.: J. Biol. Chem. **16**947), 625. **9** (1MYLREA, P. J. and HEALY, P. J.: Aust. Vet. J. **44** (1968), 570. POLISHUK, W. Z. and DIAMANT, Y. Z.: Fert. Steril. **24** (1973), 245. ROUSEL, J. D. and STALLCUPP, T.: Amer. J. Vet. Res. **27** (1966), 1572. SNEDECOR, G. W. and COCHRAN, W. G.: Statistical Methods. Iowa, 1967. TUBA, J. and MADSEN, N. B.: Can. J. Med. Sci. **30** (1952), 18. TUMBELSON, M. E., MARLYN, F. B. and WINGFIELD, W. E.: Cornell Vet. **63** (1973), 65. TURNBULL, K., SHUTT, D. A. and BRADEN, A. W. H.: Aust. J. Exp. Agric. Anim. Husb. **7** (1967), 314. VAN DER WESTHUYSEN, J. M. and VAN NIEKERK, G. H.: J. S. Afr. Vet. Med. Ass. **41** (1969), 325. VĚZNIK, Z., LOJDA, L. and NAVRATIL, S.: Vet. Med. (Prague), 1964, 321.

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ULTRASTRUCTURE OF ADRENAL MEDULLARY CELLS IN THE GOOSE AND DUCK

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In the avian adrenal unlike the corresponding mammalian organ, the cortex and the medulla do not form separate layers. The cortical cells form an irregular network, which traverses the entire structure, and the medullary cells are arranged in groups of varying sizes. The latter cells show a basophilic granulation on staining with haematoxylin and eosin, and take on a brown stain on treatment with chromium salts. The chromaffin cells are the secretors of the catecholamines, adrenaline and noradrenaline.

The cytoplasm of medullary cells characteristically contains catecholamine granules. Adrenaline-containing and noradrenaline-containing cells can be differentiated from one another by size, shape and structure. Electron micrographs showing the ultrastructure of adrenal medullary cells are nowadays found in practically all histological textbooks, atlases and monographs, but related literary data are scanty on birds: even WELLS and WIGHT (1971) gave only a brief outline of the topic, based chiefly on data presented by others, in their contribution to the monograph of BELL and FREEMAN (1971). HALL and HUGHES (1970) studied the avian adrenal structure during the stage of embryonic development. Detailed studies on the structure and innervation of adrenal medullary cells in various avian species have recently been conducted by UNSICKER (1973).

In domestic geese and ducks well-defined periods of sexual activity follow one another in both male and female birds. The structural changes taking place in the endocrine glands and reproductive organs were followed up in this laboratory in a series of experiments and light microscopic structural and histochemical studies of the adrenal were described in a previous paper (HASSAN, 1975). The ultrastructural studies of medullary cells reported here represent a further step in this series of investigations.

Materials and Methods

The geese and ducks used in the experiments were procured from a large production unit (State Farm of Tata). Four adult birds of each species, including two males and two females, were studied every month, all the year round. From the animals killed by bleeding, organ specimens were taken immediately, and were fixed either in OsO_4 or in glutaraldehyde. Both fixatives were prepared in chilled (+4 °C) 2% Millonig buffer and were adjusted to pH 7.2. The glutaraldehyde-treated specimens were post-fixed in OsO_4. The fixation time was 1.5 hours throughout. The fixed blocks were dehydrated in step-graded

ethanol and were embedded in Durcupan ACM (Fluka). Sections were cut with a Reichert Om U2 ultramicrotome. The sections were counterstained with uranyl acetate and lead citrate, and were examined in a Tesla BS type 613 electron microscope. Semi-thin sections stained with toluidine blue were prepared for light microscopic examination.

Results

The medullary tissue can be readily distinguished from the bundles of cortical tissue also in the electron micrographs. The main elements of the medullary tissue are chromaffin cells and interstitium.

Chromaffin cells

The cytoplasm of these cells contains granules of catecholamine. Four cell types can be differentiated on the basis of the shape, size and structure of the granules, *viz.*, adrenaline-containing cells, noradrenaline-containing cells, so-called "dark cells" and transitory cell forms (sympathicoblasts).

Adrenaline-containing cells. These usually contain round granules 50—200 nm in diameter, surrounded by a limiting membrane. The size and density of the granules depend, apart from variations related to the plane of section, on the degree of accumulation, and degradation or elimination of the adrenaline-containing substance. The "mature" granules are the largest, and their homogeneous, highly electron-dense body is separated from the limiting membrane only by a narrow halo. The granules are variable in appearance, many of them are vesicle-like; in these, the dense nucleus is surrounded either by an "empty" halo or by a finely granular zone, but the entire vesicle may consist of evenly distributed, fine granules.

In some cells the cytoplasmic region is almost entirely filled by granules, among which mitochondria and ribosomes, but no other cell structure can be differentiated. In other cells there are absence or paucity of granules in certain parts of the cytoplasm, *e.g.*, in the perinuclear zone, at the sinusoidal margin, or in the region of synapses. The mitochondria are oval or elongated and have lamellar cristae. The granular endoplasmic reticulum is underdeveloped, but free ribosomes and polysomes are abundantly present. The Golgi complex is composed of small, flattened saccules. The nucleus is centrally placed, round or oval, with a loose chromatin content.

Noradrenaline-containing cells. These are characterized by vesicular or vacuolar structure rather than dense granulation of the cytoplasm and remind, at first sight, of degenerated or inadequately fixed cells, the more as all membranous organelles, except the mitochondria, have a fragmented appearance.

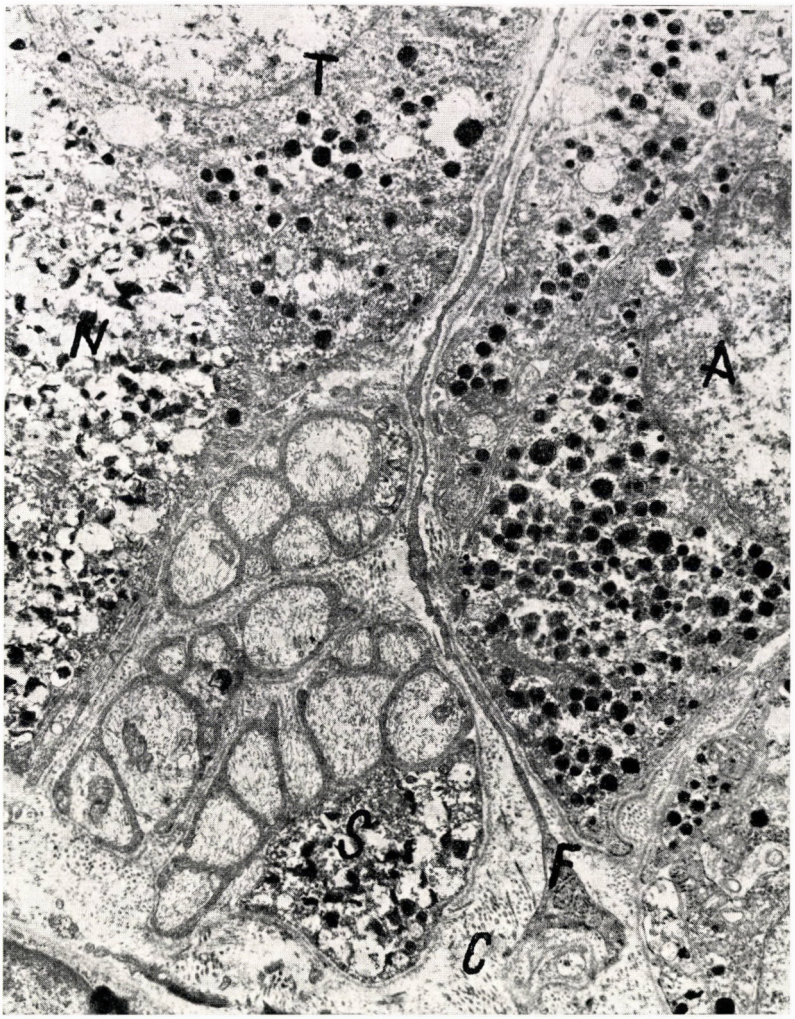

Fig. 1. Adrenaline-containing (A), noradrenaline-containing (N) and transitory (T) cells in adrenal medullary tissue. Note unmyelinated nerve fibres, a detail of a Schwann cell (S), the long, thin process of a fibroblast and collagen fibres (C) in the interstitium. (Female goose, August. OsO_4 fixation, ×9260)

The noradrenaline-containing vesicles are 150–500 nm in diameter, and only very few of them have contents similar in density to adrenaline granules. The electron-dense part of the vesicle contents varies in amount, and in part of the cells it is tightly pressed against the membrane in the form of a semilunar or sickle-shaped mass. The cross sections of the mitochondria are round or oval, and the mitochondrial cristae are of the lamellar type. In places granular endoplasmic reticulum is seen with distended spaces. The Golgi appa-

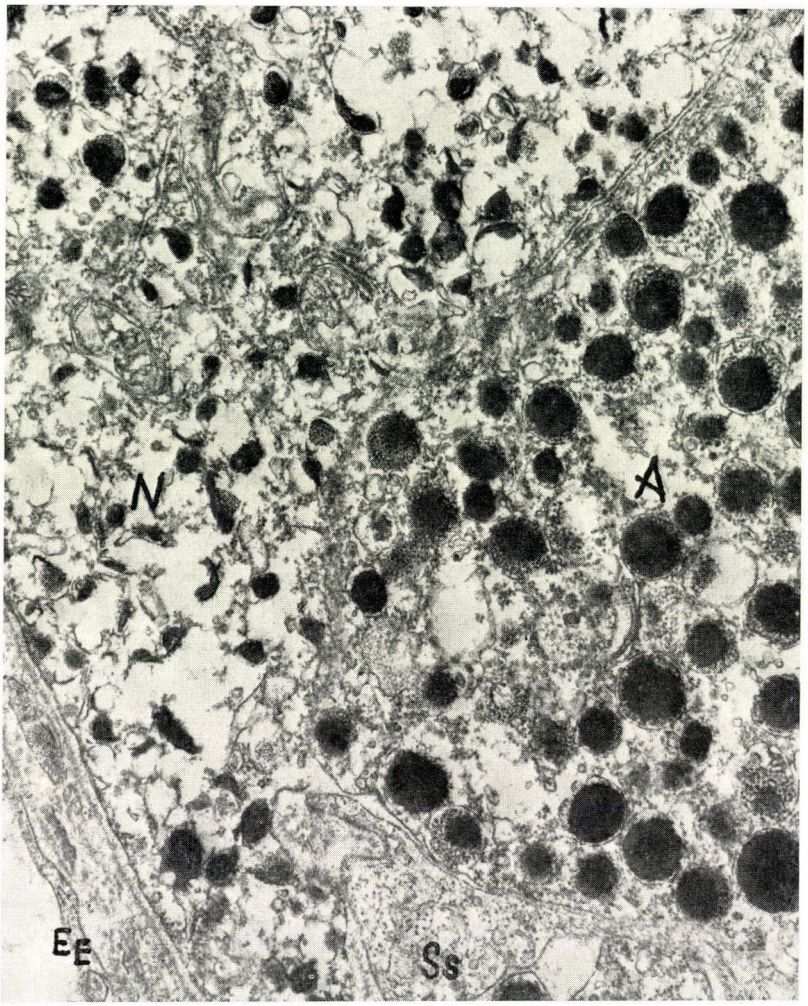

Fig. 2. An adrenaline-containing (A) and a noradrenaline-containing (N) cell in adjacent localization. The cell on the left side has a vesicular-appearing cytoplasm, that on the right contains "store" granules. Note the detail of a somatic satellite cell (Ss) between the two different medullary cells. A basement membrane separates the cells from the sinusoidal endothelium (E) and from the interstitial space. (Female duck, August. OsO_4 fixation, ×19 800)

ratus is small, but the saccules forming it are dilated, above all at the marginal parts, nearby which vesicles, comprising a substance of low electron density, are localizing. The ribosomes are generally less numerous than in the adrenaline-containing cells. The large, round nucleus contains a moderate amount of chromatin.

The so-called "*dark cells*" representing the third type of medullary elements in the avian adrenal are characterized by the presence of large, dense

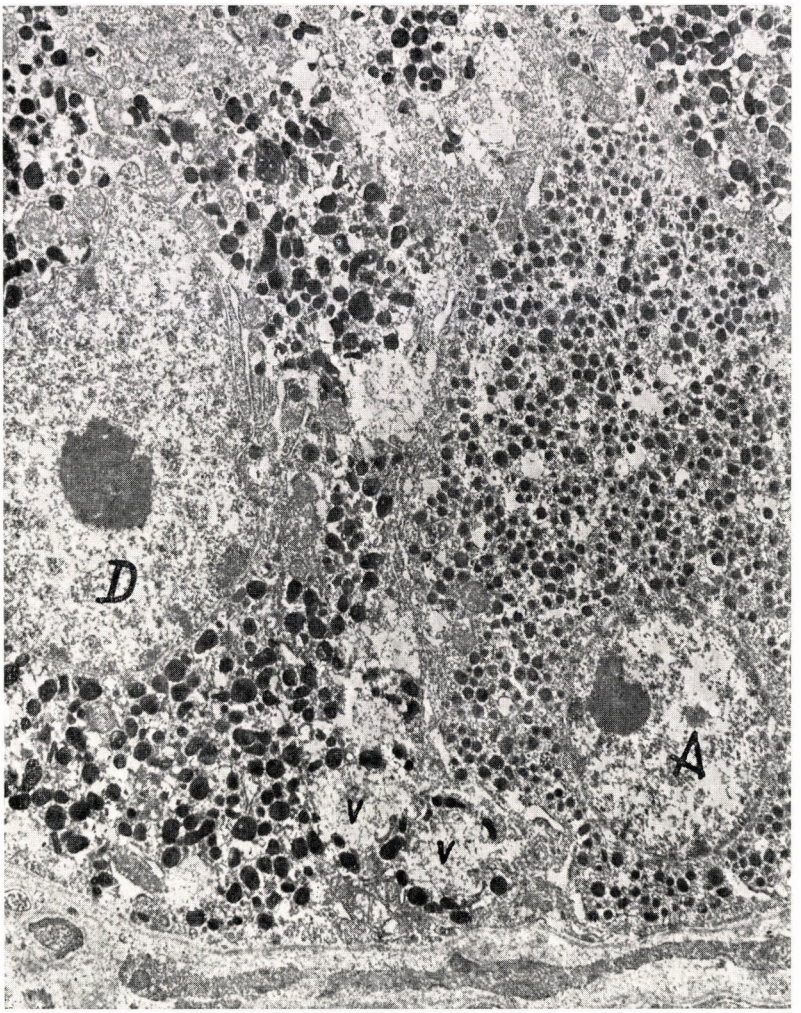

Fig. 3. A dark cell (D) with few vacuoles surrounded by granules is seen nearby an adrenaline-containing cell (A). (Drake, February. Glutaraldehyde–OsO_4 fixation, ×9260)

granules, which fill almost the entire cytoplasm, accounting for a darker shade than seen in the rest of medullary cell elements. Both the shape and the size of the dark cells are variable: some are larger than 500 nm in diameter, and round, oval, and elliptic forms were equally seen. The granules are packed with a highly electron-dense substance, which may render the limiting membrane invisible. The mitochondria are either oval or elliptic in shape, with lamellar cristae. The canals of the granular endoplasmic reticulum are as a rule dilated. Golgi apparatus was not seen in our electron micrographs, so that no information was obtained on its structue. Many free ribosomes were apparent,

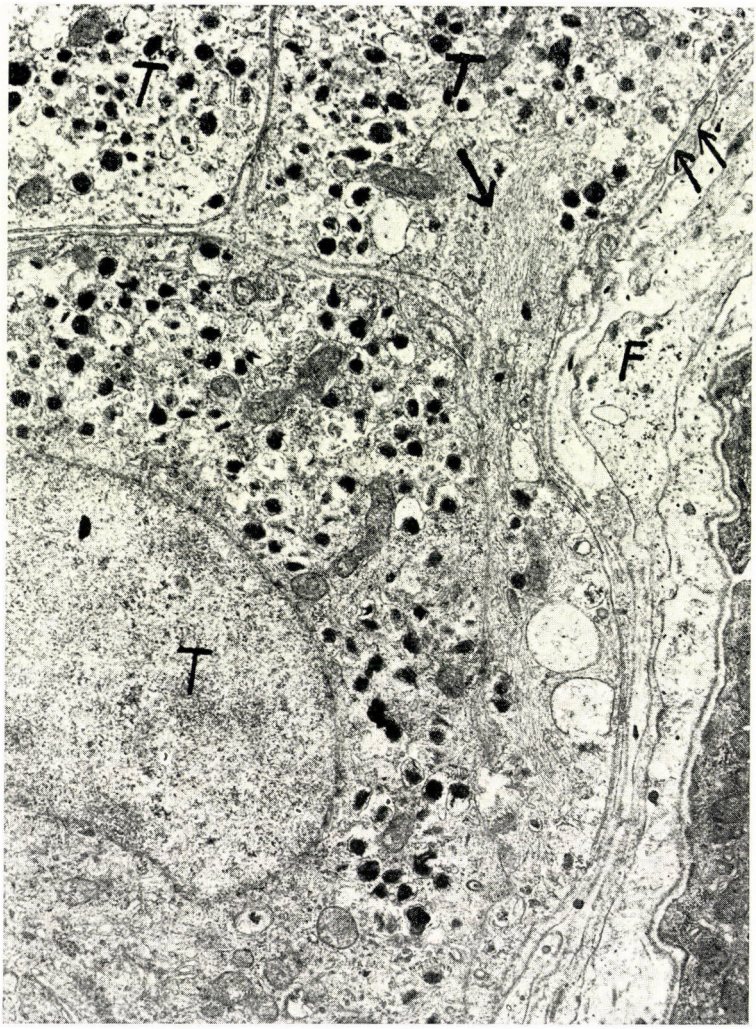

Fig. 4. One among three neighbouring transitory cells (T) extends a process in which parallelly arranged filaments are seen. Note the thin lamella of a satellite cell and the basement membrane (arrows) nearby the transitory cells, and the detail of a fibrocyte (F) in the interstitium. (Gander, February. OsO_4 fixation, ×11 600)

above all in the marginal and perinuclear regions. The chromatin was evenly distributed, and the nucleolus was large and highly electron-dense in the bulky, oval nuclei.

The transitory cell forms (sympathicoblasts) have also been classified as chromaffin cells on the basis of their cytoplasmic structure. Dense-core vesicles and catecholamine granules were either localized in one part of the cell, or were evenly distributed in the entire cytoplasm. Most vesicles were

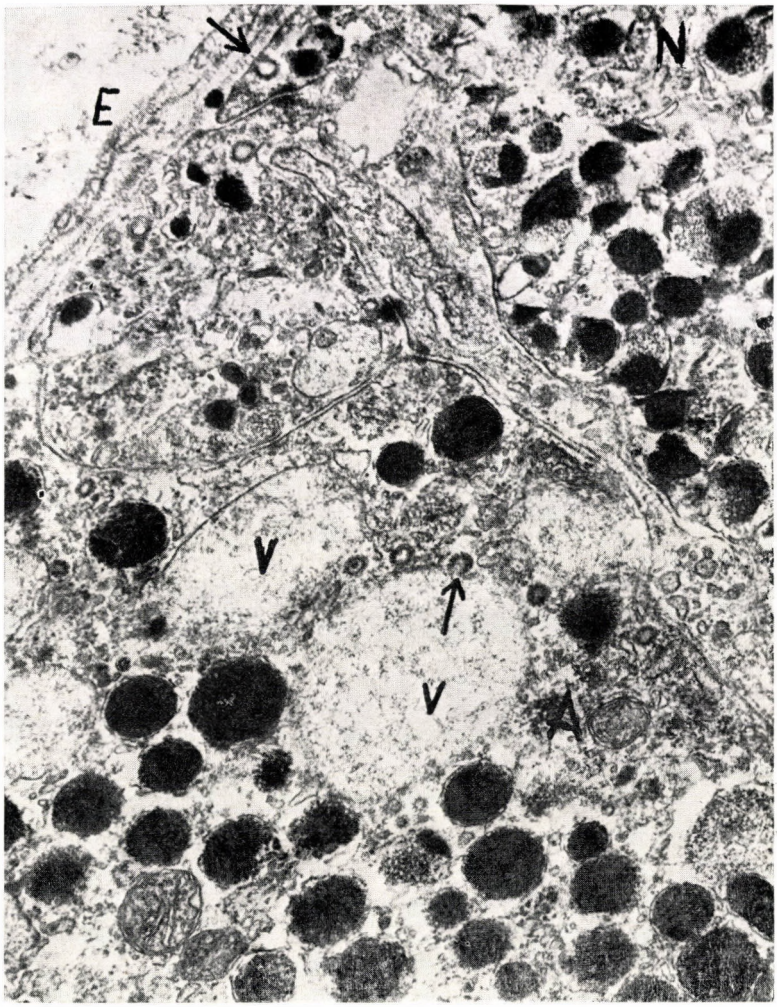

Fig. 5. An adrenaline-containing cell with vacuoles (V) and several coated vesicles in the cytoplasm; one of the vesicles opens into the vacuole, another into the extracellular space (arrows). E, sinusoidal endothelium; N, detail of a noradrenaline-containing cell. (Female duck, August. OsO_4 fixation, ×29 700)

10—50 nm in diameter, but also larger ones, filled by a substance of low electron density, were often seen. The cytoplasmic details free from granules comprised well-defined organelles, *i.e.*, a well-developed granular endoplasmic reticulum, densely arranged ribosomes, and many elongated mitochondria with cristae. Some cells were even extending processes, inside which vesicles, vacuoles, granules and parallelly ordered filaments were seen.

Vacuoles 1—1.5 μm in diameter, filled by a granular-filamentous substance of low electron density, were also present in the chromaffin cells. Some

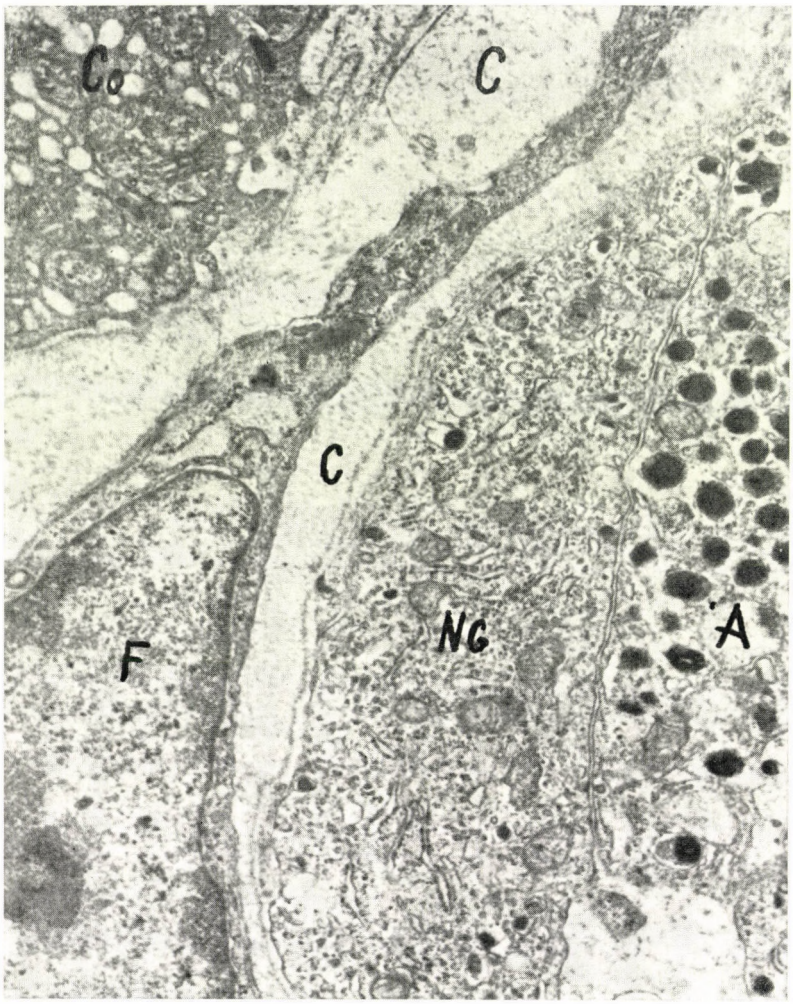

Fig. 6. Note detail of a nerve cell (Nc) nearby the adrenaline-containing cell. There are one fibroblast cell (F) and several collagen fibres (C) in the interstitium. Detail of a cortical cell (Co) with large tubular mitochondria and distended endoplasmic reticulum is also seen. (Female duck, February. OsO_4 fixation, $\times 9260$)

vacuoles were surrounded by vesicles and granules of varying size and density, others showed association of the limiting membrane with coated vesicles; the latter structures were also seen to associate with the cell membrane, or to occur freely and singly in the cytoplasm.

The medullary cells formed minor or major groups or nests of various sizes in the spaces between the bundles of cortical tissue. In accordance with their nervous origin of the medullary cells, the aggregations of these cells are surrounded by elements of glia cell origin, so-called "somatic" satellite cells.

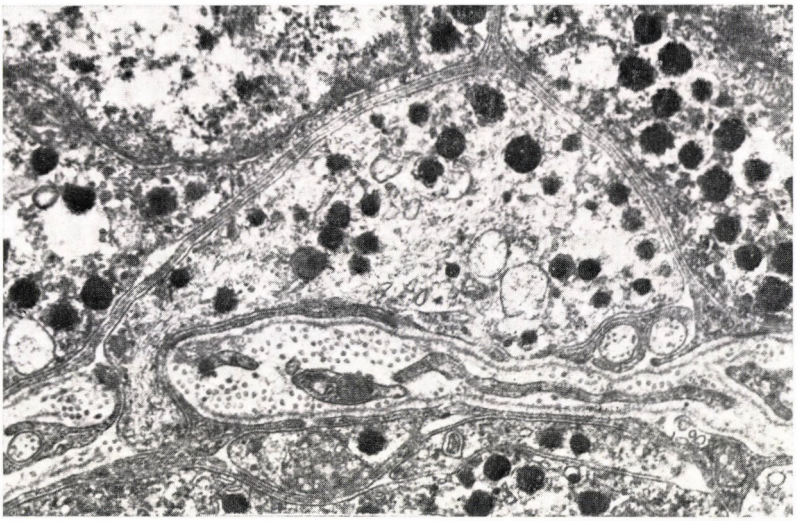

Fig. 7. Adrenaline-containing granules, vesicles and filaments in the swollen part of an unmyelinated adrenergic axon, which is surrounded by the thin lamella of a Schwann cell and by the basement membrane. (Female goose, August. OsO_4 fixation, $\times 14\,850$)

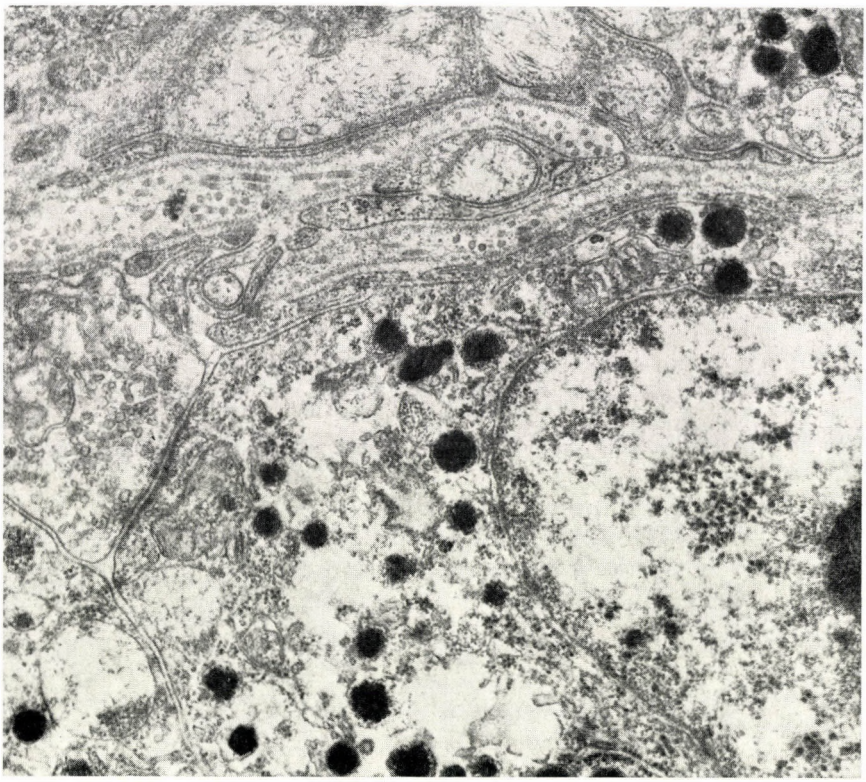

Fig. 8. Cholinergic synapsis with adrenaline-containing medullary cell. (Female goose, August. OsO_4 fixation, $\times 19\,800$)

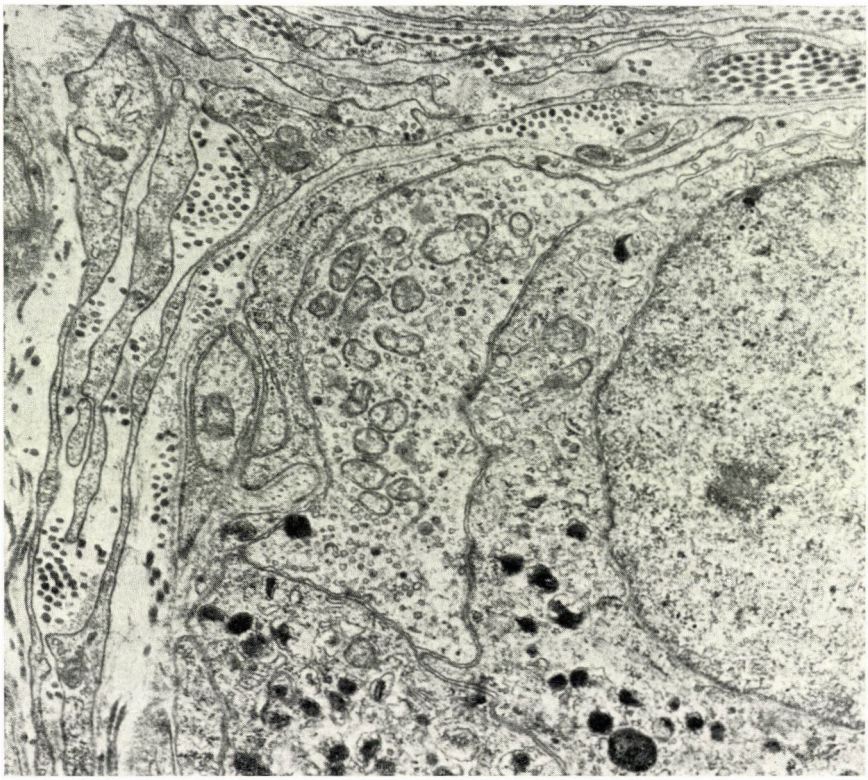

Fig. 9. Synapsis to two neighbouring transitory cells. The presynaptic plasma contains many mitochondria, cholinergic vesicles and a few dense-core vesicles. The synaptic axon terminal is separated from the interstitium by a satellite cell and by the basement membrane. Several fibroblastic processes and cut surfaces of collagen fibres are seen in the interstitium. (Female goose, August. OsO_4 fixation, ×16 200)

The thin layer of satellite cells forms a coat over the free surfaces of medullary cells and also extends between the latter to varying depths. At each discontinuity of the satellite cell coat, the basement membrane was either directly associated with the medullary cells, or extended above the satellite cells. The medullary cells were in close contact with one another over large surfaces; the intercellular space separating them was about 20 nm wide or in places wider, and interdigitations were also frequent. Many desmosomes connected the cells with one another, but zona occuludens-like structures were never seen.

Elements resembling nerve cells in structure also occur in the medullary tissue; these contain a well-developed granular endoplasmic reticulum and many ribosomes and only few catecholamine granules.

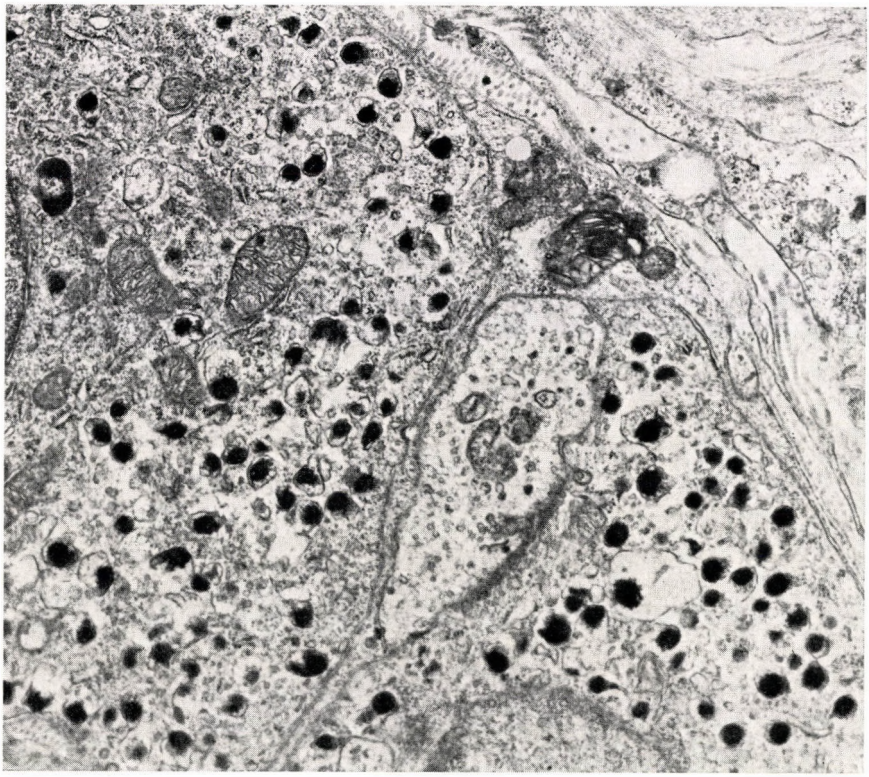

Fig. 10. Synapsis to a noradrenaline-containing cell. The presynaptic axon terminal contains cholinergic vesicles, a few dense-core vesicles and mitochondria. An autolysosome is seen in the satellite cell localizing nearby the synapsis. Note the detail of a fibroblast and several collagen fibres in the interstitium. (Female goose, August. OsO_4 fixation, ×16 200)

The interstitium

The interstitium contains fibroblasts, collagen fibres, Schwann cells, unmyelinated nerve fibres, and sinusoids lined by fenestrated endothelium. The Schwann cells may contain catecholamine granules, vesicles and vacuoles, which reside in the perikaryon and in the thick processes. The unmyelinated nerve fibres vary in thickness; a swollen — buffy — axon, containing catecholamine granules, vesicles and vacuoles, is shown in Fig. 7; this structure probably represents a detail of an adrenergic nerve fibre.

The pre-ganglional sympathetic nerve fibres form synapses with the medullary cells; these axo-somatic synapses have cholinergic-type terminals. The axon terminals are of various shapes and each communicates with two or three neighbouring cells. A few of the cholinergic vesicles in the presynaptic plasma are of the dense-core type.

Discussion

The cell elements of the adrenal medulla produce adrenaline and noradrenaline. External influences immediately elicit the secretion of these catecholamines. The relationship of the sympathetic nerve system to the adrenal medullary cells is not only functional, but also phylogenetic, the latter being differentiated from sympathicoblast cells. The functional connection between nerve system and medullary cells is maintained by direct synapses. Cells described by UNSICKER and others as sympathicoblasts, phaeochromoblasts, or various transitory forms of chromaffin cells, were also found in our material. TEHVER (1972) mentioned, with reference to similar findings of other authors, that transitory cell forms, characterized by the simultaneous presence of chromaffin cells and short processes, can also be found in mammals. Another indication of the nervous origin of the medullary cells is that they are surrounded by satellite cells of glia origin.

The sympathicoblasts differentiate to adrenaline and noradrenaline producing cells, which store these substances in the form of granules or vesicles. The adrenaline and noradrenaline granules can be differentiated from each other on the basis of shape, size and structure. Descriptions of the adrenaline-containing granules are generally unequivocal, but opinions are divergent on the characteristics of the noradrenaline granules. BLOOM and FAWCETT (1969), and also others, have described the latter as spherical or oval, with markedly electron-dense contents. SANDBORN (1970), BENEDECZKY et al. (1965) and UNSICKER (1973) characterized the medullary cells on the basis of studies in various species as elements reminiscent of degenerated or inadequately fixed cells with cytoplasmic vesiculation. Cells of such appearance were met in the present studies, too, in geese and ducks of both sexes, in sexually active and inactive periods alike. Either OsO_4 or glutaraldehyde was used for fixation.

The third type of chromaffin cells, the so-called dark cells, were found by BENEDECZKY et al. (1965) in the adrenals of the snake *Natrix natrix* and by ourselves in those of the goose and the duck. In view of the size, shape and structure of the granules seen in the dark cells, we believe that they represent a functional form of noradrenaline-storing cells, which transform to vesicular cells only when the active stage of secretion has ensued. The above-cited authors concluded from experimental observations that during the increased secretion of catecholamine, the vesicles, whereas in the resting state the granules are increasing in number.

BENEDECZKY et al. (1965) and UNSICKER (1973) hold the view that the large vacuoles present in the chromaffin cells contain the precursors (dopamine?) of adrenaline and noradrenaline. Vacuoles surrounded by granules variable in size and density were also found by us. Other vacuoles were associated with coated vesicles; the latter were present in large masses in the cyto-

plasm and part of them were merging with the membrane of the vacuole, opening into its lumen, while others were fused with the cell membrane and opened into the extracellular space. These structural details indicate that the coated vesicles either transport the contents of the vacuole into the extracellular space or *vice versa*, they transport extracellular material into the vesicle. Thus both exo- and endocytosis seem to have a certain support.

Summary

The ultrastructure of adrenal medullary cells was studied in male and female geese and ducks during the sexually active and inactive periods. Four types of chromaffin cells were found. The adrenaline-containing cells were 50—200 nm in diameter, mostly spherical in shape, and showed various degrees of electron density. The noradrenaline-containing cells had vesiculated cytoplasms; the vesicles, 150—500 nm in diameter, were spherical or ellipsoid, and comprised various amounts of a diversely arranged dense substance. The so-called dark cells, characterized by the presence of tightly-arranged, large, highly electron-dense cytoplasmic granules of heterogeneous shapes, might be regarded as a functional variant of the noradrenaline-containing cell. The fourth chromaffin cell type was represented by a transitory form (sympathicoblast), equipped with processes. Small, multipolar nerve cells were also found in the medullary tissue.

In chromaffin cells two types of large vacuoles have been described. These cells form cholinergic synapses with the preganglional sympathetic fibres. Some of the unmyelinated fibres have been identified as adrenergic. The chromaffin cells are surrounded by somatic satellite cells. The cell elements are connected with one another by desmosomes. The interstitium contains fibroblasts, collagen fibres, Schwann cells, unmyelinated nerve fibres and sinuses lined with fenestrated endothelium.

The ultrastructure of the adrenal medulla does not notably differ either between male and female birds, or between the sexually active and inactive periods.

References

Benedeczky, I., Puppi, A. and Tigyi, A.: Acta biol. hung. **15** (1965), 271. Benedeczky, I., Pupfi, A., Tigyi, A. and Lissák, K.: Acta biol. hung. **15** (1965), 285. Bloom, W. and Fawcett, W.: A Textbook of Histology. Philadelphia—London—Toronto, 1969. Hall, K. B. and Hughes, H. P.: Z. Zellforsch. **108** (1970), 1. Ham, A. W.: Histology. Philadelphia—Montreal. 1957. Hassan, A.: Acta vet. hung. **XXV** (1975), 19. Reimer, L.: Elektronenmikroskopische Untersuchungs- und Präparationsmethoden. Berlin—Heidelberg—New York, 1967. Sajonski, H. and Smollich, A.: Mikroskopische Anatomie. Leipzig, 1972. Sandborn, E. B.: Cells and Tissues by Light and Electron Microscopy. New York—London, 1970. Texbep, Й. Т.: Гистология эндокринных желез домашних животных. Тарту, 1972. Wells, J. W. and Wight, A. L.: The Adrenal Glands. In: Bell, D. J. and Freeman, B. M.: Physiology and Biochemistry of the Domestic Fowl. London—New York. 1971. Unsicker, K.: Z. Zellforsch. **145** (1973), 389. Idem: Ibidem **145** (1973), 417. Idem: Ibidem **145** (1973), 557.

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BIOMETRICAL STUDY ON EWES' GENITAL TRACT DURING POSTESTRUS

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In recent years, artificial insemination in sheep has aroused much interest. Techniques have been developed and improved, yet many problems remained unsolved. Knowledge of the normal dimensions and weights of the genital organs is necessary for an adequate diagnosis as well as for the control and treatment of infertility. However, the related literature is poor and the animals studied up to the present time were of unknown reproductive history (ewes were usually obtained from slaughterhouses). In the present study ewes of known reproductive history and slaughtered on the same day of the estrous cycle were involved.

Material and Methods

Twelve healthy merino ewes (21 months old) were slaughtered on the third day of the estrous cycle. The animals were checked for estrus by allowing a fertile ram to inseminate them. These ewes already reached puberty at 9—10 months of age and lambed at 14—15 months. They were fed on dry lot from 4.5 months till slaughter.

The genital organs were removed soon after slaughter, transported to the laboratory, where they were carefully freed from extraneous tissues and thoroughly examined. Notations on gross anatomy and biometry were recorded within 30—60 min. The techniques of De Lange (1950) and Sisson (1959) were followed when the different parts of genital organs were measured. A similar technique was used by Abdel-Rahman (1966), who studied the female sex organs of Egyptian buffaloes. The measurements were taken by means of steel callipers, except the length of the Fallopian tubes and uterine horns which were taken with a thin wire, later adjusted on a centimeter scale.

The ovarian length was represented by the dimension from tubal to uterine extremities, width from medial to lateral surfaces (at largest distance) and thickness from attached to free borders. The two ovaries were separately weighed after trimming and follicles over 2 mm in diameter were estimated. A thin longitudinal section was made to expose ovarian structures.

The external diameter of the uterine horns at the external bifurcation, and between the internal and external bifurcations at middle portion, as well as the external length of the horns along the mid-dorsum (from apices to external bifurcation) were estimated. After separating the broad ligaments and straightening the oviducts and horns, genitalia were cut open, the length of oviduct (from tubo-uterine junction to ostium abdominale), of horns (from tubo-uterine junction to internal bifurcation), of uterine body (from os internum to internal bifurcation), of cervix (from os internum to os externum including portio vaginalis), of vagina propria (from os externum to the vestibule of the vagina) and of the vestibule of vagina (from the vulvo-vaginal border to vagina propria) was estimated. The width of these organs was represented by their internal breadth. The Fallopian tube was not cut open, therefore the external diameter at mid-portion represented the width. The distance between the internal and external bifurcations, diameter of external genital opening and weight of the organs were also determined. Data were subjected to statistical analysis as described by SNEDECOR and COHRAN (1967).

Results and Discussion

Data concerning average ± standard error, range, standard deviation and coefficient of variation of dimensions and weight for ovaries and ovarian structures are shown in Table I and those for various parts of the genital tract in Table II.

Table I

Average ± S.E, range, standard deviation and coefficient of variation for dimensions and weight of ovine ovaries and ovarian structures

Observations	No.	Average ± S.E	Range	S.D	C.V, %
Length, mm	L 12	16.50±0.61	11.0—20.0	2.12	12.9
	R 12	16.62±0.66	13.0—21.0	2.30	13.9
Width, mm	L 12	9.72±0.26	8.0—11.3	0.92	9.4
	R 12	9.79±0.36	7.0—11.0	1.23	12.5
Thickness, mm	L 12	11.77±0.53	10.0—15.0	1.87	15.8
	R 12	12.57±0.44	9.8—15.0	1.53	12.1
Weight, g	L 12	0.89±0.04	0.7—1.4	0.14	15.9
	R 12	0.96±0.08	0.5—1.6	0.29	29.5
Fol. over 2 mm	L 12	10.25±1.30	3.0—20.0	4.51	44.0
	R 12	11.33±1.10	3.0—16.0	3.82	33.7
∅ larg. G. fol., mm	12	7.40±0.38	5.0— 9.0	1.31	17.7
Larg. ∅ CL, mm	12	9.20±0.39	6.0—17.0	2.98	32.3
∅ CA, mm	12	4.50±0.39	3.0— 6.0	1.37	30.4

S.E, standard error; ∅, diameter; L, left; R, right.

Table II

Average ± S.E, range, standard deviation and coefficient of variation of dimensions (cm) and weight (g) of ovine female genital tract

Observations	No.	Average ± S.E	Range	S.D	C.V, %
Fallopian tubes:					
Length	L 12	20.41±0.74	16.5—27.0	2.57	12.6
	R 12	19.39±0.36	16.9—21.0	1.25	6.4
Width	L 12	0.24±0.01	0.2— 0.3	0.03	12.6
	R 12	0.26±0.01	0.2— 0.3	0.03	11.6
Weight	L 12	0.68±0.03	0.5— 0.9	0.13	19.1
	R 12	0.67±0.02	0.5— 0.9	0.09	13.4
Uterine horns:					
Ext. length	L 12	10.79±0.84	6.7—13.2	2.90	26.9
	R 12	10.92±0.49	9.5—14.0	1.80	16.4
Ext. ∅, 1.	L 12	2.01±0.08	1.3— 2.6	0.31	15.4
	R 12	1.91±0.10	1.3— 2.8	0.37	19.3
Ext. ∅, 2.	12	3.39±0.05	3.1— 3.7	0.18	5.2
Int. length	L 10	16.69±0.72	12.3—21.0	2.28	13.5
	R 10	16.67±0.87	11.1—21.5	2.78	16.6
Int. width	L 12	3.92±0.53	3.1— 4.5	1.77	45.1
	R 12	3.42±0.10	2.7— 4.3	0.37	10.6
No. of curuncles	L 12	41.83±2.32	31.0—55.0	8.07	19.2
	R 12	45.58±1.87	35.0—55.0	6.49	14.2
Weight	L 12	17.05±0.89	12.0—22.0	3.11	18.2
	R 12	17.09±0.80	13.5—21.3	2.78	16.2
Int.-ext. bifur.	L 12	6.15±0.33	4.5— 8.7	1.17	19.0
Body of uterus:					
Length	12	3.39±0.18	2.5— 4.4	0.64	18.8
Width	12	3.04±0.18	2.4— 3.9	0.64	21.0
Weight	12	5.37±0.33	3.7— 6.9	1.14	21.0
Cervix:					
Length	12	6.21±0.35	4.2— 8.6	1.24	19.9
Width	12	2.15±0.27	1.4— 5.0	0.94	43.7
Weight	12	10.91±0.27	7.3—16.9	1.14	21.0
Vagina propria:					
Length	12	9.75±0.29	7.9—11.0	1.03	10.5
Width	12	4.96±0.26	3.5— 6.7	0.91	18.3
Vestibule of vagina:					
Length	12	4.43±0.26	3.3— 6.0	0.92	20.7
Width	12	5.83±0.15	4.7— 7.0	0.53	9.0
Vaginal weight	12	38.18±2.32	26.8—49.9	8.01	20.9
∅ ext. opening	7	4.30±0.75	3.0— 7.0	1.99	46.2

Ext. ∅, 1., external diameter at external bifurcation; Ext. ∅, 2., external diameter between the two bifurcations; L, left; R, right.

The right ovary was slightly larger (2.09 *vs.* 1.89 cm^3), heavier (0.96 *vs.* 0.89 g), and contained somewhat more follicles over 2 mm in diameter (11.33 *vs.* 10.25) than the left one. These differences, not significant statistically, might be attributed to a more frequent activity of the right ovary. The percentage of corpora lutea in the right ovary was 58.3, verifying the slight difference in ovarian size and weight indicated. The more frequent activity of the right ovary compared to the left one was also demonstrated in ruminants by HAFEZ (1968), EL-SHEIKH and ABDEL-HADI (1970) and ROBERTS (1971).

The average ovarian weight obtained in the present study is about the same as reported by SINGH et al. (1974) for goats (0.96 *vs.* 0.91 for right and left, respectively). ALLEN and LAMMING (1961) reported higher values (1.42 ± 0.09 and 1.33 ± 0.09 g) for ovarian weight of ewe lambs reared on high and low nutrition levels, respectively. HAFEZ (1968) gave a range between 3 and 4 g. He added that the ovarian weight and dimensions are influenced by age, nutrition and the reproductive phase. The ovary was found slightly longer (16.56 mm on the average) than that reported by SISSON (1959) for ewes and by SINGH et al. (1974) for goats, but within the range reported by ROBERTS (1971) from 13 to 19 mm for ewes. The right and left ovaries did not show a consistent difference in either length or width (Table I), while the average thickness for the right ovary, 12.57 mm, slightly exceeded that for the left one, 11.77 mm. This may be attributed to the higher percentage of the presence of corpora lutea protruding from the free ovarian surface.

The average of the largest diameter of CL at 3 days postestrus, 9.2 mm, is consistent with that reported by HAFEZ (1968) for mature CL. The largest diameter of the Graafian follicle, 7.4 mm, falls within the range 4.0—10 mm, given by HAFEZ also. However, the obtained values do not reach the upper limit of 10 mm. This may be due to differences in maturity of follicles (3 days postestrus *vs.* mature follicle; present and HAFEZ's data, respectively).

Twin ovulation was not recognized in the present study, although twin lambing had been recorded for these animals at the first lambing at 14—15 months of age (lambing rate per 100 ewes was 125). This indicates that the ovulation was restricted at 21 months as compared with the time at first breeding (at 9 months of age). Since ewes were a year older at the time of slaughter, this age difference would be expected to have the opposite, if any effect. It appears that the low ovulation rate may be due to confinement as suggested by DAHMEN (1969) and HULET et al. (1974). Some other modifying factors must be accounted for this consistent difference; e.g. the meterological factors were different during these two periods (September, 1973 and September, 1974) in Hungary. Furthermore, the animals were kept in the first period in the Vértesboglár, whereas in the second period in the clinic in Budapest, where the illumination was different.

The left Fallopian tube was longer than the right one (20.41 *vs.* 19.39 cm). Such an asymmetry was not found either in the width at the middle portion or in the weight of tubes. HAFEZ (1968) reported similar value for ovine tubal length; a greater difference (about 3 cm) was reported in goats by SINGH et al. (1974).

The external length and diameter, the internal width as well as the number of curuncles in uterine horns also showed asymmetry (Table II). Similar difference in length of horns was demonstrated for goats (HADI, 1961; BASU, 1961; SINGH et al., 1974). The average weight of uterine horns was approximately the same for right and left (17.09 *vs.* 16.05 g), respectively. The distance between the internal and the external bifurcations measured 6.15 cm on the average. The horns were coiled in spiral forms, curving downward, forward, outward and turning backward and upward and gradually tapering towards the Fallopian tubes. The tubo-uterine junction was not as clearly distinct as in bovine.

The average body of uterus (3.39 cm) obtained in the present study was longer than given by HAFEZ (1968) (1—2 cm in ewes) and by SINGH et al. (1974) (1.3 cm in goats). The cervical lumen was tightly closed by reciprocal prominences and depressions of the mucous membrane in 5 to 6 annular folds. The cervical length was 6.21 cm on the average and varied between 4.8 and 8.0 cm. The respective values were 4.0—10.0 cm in ewes (HAFEZ, 1968) and 3.42 cm in goats (SINGH et al., 1974).

The average length of the vagina propria was 9.75 cm and that of the vestibule of the vagina was 4.43 cm. The goat vaginal length (from vulvo-vaginal border to os externum) was only 8.0 cm as reported by SINGH et al. (1974).

Summary

The dimensions and weights of the genital tracts of 12 ewes (21 months old) were measured on the third day of the estrous cycle. The obtained values were compared with those reported in literature for ewes and goats.

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References

ABDEL-RAHMAN, H. and ABDEL-HADI, A. H.: Anatomy and histology of the female reproductive tract in the Egyptian buffaloes. Thesis. Cairo, 1966. ALLEN, D. M. and LAMMING, G. E.: J. Agric. Sci. **57** (1961), 87. BASU, S., GOSWAMI, S. K. and DE, S. K.: Indian Vet. J. **38** (1961), 302. DAHMEN, J. J.: Caldwell Bran Station. Annu. Repr., 1969. DE LANGE, A.: Onderstepoort J. Vet. Sci. **24** (1950), 125. EL-SHEIKH, A. S. and ABDEL-HADI, A. H.: Indian J. Anim. Sci. **40** (1970), 17. HADI, M. A.: Indian J. Vet. Sci. **32** (1964—65), 106. HAFEZ, E. S. E.: Reproduction in Farm Animals. Philadelphia, 1968. HULET, C. V., SHELTON, M., GALLAGHER, J. R. and PRICE, D. A.: J. Anim. Sci. **38** (1974), 1218. ROBERTS, S. J.: Veterinary Obstetrics and Genital Diseases. Michigan, 1971. SINGH, S. K., BHATTACHARYA, A. R. and LUKTUKE, S. N.: Indian Vet. J. **51** (1974), 81. SISSON, S. and GROSSMAN, J. D.: The Anatomy of the Domestic Animals. Philadelphia—London, 1959. SNEDECOR, G. W. and COCHRAN, W. G.: Statistical Methods. Iowa, 1967.

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STUDIES ON THE CYCLIC CHANGES IN PLASMA PROGESTERONE LEVEL AND PREGNANDIOL ELIMINATION IN CATTLE

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The alterations of progesterone level during the sexual cycle can be followed up either by demonstration of the biologically inactive progesterone metabolite pregnandiol in the urine, or by determination of progesterone concentration in the peripheral blood.

According to determinations by the method of FEHÉR et al. (1967), the average daily pregnandiol excretion was 2.0 mg during oestrus, 6—7 mg on days 7—8, and 4—5 mg on days 14—15, of the cycle (HARASZTI et al., 1968). New methods have markedly increased the reliability of the measurement of plasma progesterone based on gas chromatography (JANNONS et al., 1964; VAN DER MOLEN and GROEN, 1965; STABENFELDT et al., 1969) or on the determination of competitive protein binding (MURPHY, 1964 and others). E.g. ROBERTSON (1972) found 0.1—0.4 ng/ml plasma progesterone immediately before and during oestrus, 3—6 ng/ml from the 11th to 13th day after oestrus, and again 0.1—0.4 ng/ml at 72—24 hrs before the next heat. Similar average values were obtained by STABENFELDT et al. (1969), who found 0.4 ng/ml in the follicular phase and 6.6 ng/ml in the luteal phase. The differences between the values given by different authors have in all probability been due to the diversity of the methods employed.

Materials and Methods

We examined five animals, of which four were 18—20 months old heifers and one was a 3-year-old cow which had already produced one calf. The animals were kept under uniform conditions of environment and were fed on the same schedule throughout the experiment and during the preceding month. Determinations of progesterone and pregnandiol were carried out in simultaneously obtained samples of blood and urine during the cycle, in cases of corpus luteum (CL) persistens and in the early stage of gestation. The day on which the animals showed the most pronounced signs of oestrus by behaviour and on clinical and rectal examination as well, was regarded as the day of onset of the cycle. Blood samples for progesterone determination were obtained from the jugular vein, and urine excreted over 24 hrs was used for each pregnandiol determination. The determinations were carried out on five to six occasions per animal.

The plasma progesterone levels were measured by the gas chromatography method developed by FEHÉR et al. (1975). The working principle of this procedure is the gas-chromatographic analysis of a plasma extract purified by thin-layer chromatography. A Pye-Unicam 104 analytical gas chromatograph and a 3% SE-30 column and flame ionization detector were used. The quantitative determinations were carried out by a standardized method evolved by the method of FEHÉR et al. (1967), based on acid hydrolysis, extraction, thin-layer chromatography and acetylation of the test material, followed by spectrophotometric reading of the colour reaction produced with sulphuric acid.

Results

The numerical results of the progesterone and pregnandiol determinations are shown in Tables I, II and III, the dynamics of the hormone levels are presented graphically as a function of time in Figs 1—5. The continuous line represents the pregnandiol, the broken line the progesterone concentration. The hormonal cycles of the 3-year-old cow No. I and of heifer No. II are shown in Table I and Figs 1 and 2. The blood progesterone level and the urinary elimination of pregnandiol changed similarly during the cycle. Peak values were generally obtained between 8 and 14 days.

The cycles of heifers No. III and IV were arrested by persistence of CL metoestrum; with animal No. IV, interruption of this state was possible by crushing the corpus luteum by rectal intervention. The clinical state is reflected in Table II and Figs 3 and 4. Heifer No. III showed return of a high progeste-

Table I

Blood progesterone levels and urinary pregnandiol excretion during the normal cycle of the bovine female

Day of cycle	Animal No. I		Animal No. II	
	Progesterone, ng/ml	Pregnandiol, mg/24 h	Progesterone, ng/ml	Pregnandiol, mg/24 h
1	1.1 (oestrus)	0.8	1.8	2.0
6	1.7	1.8	2.8	4.0
8			4.8	5.1
9	5.4	6.1		
13	2.5	4.5		
14			6.2	7.0
16	1.4	1.7		
18			1.7 (oestrus)	1.9
19	1.2 (oestrus)	0.9		

Table II

Blood progesterone levels and urinary pregnandiol excretion during the cycle of two heifers with persistent corpus luteum

Day of cycle	Animal No. III	Animal No. IV	
	Progesterone, ng/ml	Progesterone, ng/ml	Pregnandiol, mg/24 h
1	0.2 (oestrus)		
3		0.7	0.1
7	0.7		
8		0.7	0.5
14	1.0		
16		1.4	1.6
18	1.7		
21		0.8	1.0
26	0.9		
27		1.5 (enucleation)	1.8
29		0.5	0.8
32	4.6		

Table III

Progesterone levels and pregnandiol excretion during early pregnancy of the bovine female

Day of cycle	Animal No. V	
	Progesterone, ng/ml	Pregnandiol, mg/24 h
1	0.7 (oestrus)	0.2
6	1.4	1.5
14	1.7	2.0
20	1.8	2.2
26	2.3	3.5

rone level on the 32nd day of observation. Heifer No. IV showed maximum progesterone level and pregnandiol elimination on days 16 and 27, but the increment was below the normal maxima.

The data of heifer No. V can be seen in Table III and Fig. 5. This animal had been inseminated on the day of oestrus and on the subsequent day again, so that it became pregnant. The plasma progesterone and urinary pregnandiol levels of this animal are characteristic of pregnancy both having risen continuously until reaching levels 4 to 10 times higher than the oestrus levels on the 26th day of gestation.

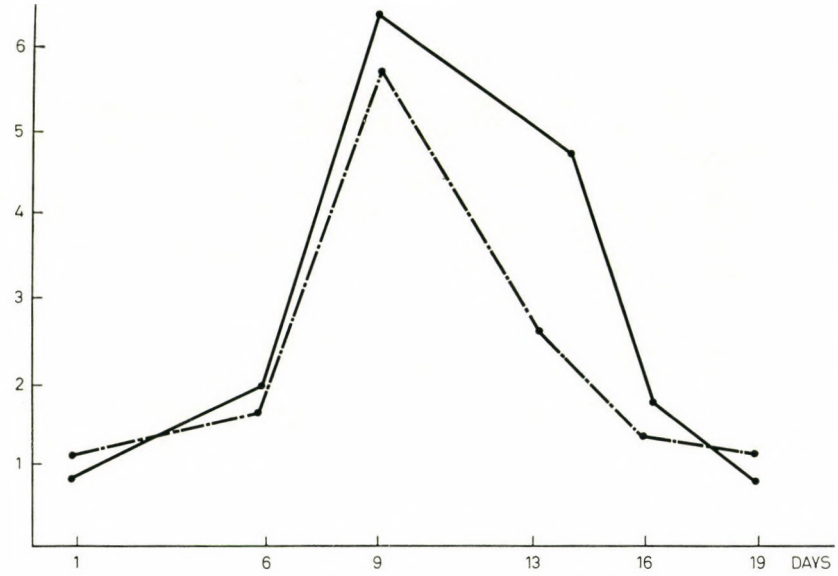

Fig. 1. Dynamics of the plasma progesterone level and urinary pregnandiol excretion during the normal cycle of a 3-year-old cow (Animal No. 1)

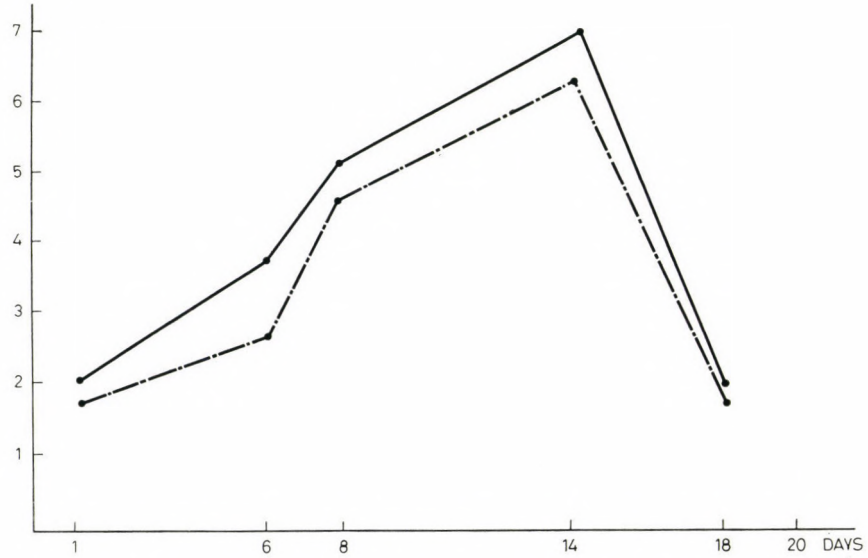

Fig. 2. Hormone levels in blood and urine of heifer No. II during a normal cycle

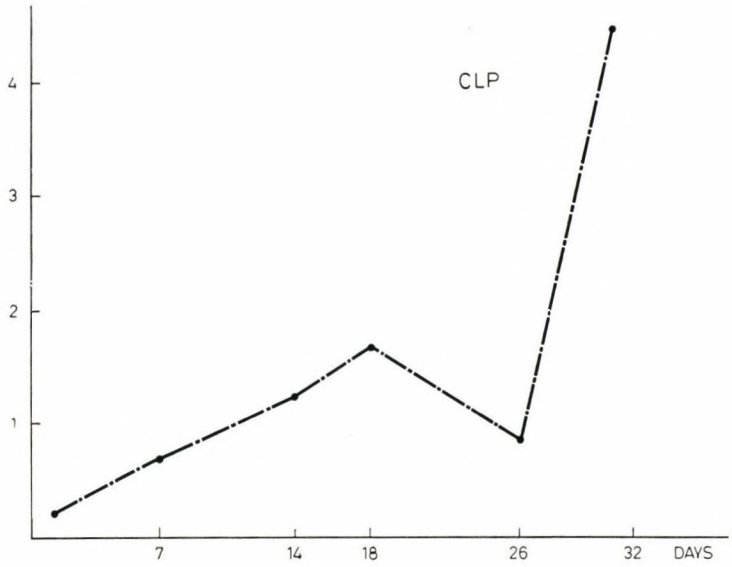

Fig. 3. Plasma progesterone levels in the case of persistent CL (heifer No. III). CLP, corpus luteum persistens

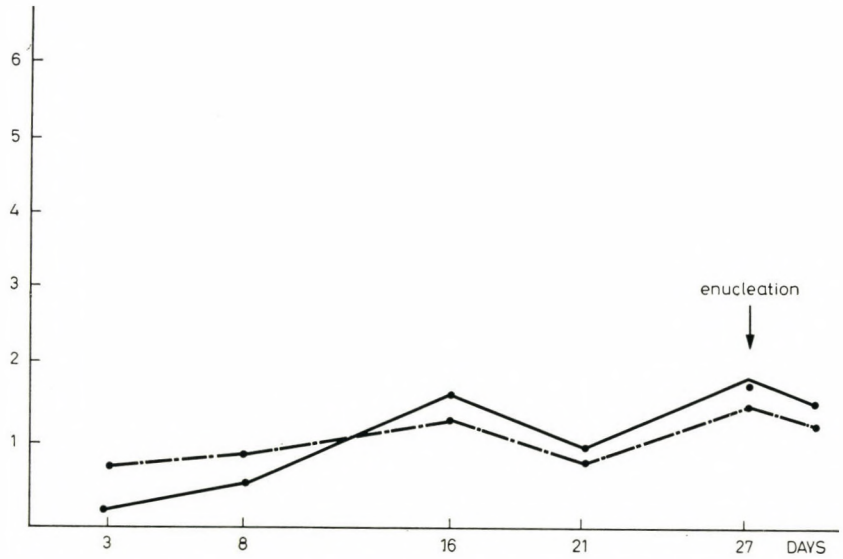

Fig. 4. Dynamics of plasma progesterone and urinary pregnandiol levels in the case of persistent CL, before and after enucleation (heifer No. IV)

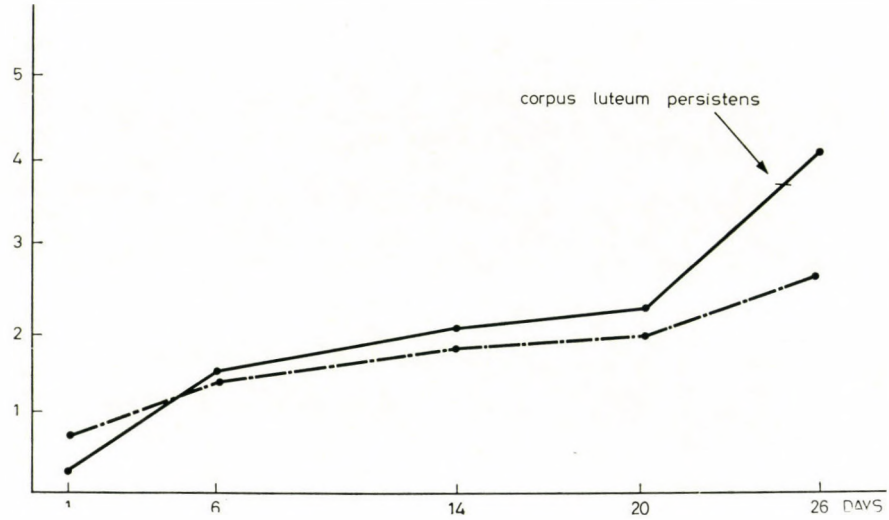

Fig. 5. Dynamics of plasma progesterone and urinary pregnandiol levels in early pregnancy, after successful artificial insemination (heifer No. V)

Discussion

The experimental results clearly reflect the parallelism of changes in plasma progesterone and urinary pregnandiol levels with the development and functional state of the CL. The progesterone levels measured in the course of oestrus were similar to those reported by GUPTA and POPE (1968), and thus generally higher than those given by STABENFELDT et al. (1969) and ROBERTSON (1972), but determinations in the later phases of the cycle were in agreement with those described by the latter authors. The clinical examinations and the laboratory tests unequivocally showed that each of heifers No. I and II had a normal cycle. Animals No. III and IV both had a persistent CL, characterized by a low progesterone level with peaks at 27 and 32 days, a tendency reminding of the rise of plasma progesterone in the early stage of pregnancy as seen in heifer No. V. Crushing of the persistent CL in heifer No. IV was followed 48 hrs later by fall of the prolonged high progesterone level and urinary pregnandiol excretion to practically oestrus levels. This accords well with the finding of EDQUIST et al. (1970) that the progesterone level fell to 0.5 ng/ml 3 days after enucleation performed at 37 days of pregnancy and followed by abortion, whereas it persisted at 4.8—5.0 ng/ml in the corresponding early stage — between 16 and 36 days — of uninterrupted pregnancy. According to ROBERTSON (1972), during early gestation the plasma progesterone level corresponds roughly to that characteristic of the CL phase of the cycle. The cyclic changes of urinary pregnandiol elimination were the same as those determined earlier (HARASZTI and FEHÉR, 1970).

As far as we are informed, parallel examinations of plasma progesterone and urinary pregnandiol levels in the bovine female have not yet been described in the literature. The data reported in this paper support the conclusion that the simultaneous determination of these two parameters of ovarial function is a valuable aid in the evaluation of the hormonal function of the ovary and for taking appropriate therapeutic measures if required.

Summary

The alterations of progesterone level during the sexual cycle of the bovine female can be followed up either by demonstration of the biologically inactive progesterone metabolite pregnandiol in the urine or by determination of the progesterone concentration in the blood plasma. Average daily pregnandiol excretion was determined as 2.0 mg during oestrus, 6.8 mg at 7—8 days and 4.6 mg at 14—15 days of the cycle. Plasma progesterone levels in the normal cycle were assessed as 1.2—1.8 ng/ml on the day of oestrus, 1.7—2.8 ng/ml at 6 days, 4.8—5.4 ng/ml at 8—9 days, 2.5—6.2 ng/ml at 13—14 days and 1.2—1.7 ng/ml at 18—19 days, when the oestrus was returning. In the cases of persistent corpus luteum and early pregnancy, the changes of both parameters were characteristic of the clinical state.

References

EDQUIST, L. E., EKMAN, L., GUSTAFSSON, B. and ASTRÖM, G.: Zbl. Vet. med. **17** (1970), 899. FEHÉR, T., BAKSAI, É. and HARASZTI, J.: Magyar Állatorvosok Lapja (MÁL), 1967, 457. FEHÉR, T., FEHÉR, G. K. and BODROGI, L.: J. Chromatogr. **111** (1975), 125. GUPTA, S. E. and POPE, O. S.: J. Endocrinol. **40** (1968), 12. HARASZTI, J. and FEHÉR, T.: Acta vet. hung. **20** (1970), 333. JANNONE, M. E., MCCOMAS, D. B. and GOLDFIEN, A.: J. Gas Chromatogr. **2** (1964), 30. MURPHY, B. E. P.: Nature **201** (1964), 679. ROBERTSON, H. A.: Canad. J. Anim. Sci. **52** (1972), 645. STABENFELDT, G. H., EWING, L. L. and MCDONALD, L. E.: J. Repr. Fert. **19** (1969), 433.

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ISOLATION OF PARAINFLUENZA-3 (PI-3) VIRUS FROM CATTLE DISEASED WITH ACUTE RESPIRATORY SYMPTOMS

By

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An acute respiratory disease, characterized by extraordinary rapid spread, has been affecting the large cattle herds in Hungary since the early 'sixties. Its incidence was initially sporadic, later it tended to rise, and occasionally cattle kept in small private farmyards were also involved. The condition has been termed "bovine contagious cough" in the Hungarian veterinary literature.

The disease is easily identified by its epizootiological and clinical characteristics, which differ from those of both infectious bovine rhinotracheitis and adenovirus-induced pnexmoenteritis. However, despite the well-defined picture and course, different types of viruses have been isolated from affected cattle; among others parainfluenza-3 (PI-3) virus (REISINGER et al., 1959) and respiratory syncytial (RS) virus (PACCAUD and JAQUIER, 1970) have been described as causal factors and type-A influenza virus has also been incriminated (TANYI, 1974).

The epizootiological characteristics, symptoms and gross lesions of the disease were studied and described by several authors (ROMVÁRY, 1961; CSONTOS, 1970; BARTHA, 1973; DOMÁN, 1974; RADNAI, 1974; TANYI et al., 1974), but aetiological investigations had failed until CSONTOS (1970) reported isolation of PI-3 virus from three of the many cases pursued by virological methods. TANYI et al. (1974) have recently isolated influenza virus from animals showing the characteristic symptoms of the disease.

We isolated PI-3 virus or RS virus from calves diseased with acute respiratory symptoms. Isolations of PI-3 virus are reported in this paper.

Epizootiology and Course

Outbreaks of an acute respiratory disease, aetiologically unrelated to either IBR virus or adenoviruses, were studied by us in 23 herds during the 'sixties and in 9 herds in the early 'seventies. In every case, the disease spread rapidly in the affected populations, above all among first-pregnant heifers and fattening cattle, but occasionally it also involved 3—6 months old calves, causing severe symptoms. The course of the disease varied according to the environmental conditions, being mild and non-lethal in some herds, but causing severe economic losses in others. According to our own experience, the out-

breaks were generally milder and economically less relevant during the 'sixties than in the 'seventies. On 14 of the 23 large herds affected during the 'sixties, there were no fatal cases. In the remaining 9 herds the average loss due to death or emergency slaughter did not exceed 0.6%. The maximum loss was 2% mortality in one of the fattening bull herds involved. In contrast, only one of the nine herds studied in the 'seventies was free from loss due to spontaneous death; in seven herds the average mortality rate was 2.3% (34 animals of 1480) and in one herd 52% of the cattle (23 of 44) were lost.

A few outbreaks are described below in detail, with special regard to differences in course.

In one state farm (H) a group of 40 half-year-old growing calves was driven over 5 km distance from one unit of the farm to another, and was placed in an isolated house. Several calves began to show acute respiratory symptoms on the day after arrival, and within further three days the entire group was ill. Five calves developed pneumonia. These animals recovered after two weeks, owing to therapeutic intervention. No symptoms of disease occurred either in the herd of origin, or in the herd joined.

In a collective farm (D) 15 pregnant heifers were purchased from private owners for replacement of the cow herd which consisted of 100 animals. Three newly-procured heifers developed respiratory symptoms on the fourth day after arrival and the remaining 12 heifers on the fifth day. Several cows of the original herd also became diseased on the fifth day and the condition spread almost to the entire herd during the subsequent week. The most characteristic symptom was frequent cough, dry on the first day, then moist. The animals simultaneously showed serous nasal discharge and lacrimation, and the body temperature rose to 40.5—41.5 °C. After 24 hours the nasal discharge became mucinous, in several cases even purulent. Some animals developed subcutaneous emphysema in the spinal region. The overwhelming majority of the diseased animals recovered spontaneously in 3—5 days, only two heifers developed pneumonia but these, too, improved after two weeks as the result of antibiotic treatment. No animal died and no emergency slaughter was necessary. Very mild symptoms of the disease were also shown by growing calves kept in a house 50 m away from the cowshed, but it did not spread to the suckling calves maintained on the cows.

In a second state farm (K) the cow herd, the fattening bull herd and the rearing calf herd, 300, 200 and 120 animals, respectively, were kept each in a different unit. An outbreak of "contagious cough" occurred in the cattle unit of the neighbouring collective farm, about 6 km away from state farm K. One week later the fattening bullocks in the state farm began to show acute respiratory symptoms, although no new animal had been introduced to the farm for two months. Three days later the disease arreared in the cow herd and another two days later among the rearing calves as well. The latter showed

milder symptoms than either the fattening bullocks or the cows: they coughed less, and none of them developed subcutaneous emphysema. Nevertheless, two 5-month-old calves died of acute pulmonary emphysema during the subsequent week and further four calves (5%) succumbed to pneumonia in the following three-week period. Two bullocks (1%) and one cow (0.3%) also died, but none of the 2-month-old calves developed the disease.

In collective farm B, 44 heifers in an advanced state of pregnancy were procured from smallholders. The animals were tied down in empty stalls at one end of the fattening bull house, at the other end of which 20 cows, remnants of the original dairy herd, had been accommodated. Six weeks before the introduction of the pregnant heifer group an outbreak of acute non-fatal respiratory disease had taken place. All 44 newly-procured heifers developed severe, acute respiratory symptoms between 5 and 14 days after establishment in the house, but none of the fattening bulls still in the stable became ill. The few calves produced during the period of the outbreak remained healthy, but several miscarriages occurred. Postmortem examination showed acute emphysema, with air bubbles up to the size of a fist. The tracheal mucosa was dark red and filled with a foamy, viscous exudation. The disease did not spread to the other cattle unit of the farm.

Virus Isolation

Nasal swabs collected from diseased animals, and organ specimens from carcases were placed in Hanks' solution and were transported to the laboratory in ice box for processing on the same day, or on the next day at the latest. Rinsing fluids of swabs and 1 : 10 diluted organ suspensions were centrifuged at 3000 r.p.m. for 10 min, the supernatants were treated with antibiotics and were centrifuged again. In every case, the second supernatant was transferred to four tube cultures of calf kidney epithelium cells, using 0.1 ml as inoculum. Each culture was examined daily for cytopathic (CP) changes. Cultures showing no change were examined by the haemadsorption test of VOGEL and SHELOKOV (1957) after removal of the tissue culture fluid. CP changes were studied in preparations stained with haemalaun and eosin after embedding in celloidin. Paired sera taken from diseased animals at an interval of 3 weeks were tested for antibodies by virus neutralization and/or haemagglutination inhibition (HI) test, using 100 $TCID_{50}$ virus and serum diluted twofold serial steps. Antiserum to the strain "Umea" was used for the identification of isolates.

Results

Among the 23 herds studied in the 'sixties on account of outbreaks of acute respiratory disease, virus was isolated from only one, the growing calf herd in state farm H. The CP changes appeared on the 5th day of the second passage of the nasal swab obtained from a diseased calf. In haemalaun-eosin-stained preparations syncytium formation as well as intracytoplasmic inclusions taking on a vivid red stain and, in a few cells, also centrally placed intranuclear inclusions were seen. The tissue culture fluid of the changed culture agglutinated guinea-pig erythrocytes. Haemagglutination did not take place in the presence of antibodies to the virus strain "Umea", which also neutralized the infectivity of the isolate.

Paired sera taken from the diseased calves in the state farm H on the day of onset and three weeks later in the convalescent stage were also examined for HI antibodies. The test was positive in about half of the first blood samples at titres ranging from 1 : 20 to 1 : 40; the titre levels showed a considerable increase in the second samples and the antibodies appeared also in the initially seronegative cases. All calves which had not developed clinical illness during the outbreak in farm H also had specific antibodies in the second blood sample and those positive already in the first sample showed a titre increase of a degree similar to that developed by the fattening bullocks.

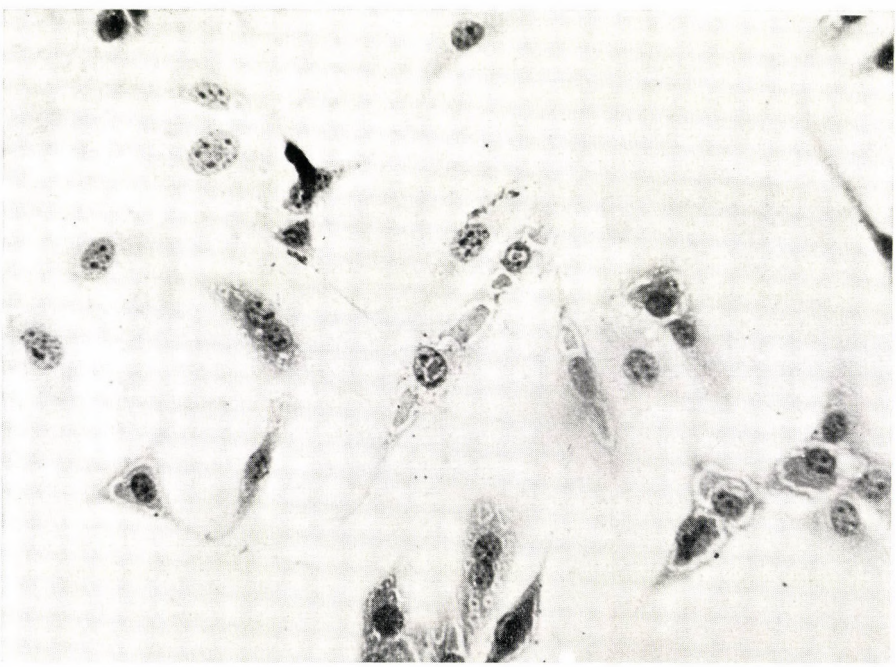

Fig. 1. Intracytoplasmic and intranuclear inclusions in calf kidney epithelial cell culture infected with PI-3 virus. H. and E., ×400

Isolation experiments failed in 22 of the 23 herds studied on account of acute respiratory disease during the 'sixties, although HI antibodies were always present in a minor or major part of the sera. However, a significant rise of the antibody level occurred only in four herds, in the remaining 18 there was no change from onset to convalescence.

Among the nine cattle herds studied for acute respiratory disease in the 'seventies, PI-3 virus was demonstrated in six, virus isolation failed in one, and respiratory syncytial (RS) virus was isolated in two (KÖVES and BARTHA, to be published).

All PI-3 virus strains were isolated from nasal swabs taken in the initial stage of the disease: all attempts to isolate virus from animals which had been ill for several days or from organs of spontaneously died or emergency-slaughtered cattle failed. The cytopathic changes characteristic of PI-3 virus appeared in the cell cultures about 96 hrs after inoculation with virus-containing nasal secretions. The cells became rounded, syncytia formed by a few cells made appearance; in the stained preparations intracytoplasmic inclusions and, in a few cells, intranuclear inclusions were also seen (Fig. 1). In the changed tissue cultures haemadsorption could be demonstrated before the CP changes became apparent, at about 48 hrs (Fig. 2). The virus grown in the tissue cultures agglutinated guinea-pig erythrocytes at low titres, *viz.* 1 : 16 or 1 : 32. Anti-

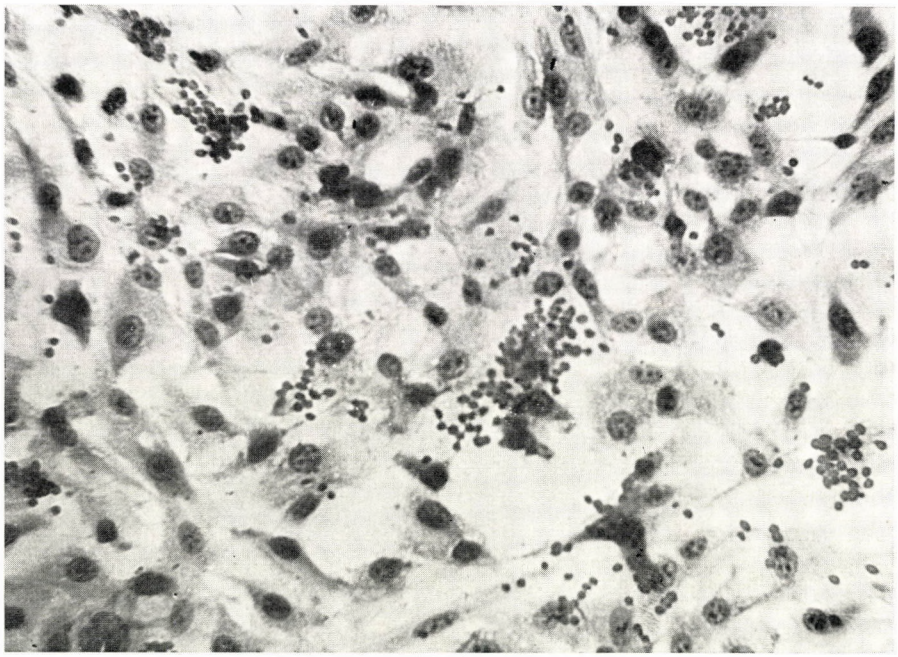

Fig. 2. Haemadsorption is taking place in cell cultures inoculated with PI-3 virus before the cytopathic changes are making appearance. H. and E., ×400

bodies to the PI-3 strain "Umea" neutralized all isolated obtained from the six herds.

Paired sera taken in the affected herds at the onset of the outbreak and three weeks later were tested by virus neutralization, because this test was in the meantime shown to be more reliable and more specific (CSONTOS, 1970) than the HI test previously applied. None of the first serum samples taken from the heifers which had been procured from private owners contained antibodies, whereas 50—80% of the animals reared in the farms had 1 : 4 to 1 : 128 titre levels of virus-neutralizing antibody in the first serum sample. The diseased fattening bullocks in the collective farm B all had antibodies in the titre range of 1 : 32 to 1 : 1024. The proportions of positive reactors and antibody levels varied between cattle groups within one and the same farm, but this had apparently no influence on the course of the disease. At the second sampling three weeks after onset, antibodies were found to appear also in those sera which had been negative at the first sampling in herds judged as positive on the grounds of virus isolation. In those herds, however, in which attempts at isolation of PI-3 virus failed, there was no rise in the antibody titres.

Conclusions

The outbreaks of bovine contagious cough were generally milder during the 'sixties than in the 'seventies. PI-3 virus was isolated from only one herd of the 23 studied in the first period, but from 6 of the 9 studied in the second one. Studies of paired sera in those herds from which virus was isolated also supported the conclusion that PI-3 virus had been the causal agent, *viz.*, specific antibodies were found in all convalescent sera and in those cases in which they had already been present in the first sample the titres showed a fourfold or still higher increases. In those herds in which attempts at isolation of PI-3 virus had failed serological evidence in support of its causal role could be obtained only in four instances, whereas in the majority of the cases there was no indication of the aetiological responsibility of either PI-3 or IBR, VD or adenoviruses. It should be remembered that PI-3 virus had been isolated from only three of the many outbreaks of "bovine contagious cough" studied in the 'sixties (CSONTOS, 1970). Since indirect evidence of the pathogenic role of PI-3 virus, *viz.*, the appearance of antibodies to this agent in the sera of the diseased animals was also lacking in part of the herds, CSONTOS arrived at the conclusion that the acute respiratory disease under study was multifactorial, *viz.*, viruses other than PI-3 may also have been involved in its aetiology. In agreement with CSONTOS's interpretation, we believe that the PI-3 virus had been responsible only for a minor part of the "contagious cough" outbreaks of the 'sixties, but it already played the main role in the more severe

enzootics occurring in the 'seventies, while RS and other viruses had a minor responsibility.

Our attempts to isolate PI-3 virus strains from organ specimens of died or emergency-slaughtered animals failed. This can probably be explained by the fact that specimens were taken in an advanced stage in which the virus may already have disappeared. In the infected cell cultures the haemadsorption test became positive well before the cytopathic changes made appearance. This phenomenon has been utilized for early diagnosis.

The course and outcome of the outbreak, as well as the extent of the losses, showed a marked individual variation. It is known that bovine respiratory diseases are greatly influenced by predisposing factors, virulence of the virus, degree of immunity, and occasional presence of secondary disease.

Fattening and gestation obviously played a major role as predisposing factors, for fattening bullocks and pregnant heifers or cows showed the most severe symptoms in almost all diseased herds. According to our own experience, transport is another important resistance-depressing factor. Nevertheless, we failed to find drastic resistance-decreasing stressors in most of the herds studied, particularly in the outbreaks of the 'seventies, which were characterized by epidemic-like spread from one area to the other, affecting even small groups of cattle in private farmyards, which are usually free of outbreaks of respiratory disease caused by other viruses.

Information about the impact of viral virulence on the severity of symptoms and losses is not emerging from the available data. However, the suspicion that more virulent strains than the indigenous ones may have been introduced into Hungary with recent cattle imports is supported by the practical observation that the spread of the disease has become epidemic-like, the symptoms were more severe and losses have considerably increased since the 'sixties.

The postinfection state greatly depends on the immune state of the organism. As previously described, HI antibodies to PI-3 virus were demonstrable in 50—80% of the serum samples in each large herd. It would be expected that animals possessing antibodies show very mild if any symptoms; notwithstandingly, the disease in herd mates of identical origin was unrelated to the presence or absence of serological antibodies and, to a certain extent also to the antibody titre. This weighs in favour of the intrepretation that virus-neutralizing and HI serum antibodies to PI-3 may play little role in the immune mechanism, competence being chiefly associated with the secretory (IgA) antibodies, not yet investigated by us. This accords well with the experience that the topical immunity of the tracheal mucosa has a decisive influence on the course of respiratory disease.

Since the symptoms did not usually reappear in the herds which had recovered from an outbreak, there was reason to postulate that active immunity

was developed in response to the infection. The duration of active immunity could not be judged from the available observations. It should nevertheless be noted that in farm B, those animals which had recovered from an outbreak, did not become ill when the newly-introduced group of susceptible (antibody-free) heifers placed with them developed severe symptoms. All animals resistant to the recurrence of the outbreak had high titres of virus-neutralizing serum antibodies.

Practitioners have reported rare relapses in individual cases. Since such cases have not yet been available for laboratory investigation, no aetiological statement can be made. The responsibility of two causal agents (PI-3, RS or other virus) for such apparent relapses seems highly possible.

The extent of losses from "contagious cough" greatly depends also on the presence or absence of secondary pneumonia. Minor resistance-depressing factors may already be sufficient to promote the development of pneumonia especially in young, 3—6 months old calves which, although develop the symptoms of the virus disease in a less characteristic form than growing or adult cattle, are as a rule more severely affected than the latter, owing to greater liability to secondary catarrhal-purulent pneumonia.

Summary

PI-3 virus was isolated from several outbreaks of a rapidly-spreading, acute bovine respiratory disease, affecting above all large cattle herds. Seronegative replacement heifers in advanced state of pregnancy developed the disease in a very severe, often lethal form, shortly after their transfer from smallholder farms to the large-production unit. In several cases the fattening bull herd and the 3—6 months old rearing calves also became ill, and many of the latter developed secondary pneumonia. Those animals which had been affected by outbreak six weeks earlier and had high serum antibody titres remained clinically healthy, while newly-introduced susceptible animals in the same stable became severely ill. Animals possessing low serum levels of virus-neutralizing antibodies developed mild to severe symptoms. It may be supposed that secretory (IgA) antibodies play a greater role in the immune response than the virus-neutralizing serum antibodies.

References

BARTHA, A.: Állategészségügyi és Takarm. Közl. **3** (1973), 4. CSONTOS, L.: Fortpfl. Haust. **6** (1970), 308. DOMÁN, I.: Magyar Állatorvosok Lapja (MÁL), 1974, 761. PACCAUD, M. F. and JAQUIER, CL.: Arch. ges. Virusforsch. **30** (1970), 327. RADNAI, L.: MÁL, 1974, 765. REISINGER, R. C., HEDDLESTON, K. L. and MANTHEI, C. A.: JAVMA **135** (1959), 147. ROMVÁRY, J.: MÁL, 1961, 241. TANYI, J., ROMVÁRY, J., ÁLDÁSY, P. and MÁTHÉ, ZS.: MÁL, 1974, 373. VOGEL, J. and SHELOKOV, A.: Science **122** (1957), 358.

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ISOLATION OF RESPIRATORY SYNCYTIAL (RS) VIRUS FROM CATTLE DISEASED WITH RESPIRATORY SYMPTOMS

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(Received June 5, 1975)

Aetiological investigations into an acute respiratory disease, showing rising incidence and causing great economic losses in the large cattle herds, resulted in demonstration of the causal role of parainfluenza-3 (PI-3) virus in part of the cases (Csontos, 1970; Bartha and Köves, 1975).

Since, however, a causal relationship was not always demonstrable between the characteristic acute symptoms, termed in Hungary as "contagious cough" and the PI-3 virus, a multifactorial aetiology of the disease has been postulated (Csontos, 1970). Recently respiratory syncytial (RS) virus has been shown to be responsible for outbreaks of an acute bovine respiratory disease both in Western Europe (Paccaud et al., 1970; Wellemans et al., 1970; Jacobs et al., 1971) and Japan (Inaba et al., 1970). In Hungary, isolation of influenza virus was also reported from similarly diseased cattle (Tanyi et al., 1974).

The causal role of RS virus has been confirmed by us in the course of studies conducted recently in nine diseased herds. RS virus was isolated from two of these herds.

Epizootiological Course

In a state farm (N) the beef bull house, accommodating 200 animals, was stocked gradually by introducing 20 fattening bullocks in each turn. Fifteen days after the introduction of the last group, part of the animals began to show acute respiratory symptoms: first the younger bullocks and within 10 days the entire herd became ill. The symptoms were characteristically the same as known from previous outbreaks of the so-called contagious cough: the body temperature rose to about 41 °C, accompanied by serous nasal discharge and frequent cough. The cough was loud on the first day, later moist. Finally dyspnoea developed. After one or two days the nasal discharge became mucous, and contained detritus. In some cases it was even purulent. Some animals developed subcutaneous emphysema in the spinal region. The overwhelming majority of the bullocks recovered spontaneously after five days of illness, but in certain cases the course lasted 10—15 days. Two of the

180 animals died with acute symptoms within two days, and one had to be emergency-slaughtered. At postmortem examination all three animals showed severe pulmonary emphysema and an extensive oedema involving almost the entire lung. The bronchi and trachea were filled by a foamy, serous-mucous exudation.

The disease did not affect other age groups in the affected cattle herds.

In another state farm (H), an acute respiratory disease was spreading rapidly among four to six months old calves kept in the rearing unit. The febrile course — 40—41 °C body temperature — was accompanied by very severe dyspnoea and nasal discharge, which was first serous, later purulent. Coughing was less frequent than in previous outbreaks of the "contagious cough". This non-characteristic respiratory disease involved the greater part of the above 3 months old calves within two weeks. The majority of the diseased animals had recovered by the 7th to 10th day of illness, but secondary pneumonia supervened in about 20% of the cases, and half of the affected calves died; the other had seemingly recovered in one month, but considerably retarted in growth. One calf died in the acute stage of the outbreak and 10 animals died of secondary pneumonia. The cattle herds kept in other units of the farm did not become affected.

Virus Isolation

Nasal swabs taken from animals showing acute symptoms, as well as changed organs and associated lymph nodes obtained at autopsy, were transferred to the laboratory in ice boxes. Rinsings of swabs in Hanks' solution and 1 : 10 diluted organ suspensions were centrifuged twice at 3000 r.p.m. for 15 min, treated with antibiotics and inoculated into calf-kidney and calf-testicle cell cultures. Each sample was transferred to four tubes of both cultures. The growth medium of the cultures did, the maintenance medium did not contain foetal calf serum. Both primary and secondary cell cultures were employed for virus isolation. Part of the cell cultures were stationary, part were rotated 12 times an hour during the period of outgrowth. Microscopic examination for cytopathic (CP) changes was performed daily. The tissue culture fluids of the monolayers not showing CP changes were collected when the spontaneous cell degeneration began to appear at 10—14 days after inoculation and were transferred to fresh cultures. At least five blind passages were performed in this manner before an experiment was accepted as negative. The changes were studied in preparations stained with haemalaun and eosin. The isolates were identified by means of a type antiserum to human RS virus.*

* Made available by the courtesy of Dr. I. Dömök, National Institute of Hygiene, Budapest

Paired serum samples from diseased animals were studied by virus neutralization test, using virus from the tissue-culture adapted 10th passage of the isolate, which had satisfactory growth properties.

Results

RS virus was isolated from one nasal discharge obtained in farm N and from a lymph node originating from the emergency-slaughtered animal in farm H. In both cases, CP changes appeared only in the third passage and exclusively in cultures which had continuously been rotated during incubation. The CP changes appearing in the third passage were focal and slight, and took 12—14 days to develop. Only few microscopically visible gaps were seen in the confluent monolayer, with syncytia formed by a few cells at the marginal parts (Fig. 1). The CP changes developed sooner, when the virus was inoculated either into a still developing monolayer, or into a cell suspension, and it appeared earlier in each subsequent passage: the eighth passage of the strain BF produced easily readable CP changes already after five days. In stained cell culture preparations syncytium formation and homogeneous acidophilic inclusions surrounded by a halo were seen (Fig. 2).

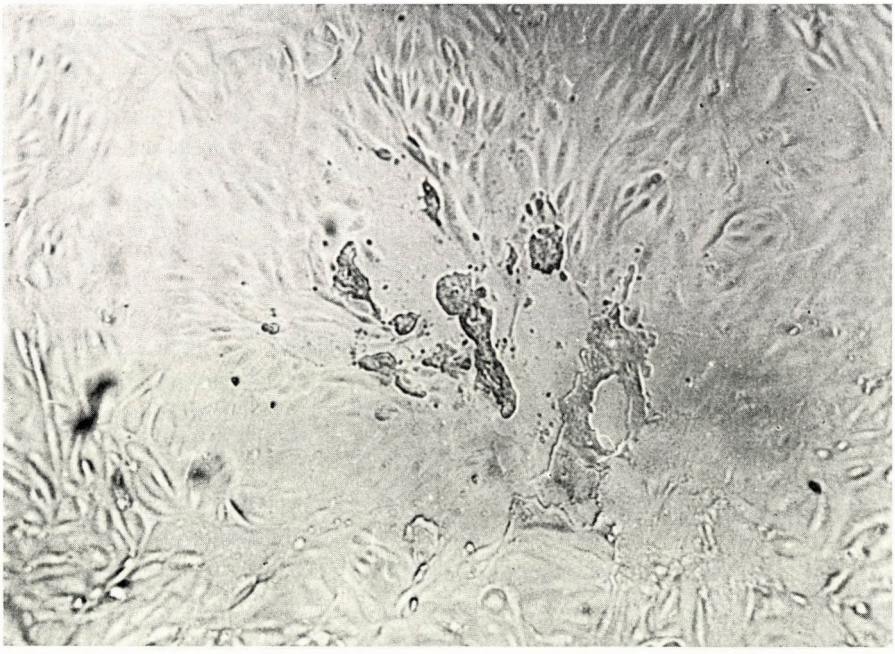

Fig. 1. Syncytia produced by RS virus in calf kidney epithelial cell culture. Unstained preparation, ×200

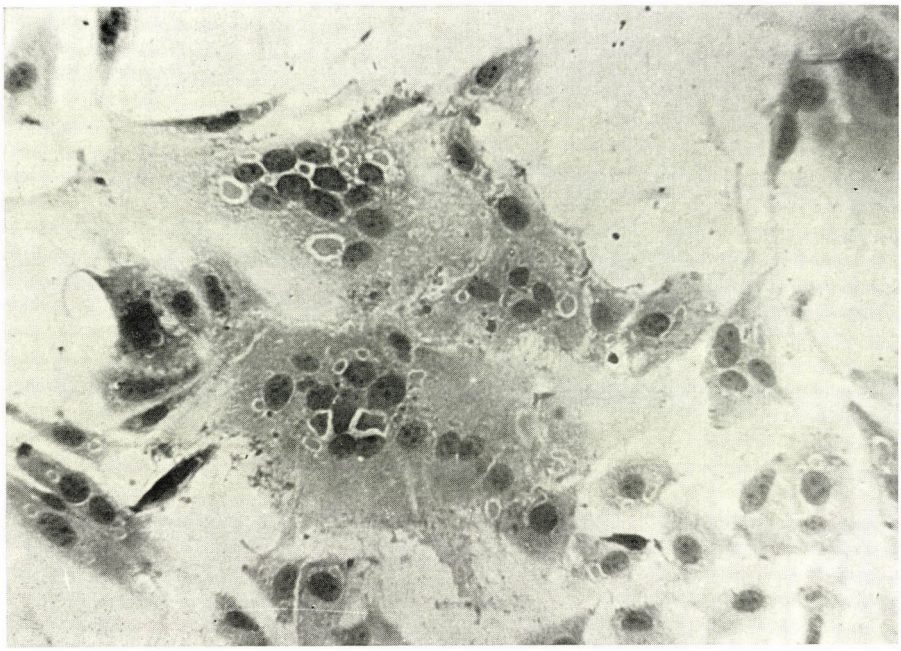

Fig. 2. Syncytia and intracytoplasmic inclusions in calf kidney epithelial cell culture infected with RS virus. H. and E.-stained preparation, ×400

Neither the virus-infected cell cultures, nor the virus-containing tissue culture fluid adsorbed the red blood cells of any animal species tested. The virus proved sensitive to chloroform.

The BF strain was neutralized by 1 : 80 dilution of the anti-human-RS virus serum, which had a homologous titre of 1 : 320, but its infectivity was not affected by 1 : 10 diluted anti-PI-3 serum, the titre of which was 1 : 620.

Paired serum samples obtained from diseased animals in both herds were tested for antibodies neutralizing PI-3, adenoviruses or the viruses of VD or IBR. The calves in herd N had both adeno- and PI-3 virus antibodies at identical levels in both samples and calves of herd H additionally had VD virus antibodies. Testing for antibodies to the isolate — strain BF — was possible only in herd N: the first serum samples did not contain specific neutralizing antibodies, but these were found to be present at titres of 1 : 4 to 1 : 16 in 16 of 20 in the second samples.

Discussion

RS virus has been isolated from one calf each in two herds affected by an acute respiratory disease ("contagious cough"). The causal role of other respiratory viruses, such as VD, IBR, PI-3 and adenoviruses could be excluded by serological evidence. The aetiological responsibility of RS virus was confirmed in one herd by demonstration of virus-neutralizing antibodies in serum samples taken during convalescence.

RS virus was isolated only from two of the diseased animals, exclusively in roller tube cultures of calf kidney cells; attempts at isolation failed when stationary cultures were used. The CP changes due to RS virus did not appear until the third passage, in which it took 12—14 days to develop. In the eighth passage the changes appeared already after five days' incubation. A satisfactory replication took place also in stationary cultures when these were inoculated before the completion of the monolayer or when the cell suspension serving for the preparation of monolayers was inoculated. Such cultures could be utilized for virus neutralization tests, too.

Since the isolation experiments were initially made only in non-rotated confluent monolayers of calf kidney or calf testicle cells, the isolation of RS virus from two cases does not reflect the true role of this agent in the outbreaks studied. Diagnostic differentiation of the epizootiological course, symptoms and gross lesions of the RS-virus disease from those of the PI-3-virus disease was not possible either because the outbreak had completely subsided by the time the RS virus was isolated, viz. 6—8 weeks after onset. However, the virus neutralization test appears to be a helpful laboratory diagnostic procedure if higher passages of the virus, producing CP changes in five days, are used.

In Western Europe great importance has been attached to the pathogenic role of RS virus: in Belgium (WELLEMANS, 1970) it has even been classified as the main causal agent of bovine respiratory disease. In view of this, close investigations into the epizootiological course, symptoms and gross lesions of the RS virus disease seem to be of immediate interest also in Hungary, with special regard to diagnostic differentiation from the similar condition caused by the PI-3-virus.

Summary

RS virus was isolated in roller tube cultures of calf kidney epithelium cells from calves showing acute respiratory symptoms. The cytopathic effect of the virus did not appear until the third passage in which it took 12—14 days to develop, but this period was gradually reduced to five days after several consecutive transfers. Virus replication also took place in non-rotated cultures, when inoculation was made either prior to the outgrowth of the confluent monolayer, or into cell suspensions serving for the preparation of monolayers. The isolate did not haemagglutinate red blood cells of various animal species. The isolates were neutralizable by antibodies appearing in the convalescence at titres ranging from 1 : 4 to 1 : 16. The aetiological role of other respiratory viruses could be excluded by serological evidence in both verified cases of RS virus infection.

References

BARTHA, A. and KÖVES, B.: Magyar Állatorvosok Lapja (MÁL), 1976, 103. CSONTOS, L.: Fortpfl. Haust. **6** (1970), 308. INABA, Y., TANAKA, Y., OMORI, T. and MATUMOTO, M.: Jap. Exp. Med. **40** (1970), 473. JACOBS, J. W. and EDINGTON, N.: Vet. Rec. **88** (1971), 694. PACCAUD, M. F. and JAQUIER, C. I.: Arch. ges. Virusforsch. **30** (1970), 327. TANYI, J., ROMVÁRY, J., ÁLDÁSY, P. and MÁTHÉ, ZS.: MÁL, 1974, 373. WELLEMANS, G., LEUNEN, J. and LUCHSINGER, E.: Am. J. Med. Vet. **114** (1970), 89.

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EFFECT ON LAYING GEESE OF FEEDS CONTAINING THE FUSARIOTOXINS T2 AND F2

By

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Experimental observations of Hungarian authors (PALYUSIK et al., 1971; VÁNYI et al., 1973) attracted attention to the depressive effect of the F2-toxin-producing fungus *Fusarium graminearum* on spermiogenesis in ganders. More recent studies (PALYUSIK et al., 1974) have shown that feeds infected with *F. sporotrichioides* or *F. poae*, the fungi known to produce the toxin T2 and other toxins of trichothecene structure, are still more powerful inhibitors of anserine spermiogenesis than F2.

The present studies were carried out to examine the effect of toxins F2 and T2 on the egg production of laying geese.

Materials and Methods

Maize infected artificially with either F2-producing *F. culmorum*, or T2-producing *F. sporotrichioides* was added to the feed of one-year-old Rhineland laying geese as a 10% ration. The toxic feeds, containing 100 ppm F2 or 3 ppm T2 were fed to the layers for 42 and 10 days, respectively, without interruption. The amount of T2 was then reduced to one half over the subsequent six days because the birds became ill, and the toxic ration was omitted completely from the 16th day. Experimental feeding was commenced in March, i.e., in the most active period of the laying season. Ten birds were used in each group. Experimental and control geese, on a total 30 birds, produced 271 eggs before the beginning of the experiment, which corresponds to a group average of 90. The birds were observed for 10 weeks. The control birds were given a 10% toxin-free maize meal ration throughout the period of experimental diet.

Quantitative determinations of the toxins F2 and T2 were carried out by thin-layer chromatography, using purified reference standards.

Detection of F2 in the thin-layer chromatograms was made either by fluorescence test or by the vivid yellowish-orange colour reaction produced with 4-bromobenzol-diazoniumfluoroborate of the fluoroborate series recommended by SARUDI (1974).

For the determination of T2, an extract was prepared from the toxic feed with dichloromethane (methylene chloride). The extract was purified by phase separation with petroleum ether, ethyl acetate and acetonitrile, and 0.05 ml of the acetonitrile fraction was applied to the chromatography plates prepared in this laboratory from Stahl's 10—40 μ Kieselgel G (Merck). After twodimensional chromatography, the silica gel plates were sprayed with 20% aqueous sulphuric acid solution and were heat-treated for 10 min at 100 °C. The petroleum ether–diethyl ether–glacial acetic acid mixture (70 : 30 : 2) recommended by OLIFSON et al. (1972) was used as first developing solvent and the commonly employed chloroform–methanol (95 : 5) mixture as second solvent. As a result of the above treatment, T2 was easily differentiated by its sky-blue fluorescence.

However, the *F. sporotrichioides*-infected toxic feed contained, apart from T2, three trichothecene toxins, which had lower Rf-values than T2, but also showed a sky-blue fluorescence. Identification of the latter three toxins was not possible for lack of reference standards, and still less information could be obtained on those unknown trichotecene toxins which did not show fluorescence.

Results

Egg production. Control birds and birds fed on the F2-containing diet regularly produced eggs for 37 and 38 days, respectively, after the beginning of the experiment, whereas the layers given the T2-containing diet ceased to produce eggs within 10 days (Fig. 1). The eggs produced during the above 38 days numbered 89, 68 and 28 in the control, F2 and T2 groups, respectively. In the meantime two birds in each group were killed by bleeding to check the status of the ovary and the oviduct, so that only 8 layers per group were alive at the and of the egg-laying period. The birds were fertilized by artificial insemination with semen from healthy ganders fed on non-toxic diet. From the control, F2 and T2 groups, 70, 56 and 13 eggs, respectively, were placed in the incubator. In the above order, egg fertility was 77, 46 and 54%.

Symptoms. Birds of the control and F2 groups did not show any sign of disease. Feed consumption and weight gain were, apart from minor fluctuations, uniform in these two groups; the birds' mean weight being 4.20 and 4.25 kg, respectively, in the eighth week of the experiment.

Layers of the T2 group, however, refused part of the toxic feed and, were gradually losing weight. At 14 days, when the toxic diet was still fed, the average daily feed consumption of the T2 birds was only 98 g, contrasted to 222 and 227 g in the control and F2 groups, respectively, and it rose little even after omission of the toxin, the maximum being 138 g daily. During the eighth week the average body weight of the T2 layers fell to 2.40 kg. One cause

of the reduced feed consumption may have been the presence of the "refusal of feed factor", at least at the beginning of the experiment, and furthermore the extensive necrotic and diphtheroid lesions of oral, glottal and pharyngeal mucosa may have interfered with feed intake and deglutition. Apart from two birds, one of which was killed at 2 weeks, the other at 4 weeks for diagnostic purposes, the remaining eigth T2-layers all died spontaneously 5 to 10 weeks after the onset of the experiment. No bacteria could be isolated from the organs of the birds.

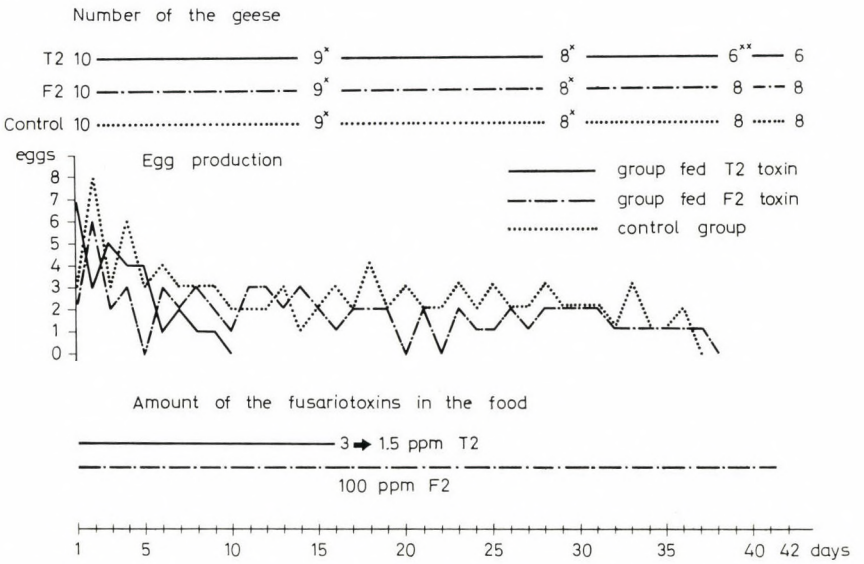

Fig. 1. Effect of fusariotoxins on the egg production of laying geese. Birds given T2-toxin-containing diet ceased to produce eggs within 10 days. ×, the missing birds were killed by bleeding; ××, the missing bird died

Gross and microscopic lesions. The organs of control and F2 birds killed by bleeding for diagnostic study did not show any abnormality.

The carcases of T2 birds showed marked emaciation, cachexy, severe myocardial degeneration, and necrotic and diphtheroid lesions in oral, glottal and pharyngeal mucosa, which had already been present while the birds had been alive. Such oral lesions were described earlier in growing geese fed on diets infected with *F. sporotrichioides* (PALYUSIK et al., 1968) or in hens fed with *F. tricinctum* (SPEERS et al., 1972). Yellowish-white lesions of similar type, but of varying severity, were found in chicks fed 4—16 ppm of purified T2 toxin (WYATT et al., 1972). This dose of T2 caused disease in the day-old chicks, but did not kill them. Postmortem examination of the T2 geese showed marked dilatation of the heart, especially of the right ventricle, the thinned wall of which was sprawling over the left ventricle like a loose sac. The spleen appeared

normal, but the liver and kidneys were degenerated, and in some cases a haemorrhagic enteritis was found.

The rapid decrease, and subsequent cessation, of egg production in the T2-group was clearly due to the alteration of the ovary and oviduct. Both

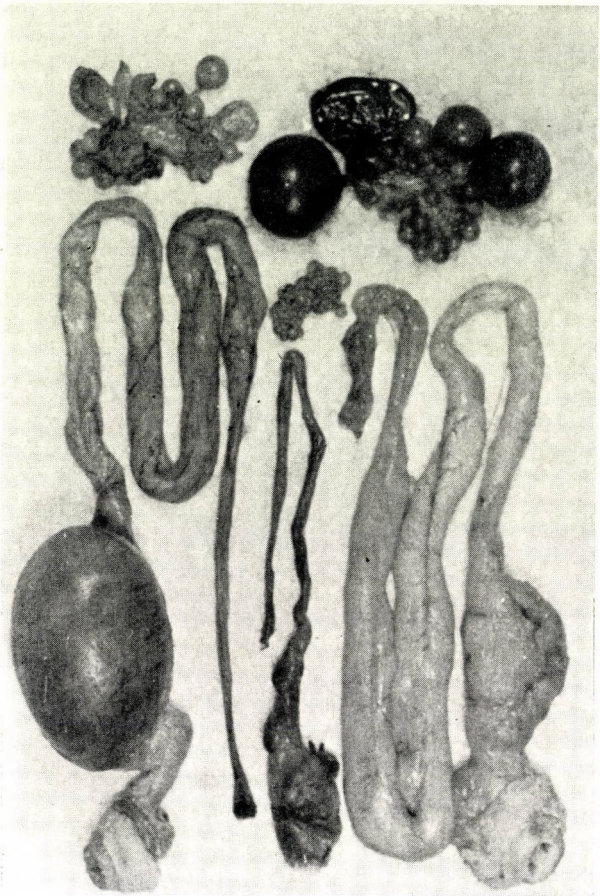

Fig. 2. Experimental fusariotoxicosis in laying goose. Centre: atrophic oviduct and ovary of a laying goose fed feed containing 3 and 1.5 ppm T2 toxin daily for 10 and 6 days, respectively; the maize was experimentally infected with *F. sporotrichioides* (28th day of experiment). Right: Ovary and oviduct of control bird fed on non-toxic diet. Left: Ovary and oviduct of laying goose fed on F2-toxin-containing diet. Each goose was exsanguinated on the 28th day of the experiment

organs exhibited a marked atrophy already at 14 days, being only half as large as in the control birds, and at 28 days only a small, shrunken mass was found (Fig. 1). At the same time the corresponding organs of control and F2-treated birds showed all morphological signs of active egg production.

Specimens obtained from the ovary, heart muscle and liver of birds

killed on the 28th day and of those spontaneously died on the 56th day of the expriment were embedded in paraffin, and the sections were stained with haematoxylin and eosin. Inflammatory cell elements were not found in the organs. The atrophic ovaries of the birds treated with T2 contained mainly atretic or atretizing follicles, but such follicles also occurred, in considerably lower numbers, in the ovaries of control and F2-treated birds on the 28th day of the experiment, *viz.* while these birds still produced eggs. The germinal epithelium was, however, intact also in the ovaries of T2-treated birds. Microscopic haemorrhages were in places found in liver and heart muscle of birds of the T2 group.

Conclusions

The F2 toxin did not notably affect the amount of egg production by the laying geese, but caused a slight, not significant, decrease in egg fertility as compared to the controls. This is in good accordance with the earlier observations of SPEERS et al. (1972) on F2-treated laying hens and of VÁNYI et al. (1973) on similarly treated laying geese.

Unlike this, consumption of feed infected with the T2-producing *F. sporotrichioides* fungus was followed first by a marked reduction, then by cessation, of egg production within 10 days, while control and F2-treated birds continued to lay eggs regularly for further five weeks. SPEERS et al. (1972) reported that laying hens fed on a diet containing *F. tricinctum* or *F. oxysporum* ceased to produce eggs within a week. In the present experiment the atrophy of the oviduct and ovary accounted for the cessation of egg laying by the geese treated with T2. Since T2-producing *Fusarium* strains commonly occur in Hungary, the T2 toxin, or a related toxic substance may well be incriminated as a factor responsible for egg production disorders in goose flocks.

According to the histological findings, apart from causing atrophy of the ovary, the T2 toxin accelerates follicle atresia, without, however, giving rise to inflammation either in the ovary or in any other organ. The fresh microscopic haemorrhages found in the heart muscle and liver of spontaneously died and exsanguinated T2-birds were clearly not due to the direct effect of the toxin, for the toxic ration was omitted for almost two weeks before exsanguination and for almost six weeks before spontaneous death. The presence of the fresh haemorrhages nevertheless supports the conclusion that the fusariotoxin may have given rise at an earlier time to the vascular wall injury responsible for the appearance of haemorrhages at exsanguination. It should be noted that WYATT et al. (1973) demonstrated in chicks vascular wall damage liver haematoma under the influence of T2.

We hold the view that, apart from T2, other factors share the responsibility for the severe lesions found in this study.

Acknowledgements

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Summary

The effect of the fusariotoxins F2 and T2 on the egg production of laying geese was studied by feeding toxic maize rations, infected with F2-producing *Fusarium culmorum*, and T2-producing *F. sporotrichioides*, respectively, to birds in the laying season.

Geese given 3 ppm T2 toxin in the feed daily ceased to produce eggs within 10 days. All birds in this group died spontaneously, except for two geese which had been killed by bleeding for diagnostic purposes. At postmortem examination marked emaciation, cachexy, atrophy of the ovary and of the oviduct, severe myocardial degeneration and necrotic and diphtheroid lesions of oral, glottal and pharyngeal mucosa were found. The mucosal lesions had already been seen in the living.

No inflammatory changes were found in the organs of the T2-treated birds by microscopic examination, but all showed premature atresia of the greater part of the ovarian follicles. Microscopic haemorrhages, found in the heart muscle and liver, may probably have been concomitant upon vascular wall injury by trichotecene toxins.

The toxic feed contained apart from T2 unidentified trichotecene toxins.

Feeding of a diet containing 100 ppm F2 did not notably interfere with either egg production or egg fertility.

References

ОЛИФСОН, Л. Е., КЕНИНА, Ш. М. and КАРТОШОВА, В. Л.: Хроматографический метод определения токсичности зерна злаковых культур (пшеница, рож, просо и др.), пораженного токсигенными штаммами грибов Fusarium sporotrichiella. Инструкция по определению токсичности зерна. Оренбург, 1972. PALYUSIK, M., KOPLIK-KOVÁCS, Éva and GUZSAL, E.: Magyar Állatorvosok Lapja (MÁL), 1971, 300. PALYUSIK, M., NAGY, GY. and ZÖLDÁG, L.: MÁL, 1974, 551. PALYUSIK, M., SZÉP, I. and SZŐKE, F.: Acta vet. hung. **XVIII** (1968), 363. SARUDI, I.: Z. Lebensm. Unters. — Forsch. **154** (1974), 65. SPEERS, G. M., MIROCHA, C. J. and CHRISTENSEN, C. M.: Poultry Science **51** (1972), 1872. VÁNYI, A., DANKO, GY., ÁLDÁSY, P., ERŐS, T. and SZIGETI, G.: MÁL, 1973, 303. WYATT, R. D., HAMILTON, P. B. and BURMEISTER, H. H.: Poultry Science **53** (1973), 1853. WYATT, R. D., WEEKS, B. A., HAMILTON, P. B. and BURMEISTER, H. R.: Applied Microbiology **24** (1972), 251.

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SEASONAL VARIATION IN THYROIDAL ^{131}I UPTAKE AND THYROXINE SECRETION RATE IN BUFFALO (*Bubalus bubalis*)

By

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The importance of environmental influences and seasonal fluctuations in thyroid activity of the male rat have been attributed to the action of heat and cold on thyroid function (KNIGGE, 1960). STAHL and TURNER (1961) reported the changes in thyroxine secretion rate (TSR) in two strains of New Hampshire fowls and observed a decline by 41.6% from winter to summer, while PREMACHANDRA et al. (1958) described a variable extent of depression between 30 and 40% in cows and calves. SORENSEN (1961) reported a tenfold increase in TSR of pigs maintained at a temperature of 3 °C as compared with pigs maintained at 24 °C. However, no such experiments have been reported in buffalo.

The present investigation is, therefore, an attempt to determine the extent of fluctuation in thyroidal ^{131}I uptake (UT) and TSR during summer, autumn and winter seaons in buffalo.

Materials and Methods

A total of 180 determinations of UT and TSR were made in buffalo calves of both sexes in three age groups, *viz.*, under 6 months, 1—1½ years ans 2½—3 years during sum-mer (May–July), autumn (Feb.–April) and winter (Nov.–Jan.) seasons.

Thyroidal ^{131}I uptake

^{131}I was injected subcutaneously, caudal to the base of the ear, 400 μCi/100 kg body weight in each animal. The measurements of thyroidal uptake were made for 12 days with a gamma-ray spectrometer. In order to immobilize experimental animals while measuring thyroidal ^{131}I activity, a special restraining chute was constructed with a head collar for securing the head, neck and shoulder regions. The distance between the thyroid and the scintillation head was maintained at 30 cm. A thyroid phantom was prepared with the same dose of ^{131}I, as was injected into buffalo calves and was used as a standard. All readings were corrected for background radiation, which

was determined immediately prior to each reading period. The UT was expressed as percentage (UT %) and was calculated as follows:

$$UT\ \% = \frac{\text{Neck cpm} - \text{thigh cpm}}{\text{Net standard cpm}} \times 100.$$

Thyroxine secretion rate determination

For determination of TSR, exogenous L-thyroxine, 0.1 mg/100 kg body weight, was first injected 48 hrs after administration of ^{131}I and repeated injections were then given every 24 hrs. Thiouracil, a goitrogen, was also mixed with the ration (33 g/100 kg ration) and placed in the feeders 48 hrs following the injection of ^{131}I until completion of the experimental period in an effort to induce a sharper ^{131}I release rate. The level of exogenous L-thyroxine was increased at 48 hrs intervals by increments of 0.05 mg. This procedure was continued until the level of thyroxine which apparently blocked the release of thyroidal ^{131}I to achieve equilibrium represented thyroxine secretion rate of the buffalo calves.

Results

UT curves for male and female buffalo calves of the three age groups during winter, autumn and summer seasons are shown in Figs 1 to 3. Table I presents the maximum UT % in both male and female calves during different seasons.

During winter season maximum UT occurred in all the animals of different age groups at 36 hrs, whereas during autumn and summer seasons the peaks were noted at 42 hrs. The uptake was higher in the young animals of both sexes than in older ones and slightly higher in the males than in the females.

Table I

Thyroidal ^{131}I uptake in buffalo

Age group	Sex	Summer	Autumn	Winter
Under 6 months	Male	33.2	36.2	40.0
	Female	30.6	32.8	37.2
1—1½ years	Male	29.6	31.2	35.8
	Female	27.8	29.6	31.8
2½—3	Male	28.2	29.4	31.2
	Female	25.0	27.6	29.4

Data show mean of peak values in per cent.

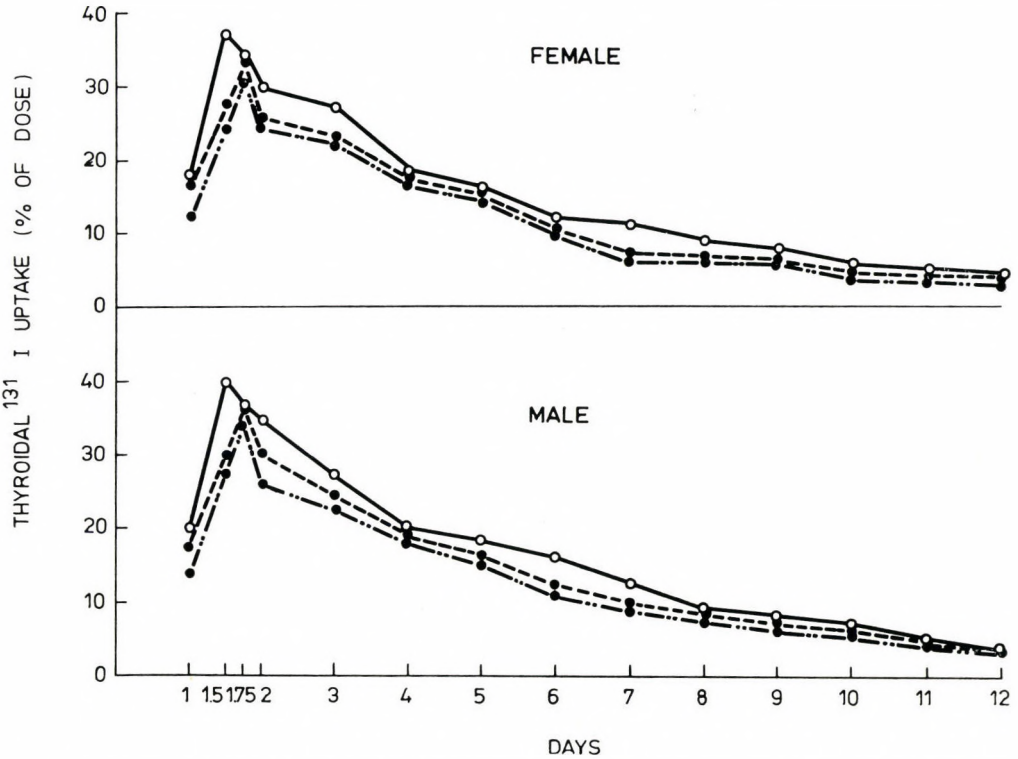

Fig. 1. Thyroidal ^{131}I uptake in buffalo calves under 6 months of age during winter ○——○, autumn ● — — — ● and summer ● — ·· — ·· — ●.

Table II

Daily thyroxine secretion rate in buffalo

Age group	Sex	Mean TSR values in mg/100 kg body weight		
		Summer	Autumn	Winter
Under 6 months	Male	0.36±.03	0.44±.05	0.60±.07
	Female	0.38±.04	0.48±.04	0.62±.06
1—1½ years	Male	0.31±.03	0.42±.04	0.46±.03
	Female	0.32±.04	0.44±.03	0.48±.02
2½—3 years	Male	0.29± 02	0.33±.03	0.40±.02
	Female	0.30±.03	0.34±.04	0.42±.03

Data show means ± S.D.

The UT was found to be the highest during winter and the lowest during summer.

Table II shows the mean TSR values for male and female buffalo calves of different age groups during winter, autumn and summer seasons. The

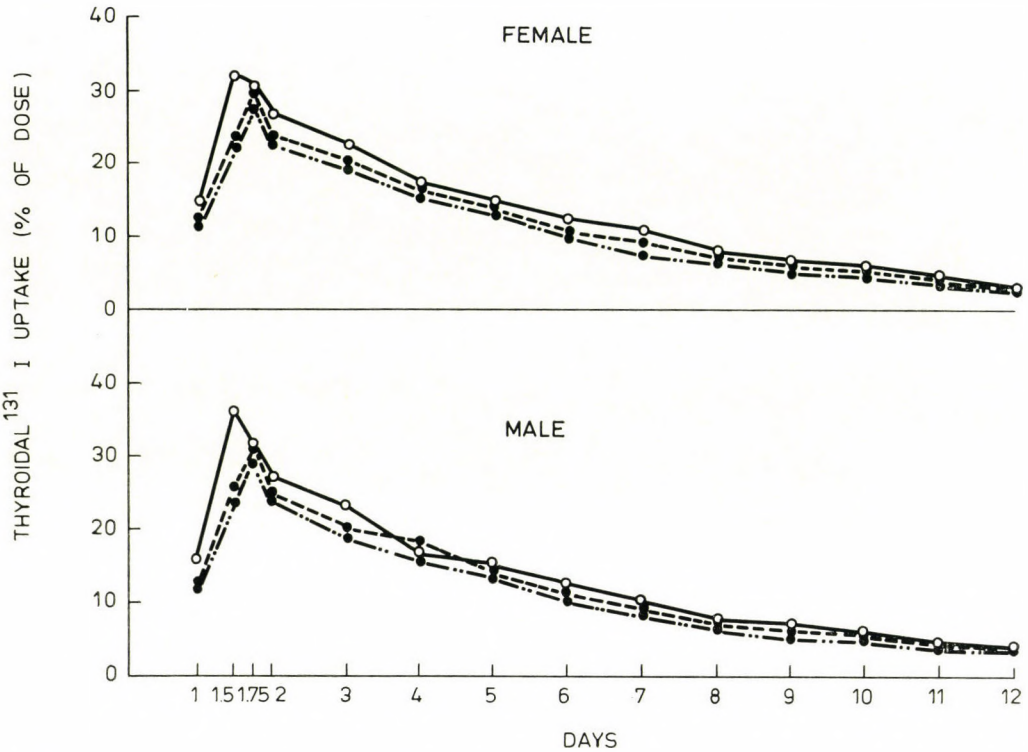

Fig. 2. Thyroidal ^{131}I uptake in buffalo calves 1—1 $^1/_2$ years of age during winter ○——○, autumn ●— — — —● and summer ●— · · —· · —●.

average daily TSR was slightly higher in female than in male buffalo calves. This difference was statistically non-significant. A decline in seasonal TSR values with advancing age was noticed in both male and female calves. The mean daily TSR values for calves under 6 months were significantly higher ($P < 0.05$) than for those of 2½—3 years.

In the group of male buffalo calves under 6 months of age the mean daily TSR was 0.6 mg/100 kg body weight during the winter season. The corresponding autumn and summer values were lower by 27% and 40%, respectively. The mean winter TSR value for the female calves of the same age was 0.62 mg/100 kg body weight. The corresponding summer and autumn values were lower by 39% and 23%, respectively. This decline was found to be statistically significant ($P < 0.01$).

In the 1—1½-year age group, the mean winter TSR value per 100 kg body weight was 0.46 mg for the male buffalo calves and 0.48 mg for the female calves, respectively; the summer values were lower by 33% for both sexes ($P < 0.01$). The reduction of TSR from winter to autumn was statistically non-significant.

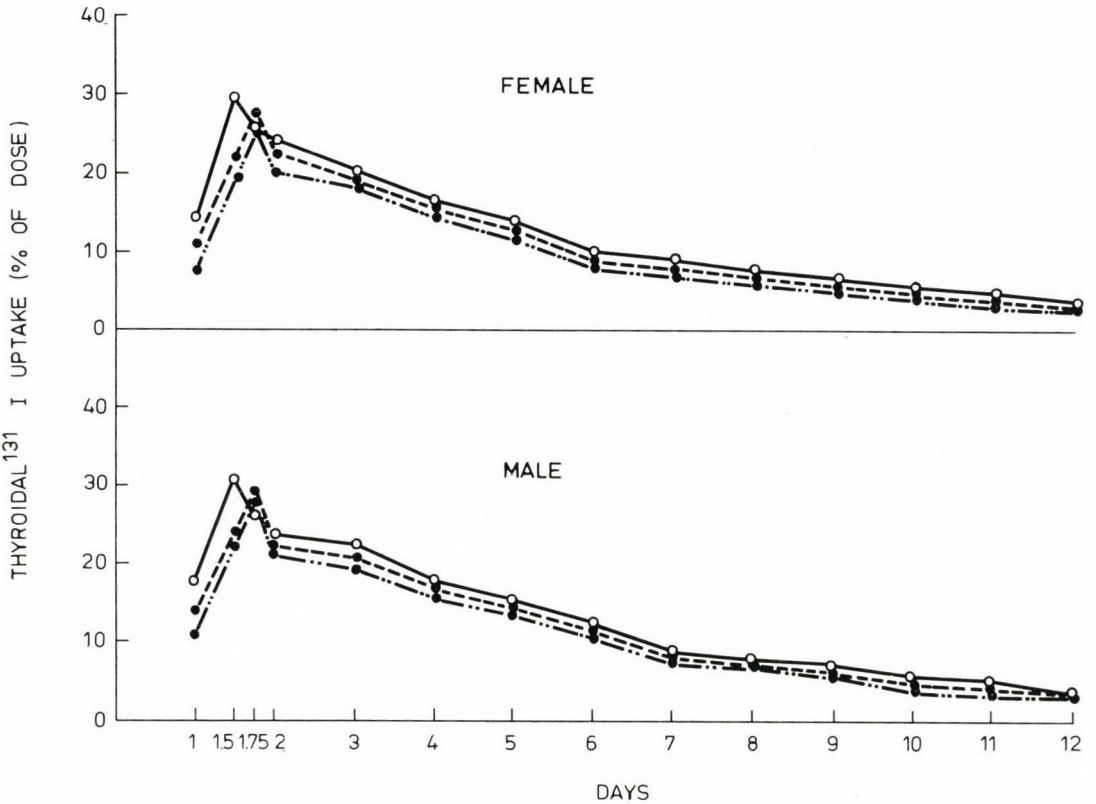

Fig. 3. Thyroidal ^{131}I uptake in buffalo calves 2 $^1/_2$—3 years of age during winter ○——○, autumn ●— — —● and summer ●— · · —●.

The mean winter TSR values for the 2½—3 years age group of male and female buffalo calves were 0.40 mg and 0.42 mg per 100 kg body weight, respectively. The respective declines were from winter to summer season 28% and 29% ($P < 0.01$) and from winter to autumn 18 and 19% ($P < 0.05$).

Discussion

In the present study on buffalo calves the peak for the maximum UT occurred between 36 and 42 hrs during different seasons of the year. The UT % was higher for young calves of both sexes than for older ones. The higher values in the young calves are evidently due to higher basic metabolic rates. This is in conformity with our earlier observations in the goat (Prakash and Sharma, 1972).

The time at which UT reaches a peak value differs in different species. In dog, the peak occurs at 72 hrs (Kaneko et al., 1959). Keating and Albert

(1949) found maximum UT between 24 and 28 hrs in man. In the bovine, HOWES et al. (1961) observed maximum UT at 72 hrs and reported that it was not affected by the dose level, whereas in swine a period of 48 hrs for maximal UT was suggested by ROMACK et al. (1964).

These findings in the buffalo calves show an increase in UT values from summer to winter, which indicates that high temperature depresses UT. Cold environment may be a greater stimulus to an increase in thyrotrophic hormone output than a warmer one, which in turn increases the activity of the thyroid gland during the winter months (PREMACHANDRA et al., 1960). LEBLOND et al. (1944) and CATZ et al. (1953) also noted decreased UT in rats exposed to high temperature. Similar correlation between UT and season was reported by BLINCONE and BRODY (1955) in cattle.

It is evident from this investigation that there was a marked decline in TSR values from winter to summer as also from winter to autumn in the buffalo calves (under 6 months, 1—1½ years and 2½—3 years) of both sexes. DEMPSEY and ASTWOOD (1943) reported a marked influence of environmental temperature in TSR values of rats. STAHL and TURNER (1961) showed a similar relation in fowls. Study of the TSR in dairy cattle has indicated considerable variation due to seasonal changes in environmental temperature. During the summer months, the mean secretion rate was about 1/3 of the winter rate (PREMACHANDRA et al., 1958). The reduced intake of food by the animals in summer may be one of the factors for reduction in TSR values and *vice versa*. It is assumed that a part of the thyroid gland becomes inactive during summer while the remainder of the gland tries to compensate the thyroxine release, but the absolute amount of the hormone secreted is less, owing to the temporary impairment of some thyroid cells, resulting in the decrease of TSR values. It is well documented that thyroid cell impairment leads to a temporary interruption of iodide-concentrating processes and hormone synthesis (GREENE, 1971). AFIEFY (1967) reported that serum thyroid hormone levels in buffaloes and cattle during summer were only 25% of the winter values. He concluded that the low hormone level produced by high ambient temperatures was responsible for decreased fertility in summer.

Our results show a decline in TSR due to advancing age. The difference between the average TSR values of male and female animals was not significant. These observations are in agreement with the findings of ROMACK et al. (1964) in swine. The reduction in TSR in summer and autumn as compared with the winter values implies that animals with potentially high thyroid activity should be selected for breeding purposes in winter season only. Moreover, these studies have also established the feasibility of measuring daily TSR of buffalo by replacement technique with thyroxine during thiouracil administration (to prevent recycling of ^{131}I).

Acknowledgements

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Summary

The seasonal variations in thyroidal uptake of ^{131}I (UT) and thyroxine secretion rate (TSR) in buffalo calves of different age groups of both sexes have been determined. The per cent UT in the male buffalo calves recorded an increase from 28 to 40 from summer to winter; in female calves the variation was from 25 to 37. The peak UT values were obtained at 36 hrs during winter, and at 42 hrs during autumn and summer. The mean values of daily TSR showed an increase from 0.29 to 0.60 mg/100 kg body weight from summer to winter in the male buffalo calves of different age groups. Similarly, an increase from 0.30 to 0.62 mg/100 kg body weight was observed in the female calves. A reduction in TSR values due to advancing age was noticed in buffalo calves of both sexes during different seasons.

References

AFIEFY, M. M.: Vet. med. J. **13** (1967), 73. BLINCOE, C. R. and BRODY, S.: Res. bull. Mo. agri. exp. Stn. No. 579 (1955). CATZ, B. I., RAVI, E. and GEIGER, E.: Am. J. Physiol. **174** (1953), 29. DEMPSEY, E. W. and ASTWOOD, E. P.: Endocrinology **32** (1943), 509. GREENE, J. N.: Am. J. Med. **51** (1971), 97. HOWES, J. R., HIGGINBOTHAM, W. A., GENNARO, J. F. and FEASTER, J. P.: Am. J. vet. Res. **22** (1961), 887. KANEKO, J. J., TYLER, W. S.. WIND, A. and CORNELLIUS, C. E.: JAVMA **135** (1959), 516. KEATING, F. R. Jr. and ALBERT, A.: Recent Progress in Hormone Research. N. Y., 1949. KNIGGE, K. M.: Proc. Soc. exp. Biol. (N. Y.) **104** (1960), 368. LEBLOND, C. P., GROSS, J., PEACOCK, W. and EVANS, R. D.: Am. J. Physiol. **140** (1944), 671. PRAKASH, P. and SHARMA, D.: Ind. J. exp. Biol. **10** (1972), 20. PREMACHANDRA, B. N., PIPES, G. W. and TURNER, C. W.: J. Dairy Sci. **41** (1958), 1609. PREMACHANDRA, B. N., PIPES, G. W. and TURNER, C. W.: Res. bull. Mo. agric. exp. Stn. No. 727 (1960). ROMACK, F. E., TURNER, C. W., LASLEY, J. F. and DAY, B. N.: J. Animal Sci. **23** (1964), 1143. SORENSEN, P. H.: Conference on the Use of Radioisotopes in Animal Biology and Medical Sciences. N. Y., 1961. STAHL, P. and TURNER, C. W.: Poultry Sci. **40** (1961), 239.

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TWO SOLID-PHASE RADIOIMMUNOASSAYS FOR THE QUANTITATIVE DETERMINATION OF *Clostridium botulinum* TYPE-A ANTIBODIES

By

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A radioimmunoassay similar to that of YALOW and BERSON (1960) has been developed for the determination of type-A toxin of *Clostridium botulinum* by BOROFF and SHU-CHEN (1973). It is based on the competition between ^{131}I-labeled and unlabeled toxin for binding to specific antibody.

We have recently developed two solid-phase radioimmunoassays for the quantitative determination of *Clostridium botulinum* type A antibodies according to the principle used for the measurement of tetanus antibodies (HABERMANN and WIEGAND, 1973; BERNÁTH and HABERMANN, 1974). In both methods the antibody is determined by reversed solid-phase radioimmunoassay (HABERMANN and WIEGAND, 1973) in which the investigated and the solid-phase-bound antibody compete for ^{125}I labeled toxin. The radioactivity bound to the solid phase is inversely related to the quantity of the antibody.

Materials and Methods

Crystalline botulinus A toxin was labeled by the chloramine T method as described previously (HABERMANN, 1974). The specific activity of the material was about 7 mCi/mg. The neurotoxin and haemagglutinin components were separated by gel filtration (HABERMANN, 1974). The labeled, purified neurotoxin was used for the tests.

Botulinus antitoxin from horse (Behringwerke, Marburg) containing 750 IU/ml type A, 500 IU/ml type B and 50 IU/ml type E antitoxin was used for preparing bromoacetylcellulose (BAC)-bound antibody, for coating the tubes and for standardization.

BAC (Serva) solid-phase antibody was prepared according to ROBBINS et al. (1969), except that 0.5 *M* sodium hydrocarbonate buffer of pH 9.7 was used instead of 0.1 *M* buffer of pH 8.9.

The pre-incubation and the incubation were made in polyethylene vessels (System Eppendorf, Hamburg), and Eppendorf tubes were used for coating,

too. The tubes were coated overnight at room temperature with the above-mentioned serum in saline (1 IU/ml type-A antitoxin). The 0.5 ml coating solution was sucked off, and the tubes were washed, dried and stored as described previously for tetanus antitoxin (BERNÁTH and HABERMANN, 1974).

The testing mixture for the coated-tube method was prepared in the following order in untreated tubes:

0.3 ml phosphate (0.05 *M*, pH 7.5)-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA);
0.1 ml normal rabbit serum;
0.1 ml serial dilution (1 : 1, between 0.0005 and 1 IU/ml) of standard serum or dilutions of serum under testing;
0.1 ml ^{125}I-labeled toxin dilution, about 7000 cpm.

The mixture for BAC-bound antibody method was:

0.2 ml PBS with BSA;
0.1 ml normal rabbit serum;
0.1 ml serial dilution of standard serum or dilutions of serum under testing;
0.1 ml ^{125}I-labeled toxin dilution.

All dilutions were made in PBS with BSA if not otherwise stated.

The mentioned mixtures were pre-incubated for 16 hrs at room temperature and a 0.5 ml aliquot was transferred into the coated tube. Alternatively, 0.1 ml of washed and suitably-diluted BAC-bound antibody was added to the mixture. The time of incubation was 4 hrs at room temperature, in the case of coated tubes in upright position. Vessels containing BAC-bound antibodies were rotated around their horizontal axis. In every experiment positive control tubes were attached without dissolved antibodies. The incubation in case of negative control was made either in uncoated tubes or in the presence of BAC-bound BSA.

When working with antibody-coated tube, we sucked off the liquid after the incubation, the vessels were then washed with saline, and the radioactivity adhering to the vessel was counted in a Packard Autogamma Spectrometer. In case of the BAC method the tubes were centrifuged (2.5 min, about 8000 *g*), an aliquot of the supernatant (0.2 ml) was transferred to another vessel and counted.

Results

Different reaction conditions were chosen to fin dout the optimum parameters. In the first incubation the antibody under testing reacted with the ^{125}I-labeled toxin. In the second incubation the solid-phase-bound antibody

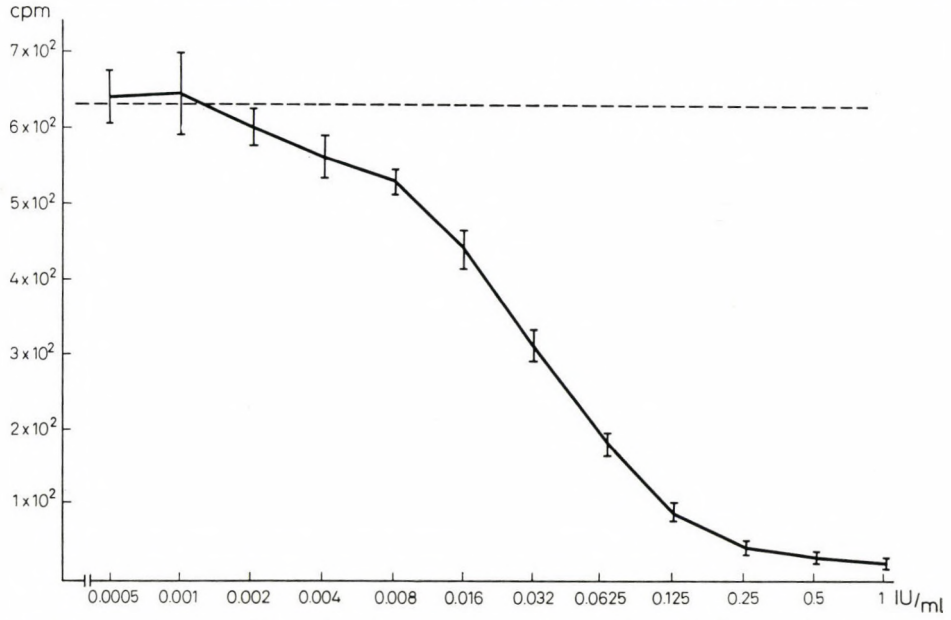

Fig. 1. Measurement of *Clostridium botulinum* A antibodies by antibody-coated tube method (16 hrs first incubation, 4 hrs second incubation). Ordinate: radioactivity adhering to the vessels in cpm. Abscissa: concentration of the serum used for standardization in IU/ml. Each dot represents the average of 4 determinations. The vertical bars represent the standard deviations. The horizontal line represents the positive control value

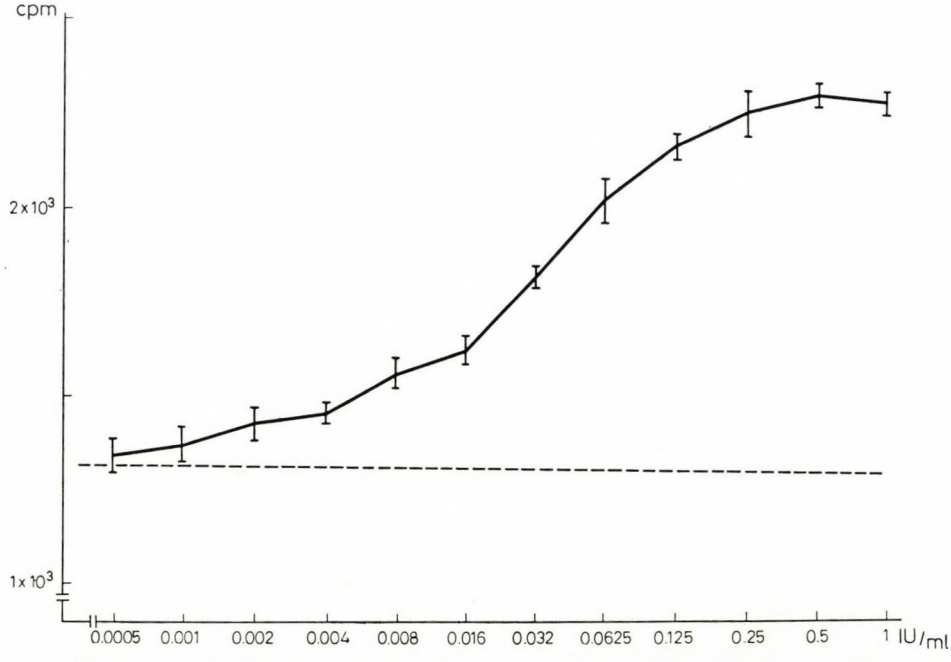

Fig. 2. Measurement of *Clostridium botulinum* A antibodies in serum dilutions used for standardization by the bromoacetylcellulose-bound antibody method. Ordinate: radioactivity of 0.2 ml supernatant in cpm. Abscissa: concentration of standard serum in IU/ml

was present, too. The times of both incubations were varied between 1hr and 16 hrs. A first incubation of 16 hrs followed by a second incubation of 4 hrs was found to be the most sensitive and convenient. Without the first incubation the limit of detection both methods is about 0.08 IU/ml. This limit becomes doubled by 16 hrs pre-incubation. The satisfactory serum antitoxin level in man is about 0.02 IU type-A antitoxin/ml (FIOCK et al., 1962). The methods can therefore be declared sensitive even without pre-incubation.

The parallel tests as well as the repeated experiments indicate the reproducibility by both methods (Figs 1, 2). The investigation of some human serum samples yielded data which are in good agreement with the results of mouse assay (Table I). The immunization was made by aluminium phosphate-absorbed A, B, C, C and E toxoids (Communicable Disease Center, Atlanta, Ga., USA). The B (0.4 U) and E (2.5 U) antibody values of serum IX were determined by mouse assay. The principles of our methods are valid for the quantitative determination of not only antitoxin A, but also other antitoxins.

Table I

Determination of *Clostridium botulinum* A antibody level of sera by different methods

Serum	IU/ml		
	coated tube method	BAC-antibody method	Mouse assay
I—V	0	0	0
VI	0.026	0.02	0.02
VII	0.075	0.067	0.07
VIII	4.6	4.9	4.5
IX	4.8	4.8	4.5

Acknowledgements

We are grateful to Dr. K.-O. RÄKER for his help in labeling the toxin, and to Mrs. Irmtraud HELLER for skilled technical assistance.

Summary

Two radioimmunoassays based on the competition between free and solid-phase-bound antibodies for labeled toxin were applied for the quantitative determination of *Clostridium botulinum* type A antibodies. In the first type of assay the solid-phase antibodies were prepared by coating plastic tubes; in the second type the antibodies were covalently bound to bromo-acetylcellulose. When the antibodies of the investigated sample and the ^{125}I-labeled toxin were incubated together with the solid-phase antibodies the limit of detectability was about 0.008 IU/ml for both tests. By a pre-incubation of the antibodies to be tested with ^{125}I-labeled toxin the sensitivity increased about two times.

References

BERNÁTH, S. and HABERMANN, E.: Med. Microbiol. Immunol. **160** (1974), 47. BOROFF, D. A. and SHU CHEN, G.: Appl. Microbiol. **25** (1973), 545. FIOCK, M. A., DEVINE, L. F., GEARINGER, N. F., DUFF, J. T., WRIGHT, G. G. and KADULL, P. J.: Immunol. **88** (1962), 277. HABERMANN, E.: Naunyn-Schmiedeberg's Arch. Pharmacol. **281** (1974), 47. HABERMANN, E. and WIEGAND, H.: Naunyn-Schmiedeberg's Arch. Pharmacol. **276** (1973), 321. ROBBINS, J. B., HAIMOVICH, J. and SELA, M.: Immunochemistry **4** (1967), 11. YALOW, R. S. and BERSON, S. Y.: J. Clin. Invest. **39** (1960), 1157.

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PRESENCE OF REOVIRUSES IN CERTAIN GOOSE EMBRYO ISOLATES OBTAINED FROM OUTBREAKS OF VIRAL GOSLING DISEASE AND IN CHICKEN EMBRYOS

By

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The agent responsible for the viral gosling disease, a disease very important economically was identified by Schettler (1971), and later by others (Dannacher et al., 1974; Kisary and Derzsy, 1974; Nadtochey and Kontrimavichus, 1975), as a previously unknown parvovirus. The host range of the parvovirus is narrow: apart from young geese it affects exclusively Muscovy ducklings, and it can be grown only in embryos or cultured cells of these two species. Earlier attempts at adaptation to chick embryo of strains isolated in goose embryo failed also in our hands (Derzsy et al., 1970; Kontrimavichus, 1971, 1975).

Our institutes have regularly exchanged isolates from goose organs since 1972, for the purpose of comparative examination, and adaptation to the chick embryo. The experimental results are reported in this paper.

Materials and Methods

Virus strains. The strains were isolated from organ homogenates obtained from goslings which died of the viral disease. Isolation and maintenance were both made by inoculation into the allantoic cavity of embryonated goose eggs pre-incubated for 9—10 days (Derzsy et al., 1966, 1970).

The strains isolated in the Soviet Union were designated as Yu, KS, BS, Kl, DK, LI, those isolated in Hungary as H, Csősz, Apaj, Rác, TA, SzE, F, L and B.

The strain ITA was isolated in Budapest from a freeze-dried organ homogenate supplied by Professor Mandelli, Italy, whose courtesy is gratefully acknowledged.

Physicochemical properties of the isolates propagated in chick embryo

Each isolate was treated with chloroform as follows: Virus-containing allantoic fluids were centrifuged and an equal volume of chloroform was added to each. The mixture was incubated for one hour at room temperature

during which it was shaken several times. Then the mixture was incubated overnight at +4 °C and centrifuged again. The virus in the supernatant was titrated parallel with a sample of the allantoic fluid.

Certain isolates were tested for heat resistance by exposure to 50 °C for one hour, to 56 °C for 30 min and to 60 °C for 30 min.

Inoculum. Part of the isolates were transferred to chick embryos after one or two passages in goose embryo, others after 5—30 passages. The visceral organs of the infected goose embryos and fragments of the chorioallantoic membrane were homogenized in the allantoic fluid, and the organ suspensions so obtained were centrifuted at 4000 r.p.m. for 20 min. The supernatant was diluted 1 : 2 or 1 : 5 in buffered saline, or infusion broth. In the first passage in chick embryo, the inoculum was prepared similarly. The eggs in which the embryos had not died by the fifth day after inoculation were cooled before opened. 0.2 ml of the 1 : 5 or 1 : 10 diluted suspension was used as inoculum in the allantoic sac. In the later passages more diluted inocula were used. From the third to the fifth passage the allantoic fluid of dead embryos was used as inoculum. Five chick embryos each selected at random from two large groups not inoculated with virus material were processed as above and used for serial blind passages.

Chick embryos. Embryonated hen's eggs pre-incubated for 7—12 days werepurchased from conventional broiler-chicken-producing plants. The health status of the flocks of origin was not known to us.

Antisera. One- to 3-year-old cocks were given either two intramuscular doses or 4—5 alternate intravenous and intramuscular doses of one of the isolats H, Csősz, ITA, KL, BS, Yu, KI or ITA. We exsanguinated two cocks immediately after their arrival to obtain control sera.

Experimental and commercial batches of hyperimmune serum, prepared with strain "B" (DERZSY et al., 1970) in goose broilers, were also used.

Virus neutralization tests. The centrifuged supernatant of allantoic fluid or organ suspension was diluted in tenfold serial steps (1 : 10—1 : 10^6) and to each dilution an equal volume of undiluted or 1 : 2—1 : 4 diluted inactivated (56 °C, 30 min) serum was added.

In the control series either saline or unfusion broth was used instead of serum. The mixtures were thoroughly shaken, incubated for two hours at room temperature, and subsequently 0.2 ml of each were injected into the allantoic cavity of five embryonated hen's eggs pre-incubated for seven days.

Cell culture. Monolayer cultures of renal epithelial cells were prepared from the kidneys of 11-day-old chicks by the usual method, after digestion with trypsin. The cell suspension contained 10^6 cells/ml. The cell suspensions were infected with 0.1 ml allantoic fluid, in further transfers with 0.1 ml tissue culture fluid, and were incubated in Petri dishes (∅ 35 mm) at 40 °C, in CO_2-containing atmosphere.

Concentration of the virus by ultracentrifugation. Allantoic fluids from chick embryos, inoculated with chick-embryo-propagated strains of high titre, were purified by repeated fractional centrifugation, and were subsequently sedimented at 120,000 *g* for three hours in a Beckman Spinco Model L2-65B ultracentrifuge, using a Ti60 rotor. The sediment so obtained from 300 ml material was resuspended in 0.3 ml PBS.

The tissue cultures were concentrated as above, after freezing and thawing.

Electron microscopic examinations. These were carried out in a TESLA BS613 and a JEM7A electron microscope, after negative staining of the concentrated material with a 4% phosphotungstic acid solution.

Results

The strains BS, Csősz and ITA killed the chick embryos within five days already in the first passage. The titre of these strains was above 10^3 from the beginning. The strain Apaj also seemed to be easily adaptable to the chick embryo: 1 : 5 and 1 : 10 diluted material from the first passage killed all chick embryos in the second passage within 3 days, and material from the second passage was similarly lethal in the third chick embryo passage, up to 1 : 1000, the highest dilution tested. The strains Yu and KL killed only 10—50% of the chick embryos in the first two or three passages, but in further transfers the embryo mortality increased rapidly, while the latency period was decreasing until the virus titre reached, or even surpassed, 10^5 in the fifth passage. The strains H and KS similarly reached high titre levels and killed a substantial proportion of the chick embryos after three or four passages.

Adaption was promoted, and rise of the virus titre was enhanced when the preincubation of the chick embryos was reduced from 10 days to 7—8 days. Twelve-day-old chick embryos were even less susceptible than the 10-day-old ones. The experimental data obtained with strain Yu in the two institutes are shown in Table I.

Table I shows that in the group of the older embryos both the mortality and the virus titre were lower and the latency was longer than in the group of the younger embryos. The titres of the virus strains propagated in chick embryo were 10^5—10^6 as determined in eggs pre-incubated for 7—8 days and about 10^4 in those pre-incubated for 10 days.

The lesions found in the chick embryos killed by the virus within 4—5 days after inoculation were similar to those found in goose embryos. Oedema of the chorioallantoic membrane caused its detachment from the shell membrane: this change was occasionally seen already at candling, while the embryo was still alive. The affected embryos regularly showed subcutaneous oedema

Table I

The effect of the age of chicken embryos on the cultivation of strainnYU (Summary of results independently obtained in two institutes)

Virus dilution	Age of chick embryo (days)		
	7—8	10	12
10^{-1}	9/9 (2—3)	10/10 (3—8)	4/4 (4—7)
10^{-2}	9/9 (2—3)	8/10 (3—5)	1/4 (5)
10^{-3}	9/9 (2—4)	8/10 (2—7)	1/4 (5)
10^{-4}	9/9 (3—4)	4/10 (3—6)	1/4 (5)
10^{-5}	9/9 (3—5)	4/10 (3—4)	0/4

Numerator: the number of inoculated embryos; denumerator: the number of killed embryos; figures in brackets: the shortest and the longest time in days required by the virus to kill the embryo.

and hyperaemia in the regions of abdominal wall, lungs, head and neck, causing a roundish conformation of the entire body. In most cases haemorrhages were found in the skin, first of all on the head, neck and toes. Furthermore, the heart was often pale, occasionally yellowish white or white, and its wall was distended. Many embryos had a swollen liver, hyperaemic, or occasionally ochre yellow in colour. Sometimes one lobe exhibited a partial or complete necrosis.

In the embryos which died 5—6 days after inoculation or later, the oedema was less pronounced, but these embryos were retarded, had a deficient feathering and deformed, crooked legs and/or toes. Paleness and/or distension of the heart also were rare, but the liver was either gray or greenish-gray, or of a variegated appearance, owing to mosaic-like yellowish spots of necrosis. Some embryos in this category had a light green allantoic fluid.

Two isolates from the Soviet Union (DK, Li) and six from Hungary could not be adapted to the chick embryo even by 6—8 blind passages.

Although more than half of the strains isolated in goose embryo could not be adapted to the chick embryo, and the degree of adaptation of the apparently adapted strains was variable, we considered the possibility that the agent demonstrated in the course of the blind passages may have been a latent virus resident in the chick embryo. For this reason, 10 chick embryos not inoculated with the goose virus were killed and homogenized, the homogenates were centrifuged and parts of the supernatant were inoculated into the allantoic cavities of embryonated hen's eggs pre-incubated for seven days. The results of this experiment are shown in Table II. Latent virus infection was indeed present in part of the chick embryos, and the titre of this virus (termed KI) considerably increased during three to four transfers so that it reached nearly the same level

Table II

Results of blind passages of homogenates obtained from chick embryos not inoculated with virus

Passage No.	Dilution of inoculum	No. of killed/inoculated embryos	Time required by the agent to kill the embryo (days)
1	1 : 2	0/10	—
	1 : 5	2/10	8
2	1 : 5	1/5	7
	1 : 10	1/5	7
3	1 : 5	6/10	3—4
4	1 : 5	6/10	3—7
	1 : 10	4/5	3—7
	1 : 100	5/5	3—7
5	1 : 10	4/5	2—3
	1 : 100	5/5	2—3
	1 : 1 000	4/5	3—4
6	1 : 10	5/5	3—7
	1 : 100	5/5	3—6
	1 : 1 000	3/5	3—8
	1 : 10 000	3/5	7—8
7	1 : 100	5/5	2
	1 : 1 000	5/5	2—4
	1 : 10 000	3/5	3
8	1 : 1 000	5/5	3—6
	1 : 10 000	5/5	3—7
	1 : 100 000	5/5	4
	1 : 1 000 000	4/5	4—8

Average ± S.E, range, standard deviation and coefficient of variation of dimentions (cm) and weight (g) of ovine female genital tract

and the same latency periods as the strains having initiated from infected goose embryos. Even the lesions were similar in every respect.

All chick embryos which died in the course of the experiments were examined bacteriologically, using agar plates containing horse serum. Outgrowth of bacteria took place in less than 1% of the embryos. These were excluded from further passages.

All virus strains which could be propagated in the chick embryo proved to be resistant to chloroform. The virus titre did not change on one-hour exposure at 50 °C, it decreased on the average by three logarithmic orders on 30 min exposure at 56 °C and the infectivity was lost after 30 min exposure at 60 °C.

A cytopathogenic chloroform-resistant agent producing intra-cytoplasmic inclusions was isolated in chick kidney epithelial cell culture after several blind passages of the chick-embryo isolates KI, Csősz, ITA, YU and BS, and of the goose-embryo isolate H. Neither CPE, nor virus was demonstrable in the chick kidney cell culture itself after several blind passages. Electron microscopic examination of tissue culture concentrates obtained by fractional ultracentrifugation showed the presence of virions 65—70 nm in diameter. The virions of the strain Csősz, supposed to be of goose origin, proved to be closely similar to those of the verified chick embryo isolate KI (Fig. 1).

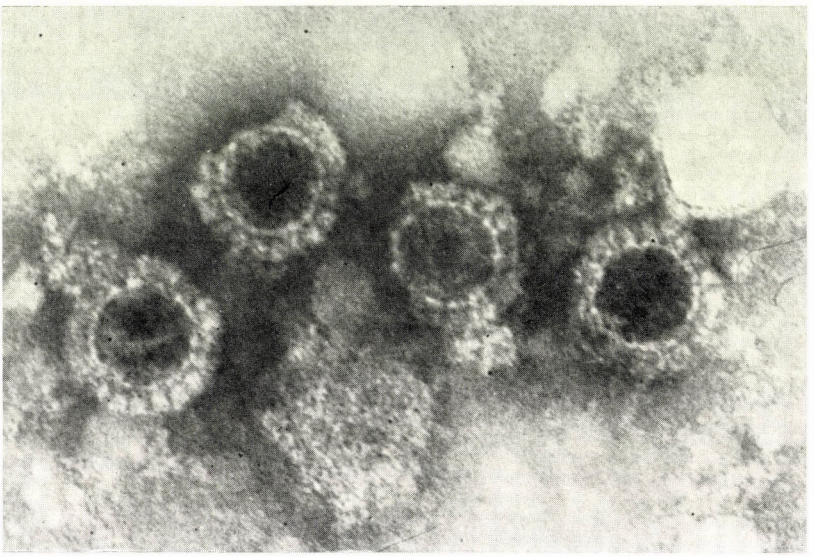

Fig. 1. Electronmicrograph on strain KI. Negative-staining, ×300 000

The antisera prepared in roosters with goose reovirus strain capable of growing in the chick embryo had a uniform neutralizing effect against all such strains. However, low antibody titres were demonstrable in part of the pre-immunization cock serum samples, and certain batches of hyperimmune serum contained, apart from parvovirus antibodies, neutralizing antibodies to the reovirus.

Discussion

A chloroform-resistant agent, capable of growing in the chick embryo, was demonstrated in strains isolated from endemic outbreaks of a lethal gosling disease in the Soviet Union, Hungary, and Italy. A similar agent was isolated after several blind passages from chick embryo homogenates not infected with goose virus. Some isolates could be adapted to chick kidney epithelial

cell culture; out of these the goose isolate Csősz and the chick embryo isolate KI were found to contain non-enveloped hexagonal virions, 65—70 nm in diameter. On the basis of certain important physicochemical properties, the chick-embryo-adapted virus strains as well as the chick embryo isolate KI were classified as reoviruses.

It was often difficult to establish whether one or the other isolate corresponded to chick-embryo-adapted virus strain or to a reovirus strain responsible for latent infection in the chick embryo. At all events, no doubt was left in respect of the origin of strain H isolated directly in chick kidney epithelial cell culture from a goose embryo material; the contamination with a reovirus of the cell culture could be excluded. The isolate KI, having been isolated from chick embryos not infected with goose virus, must be considered of chicken origin. The strains BS, Csősz and ITA, which grow readily in the chick embryo already in the first passage, are in all probability goose viruses. The strain ITA was isolated from the organs of a goose which had groups of characteristically reovirus-like virions in ultra-thin sections of heart muscle (MANDELLI et al., 1971). The origin of those isolates which required several blind passages to grow in the chick embryo at high titres is doubtful, but there are certain indications in support of their goose origin. It should be noted that part of the isolates obtained from endemic outbreaks of the viral gosling disease required 5—8 days to kill the goose embryos, while others required only 2—4 days. Exclusively the strains belonging to the latter category were adaptable to the chick embryo, and killed it after a similarly short period of latency, *viz.* after 2—4 days. It is remarkable that the goose embryos inoculated with pure parvovirus or mixed parvovirus + reovirus isolates showed the same gross lesions as did the chick embryos on infection with reovirus, *viz.*, oedema of the embryo and membranes, hyperaemia, haemorrhages, cardiac and liver lesions. Thus, the lesions are in themselves not conclusive of the nature of the causal agent.

The inconsistent results obtained in goose embryo cross neutralization tests with goose embryo isolates (DERZSY et al., 1970) can be explained by postulating that the goose embryo isolate H was a mixed strain, containing besides the parvovirus (KISARY, 1974), a reovirus. The parvovirus component proved to be antigenically identical with the Hungarian B strain, and with the parvovirus isolates obtained in the Netherlands, in France and in the Soviet Union as well (KISARY, 1974; KISARY and DERZSY, 1975). Although indications of a serological relationship between all examined strains are already emerging from the results of antigenic analysis performed in the course of the present studies, the final evidence of this requires further studies with antisera prepared in such species (*e.g.*, in the rabbit) in which the presence of avian reoviruses can be excluded. The contaminating avian reoviruses can account for the significant virus-neutralizing effect of pre-immunization cock sera.

This also applies to the hyperimmune sera prepared in broiler geese with the goose parvovirus strain "B". The marked reovirus neutralizing potency of such sera has attracted attention to the great frequency of reovirus infection in young, 1—2 months old geese. This conclusion is supported by the observation that the hyperimmune sera prepared in roosters with the examined reovirus isolates had no neutralizing effect whatever on the tissue-culture-pro pagated goose parvovirus strain "B" (DERZSY and KISARY, unpublished data).

The basic information available on the pathogenicity of reovirus strains for goslings is still that reported by CSONTOS and KIS CSATÁRI (1967), *viz.*, that the virus-containing tissue cultures killed about 20% of the inoculated goslings. The related investigations of GAUDRY and TEKTOFF (1972) are not fully conclusive because they tested their reovirus isolate on Muscovy ducklings, of which 25% succumbed to the infection. It would be of immediate interest to obtain precise information on the actual role of simultaneous reovirus infections in the variation of symptoms, gross lesions and mortality between endemic outbreaks of the goose parvovirus disease in the different countries or even within the same country.

Reovirus infections have been studied in greater detail in chickens than in geese and evidence has been accumulating that at least some reovirus types are transmissible through the egg (DESHMUKH and POMEROY, 1969; MENENDES et al., 1975). It was also reported that a latent reovirus infection may be present in chick kidney epithelial cell cultures (ACHMED and SCHETTLER, 1974), but for the present studies this possibility could be excluded by the unsuccessful serial blind passages of the culture. The verified goose reovirus and verified chicken reovirus strains isolated in this study were shown to be antigenically related to one another in cross neutralization tests. Further studies are, however, required to clarify whether the reovirus strains found in goslings represent a given type or types and whether they have any relationship to one or more of the known avian reovirus types, which differ from one another in respect of antigenic pattern and pathogenicity.

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Summary

Propagation of chloroform-resistant virus strains in chick embryos inoculated with goose embryo isolates obtained from endemic outbreaks of viral gosling disease in the Soviet Union, Hungary and Italy is reported. Similar strains were also isolated in serial blind passages of homogenates obtained from uninoculated chick embryos. The agent capable of growing in the chick embryo was identified as a reovirus on the basis of physico-chemical properties and electron microscopic appearance.

The chick embryos killed by reovirus infection showed changes (membrane oedema, cutaneous oedema, hyperaemia, haemorrhages, cardiac and liver lesions) similar to those shown by the goose embryos killed by the goose parvovirus.

The antisera prepared in geese with certain goose parvovirus strains neutralized the chick-embryo-propagated reovirus, but hyperimmune sera prepared with the latter failed to neutralize the goose parvovirus strains.

Further investigations are required to clarify the pathogenicity of the reovirus strains for the goose as well as their precise role in endemic outbreaks of the goose parvovirus disease.

References

ACHMED, A. A. S. and SCHETTLER, C. H.: Berl. Münch. Tierärztl. Wschr., 1974, 477. CSONTOS, L. and M. KIS CSATÁRI, Márta: Acta vet. hung. **XVII** (1967), 107. IDEM: Ibidem **XVII** (1967), 115. DANNANCHER, G., FONILETT, X., COUDERT, M., FEDINA, M. and PEILLON, Myriam: Recueil Med. Vet. **150** (1974), 49. DERZSY, D., SZÉP, I. and SZŐKE, F.: Magyar Állatorvosok Lapja (MÁL), 1966, 388. DERZSY, D., DRÉN, Cs., SZEDŐ, Mária, SURJÁN, J., TÓTH, B. and IRÓ, Emma: Acta vet. hung. **XX** (1970), 419. DESHMUKH, D. R. and POMEROY, B. S.: Avian Dis. **13** (1971), 427. GAUDRY, D. and TEKTOFF, J.-M. CH.: Bull. Soc. Sci. Vét. et Méd. comparée (Lyon) **74** (1972), 137. HANH, N. V.: Acta vet. hung. **XXIV** (1974), 45. KISARY, J. and DERZSY, D.: Acta vet. hung. **XXIV** (1974), 287. IDEM: MÁL, 1975, 199. KISARY, J.: Avian Pathology **3** (1974), 293. KONTRIMAVICHUS, L. M.: Viral enteritis in goslings. In: Boleznyi ptic. Moscow, 1971. IDEM: Acta vet. hung. **XXIV** (1974), 235. MANDELLI, G., VALERI, A., RINALDI, A. and LODETTI, E.: Folia vet. Latina **1** (1971), 122. MENENDEZ, N. A., CALNEK, B, W. and COWEN, B. S.: 20th World Veterinary Congress. Summaries, Vol. 2 (1975), 1062. NADTOCHEY, G. A. and KONTRIMAVICHUS, Lilia M.: In press (1975).

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РЕЗЮМЕ

ИЗБИРАТЕЛЬНЫЙ ЭФФЕКТ D-ЦИКЛОСЕРИН БИТАРТАРАТА В КУЛЬТИВИРОВАНИИ КЛОСТРИДИЙ

Я. ТАКАЧ и Е. З. ИМРЕХ

Авторами изучено торможение D-циклосерин битартаратом роста разных видов клостридий на кровяном агаре и сульфит редукционной среде с яичным желтком. D-циклосерин в концентрации 800 μг/мл сильно тормозил развитие штаммов **C. perfingens**, таковые же **C. histolyticum** совсем не развивались в этих условиях. При концентрации D-циклосерина 600 μг/мл в сульфитном агаре, содержащем взвесь яичного желтка и 20 г/л агара, все виды рода **Clostridium** за исключением **C. perfringens** росли дружно и развивали лецитиназную, липазную и сульфит редуцирующую активности, характерные для данных видов. Штаммы **C. perfringens** развивались только в том случае, если концентрацию ингибитора снизили до 400 μг/мл и культура содержалась при температуре 46 ± 0,5°C; развитие сопровождаюшей флоры тормозилось ингибитором.

C. botulinum и **C. perfringens** легко определимы, если пластинки кровяного агара с 600 μг/мл D-циклосерина и пластинки сульфитного агара с эмульсией яичного желтка и 600 или 400 μг/мл D-циклосерина (**C. perfringens**) будем применять симультанно. Морфология колоний, характер гемолиза следует проверять на кровяном агаре, активность же лицитиназы и липазы — на сульфитном агаре с яичным желтком.

ОБНАРУЖИВАЕМОСТЬ ЗАСОРЯЮЩИХ ВИРУСОВ В ШТАММАХ ВИРУСА ЧУМЫ СВИНЕЙ

II. Изучение цитопатогенного штамма Джилепси типа А и других зарубежных штаммов вируса чумы свиней

Л. БОДОН

Изученный нами цитопатогенный вирус чумы свиней PAV-1 из США в наших условиях не обладал цитопатогенным эффектом. Равным образом не были цитопатогенными вирусные штаммы из Румынии и Болгарии. Наблюдавшиеся изменеия в постоянных клеточных культурах РК-15 не были характерными ни для одной вирусной группы. На основании их характера и подобных изменений в незараженных контрольных клеточных культурах можно заключить, что в данном случае имели место старение клеток или их спонтанная дегенерация.

УДАЛЕНИЕ ЯИЧНИКОВ У СВИНОМАТОК ОПЕРАТИВНЫМ ПУТЕМ

Ф. ТОРДАИ

Гормональная овариэктомия поставленных на откорм свиноматок в Венгрии запрещена, поэтому практикуется способ удаления яичников оперативным путем. В стране очень часто пользуются этим мероприятием особенно в приусадьбенных хозяйствах, откармливающих свиней для личных нужд.

В 30—40-х годах распространился так наз. китайский способ овариэктомии, страдающий некоторыми недостатками, благодаря чему стало необходимым модифицировать старую операционную методику.

У 10-недельных неполовозрелых свинок веса в 30—40 кг автор рекомендует удалить яичники и рога матки (овариогистерэктомия). Операцию можно провести на животном, лежащем или на левой, или на правой стороне, ибо нижний яичник легко доступен. Поочередным втягиванием в операционную рану отдельных участков рогов матки отыскиваем яичник другой стороны, или один палец опускаем в брюшную полость под корпус матки и одновременно вынимаем оба рога с яичниками.

У половозрелых свиноматок возраста 3—4 месяцев и больше операция осуществляется на столе в лежащем на правой стороне положении. Указательный и средний пальцы вдоль прямой кишки протакскиваем в кранио-кавдальном направлении. Между пальцами застревает свободный край lig. **suspensorium ovarii** на конце которого имеются яичники.

ВЫЯВЛЕНИЕ САЛМОНЕЛЛ В МЯСНЫХ ПРОДУКТАХ С ВЫСОКИМ СОДЕРЖАНИЕМ ЖИРА

Я. ТАКАЧ и ДЬ. Б. НАДЬ

В трех экспериментальных сериях сравнивались методы выявления салмонелл и эффективность обогающих сред при температурах 37 и 43°С в мясных продуктах с высоким содержанием жира, готовленных без температурной обработки, консервированных посолами и сушкой. Обнаружено, что наиболее надежным методом является тот, при котором 25 г гомогенизированного колбасного материала вносят в 225 мл тетратионатной питательной среды Бирбрауера, содержащей 1% твин 80 и содержат в течение 24 $\pm$ 2 часа при температуре 42—43°С. Согласно исследованиям авторов ни предварительные обогащения лактозой или буфериро ванной пептонной водой, ни инкубация при температуре 37°С и 42—43°С, ни их вариации далеко не так эффективны, как описанный метод. Преимуществом последнего является еще и то, что в течение 2х24 $\pm$ 2 часов можно определить благополучие образца по салмонеллам, а неблагополучные по салмонеллам производственные партии можно в течение 3$\times$24 $\pm$2 часов выбраковывать; в практике это — благодаря быстроте метода— является благоприятным.

ВЛИЯНИЕ ВОЗРАСТА, КОРМЛЕНИЯ И ОБРАБОТКИ ГОНАДОТРОПНЫМ ГОРМОНОМ НА АКТИВНОСТЬ ЩЕЛОЧНОЙ ФОСФАТАЗЫ СЫВОРОТКИ ЯРОЧЕК

Х. АБДЕЛ-РАХМАН

Определялась активность щелочной фосфатазы в сыворотке ярочек с промежутками времени в 4 недели. Средняя активность энзима в возрасте 19—31, и 45 недель равнялась 6,68, 12,48 и 17,62 BU/100 мл сыворотки соответственно. Повышение активности к возрасту 35 недель совпало с началом овариальной активности, на наличие которой указывало колебание содержания хлора в слизи шейки матки.

Второе усиление активности наступило к моменту оплодотворения (71% животных оплодотворено). Обработка гонадотропным гормоном тоже усилила активность щелочной фосфатазы. Животные, кормившиеся сеном из люцерны и смесью концентратов, прибавляли лучше на весе и показывали высшую активность, чем животные, получавшие только сено из люцерны или луговое сено и кукурузную дерть. В опыте не отмечено влияния возраста на энзимную активность.

УЛЬТРАСТРУКТУРА МОЗГОВОГО ВЕЩЕСТВА НАДПОЧЕЧНЫХ ЖЕЛЕЗ ГУСЯ И УТКИ

Э. ГУЖАЛ и А. ХАССАН

Авторами изучалась ультраструктура мозгового вещества надпочечников гусей и уток обоих полов в активный и неактивный периоды функции половых желез. Описывают четыре типа хромафинных желез. Гранулы адреналин содержащих клеток размера 50—200 μ, в большинстве случаев сферически и разной плотности. Цитоплазма норадреналин содержащих клеток везикулярного характера, сферические или эллипсоидные везикулы размера 150—500 μ и содержат вещество разной плотности с разным распределением.Так называемые темные клетки, содержащие гетерогенной формы гранулы с плотным веществом, согласно авторам являются функционирующей формой норадреналин содержащих клеток. Четвертая форма хромафинных клеток представлена переходными клетками, обладующими отростками (симпатикобласты). В мозговом веществе имеются и мультиполярные клетки.

Описывают два типа крупных вакуолей хромафинных клеток. Синаптические преганглионарные волокна образуют с хромафинными клетками холинэргные синапсы. Среди безмякотных волокон имеются и адренергные таковые. Хромафинные клетки граничат с соматическими сателитными клетками. Клетки спаиваются десмозомами. В интерстиции имеются фибробласты, коллагеновые волокна, Шванновские клетки, безмякотные нервные волокна и синусы, выстланные фенестральным эндотелием.

Авторами не обнаружено существенной разницы между ультраструктурой мозгового вещества надпочечных желез гуся и утки обоих полов и при разном состоянии половых желез.

БИОМЕТРИЧЕСКОЕ ИЗУЧЕНИЕ ГЕНИТАЛЬНОГО ТРАКТА ОВЦЕМАТОК В ПОСТЭСТРУСНОЙ ФАЗЕ

Х. АБДЕЛ-РАХМАН

Размеры и вес генитального тракта 12 овцематок 21-месячного возраста определялись в 3-й день полового цикла. Полученные данные сравниваются с таковыми литературы, полученными на овцах и козах.

ИЗУЧЕНИЕ ДИНАМИКИ ИЗМЕНЕНИЯ УРОВНЯ ПРОГЕСТЕРОНА В СЫВОРОТКЕ И ВЫДЕЛЕНИЯ ПРЕГНАНДИОЛА У КОРОВ

Я. ХАРАСТИ, К. Г. ФЕХЕР и Т. ФЕХЕР

Следить за изменениями уровня прогестерона при половом цикле коров можно двумя путями. Первый путь, это определение количества биологически неактивного прегнандиола в моче, другой — это определение уровня прогестерона в сыворотке периферийной крови. При эструсе выделяется в среднем 2,0 мг/сутки; к 7—8-у и 14—15-у дням цикла — 6,8 и 4,6 мг/сутки соответственно. Уровень прогестерона в сыворотке в день эструса, потом к 6-у, 8—9-у дням формировался следующим образом 1,2—1,8 γ, 1,7—2,8 γ, 4,8—5,4 γ и 2,5—6,2 γ/мл соответственно; к 18—19-у дню т. е., к моменту следующего эструса его показатели равнялись 1,2—1,7 γ/мл-ам. При наличии персистентного желтого тела и ранней беременности изменения этих двух параметров четко отражают клиническое состояние.

ОБНАРУЖЕНИЕ ВИРУСА ПАРАИНФЛЮЭНЦЫ-3 В КРУПНОМ РОГАТОМ СКОТУ С ОСТРЫМИ РЕСПИРАТОРНЫМИ СИМПТОМАМИ

А. БАРТА и Б. КЁВЕШ

Повторно изолирован вирус параинфлюэнца-3 (ЛИ-3) из крупного рогатого скота, страдающего болезнью с острыми респираторными симптомами, быстро распространяющейся в крупных поголовьях. Серологически отрицательные нетели на поздней стадии беременности, поступившие из благополучных приусадьбенных хозяйств в неблагополучное крупное хозяйство очень скоро тяжело заболели, часто с летальным исходом. В многих случаях болели откормочные бычки и 3—6-месячные телята; у последних обычно возникла вторичная пневмония. Животные, которые переболели заразой 6 недель раньше и обладали высоким титром антител, при вспышке заразы клинически не болели, зато у заново поступивших восприимчивых животных развилась тяжелая болезнь. Животные с низким уровнем вируснейтрализирующих антител переболели легко. Можно предполагать, что секреторные антитела (IgA) играют более важную роль в иммунологической защитной реакции, чем вируснейтрализирующие антитела.

ИЗОЛИРОВАНИЕ РЕСПИРАТОРНОГО СИНЦИЦИАЛЬНОГО ВИРУСА ИЗ КРУПНОГО СКОТА, БОЛЕЮЩЕГО РЕСПИРАТОРНЫМИ СИМПТОМАМИ

Б. КЁВЕШ и А. БАРТА

Изолирован респираторный синцициальный (РС) вирус из телят с острыми респираторными симптомами в вращающихся пробирочных культурах из телятиного почечного эпителия. Цитопатогенный эффект вируса наступил только после трех пассажей и то к 12—14-у дню последнего. Дальнейшими пассажами удалось укоротить срок наступления цитопатогенного эффекта до 5 дней. Репликация вируса наступила в невращающихся культурах, если инокуляция наступила еще до возникновения сплошного одноклеточного слоя или вирус вводили в взвесь клеток, содержащихся для получения однослойной клеточной культуры. Изолят не гемагглютинировал эритроциты разных видов животных. Изолят нейтрализировался антителами из переболевших животных при титрах от 1 : 4 до 1 : 16. Этиологическую роль других респираторных вирусов удалось исключить на основа нии серологической идентификации.

ДЕЙСТВИЕ КОРМА, СОДЕРЖАЩЕГО ФУЗАРИОТОКСИНЫ T2 и F2 НА КЛАДКУ ЯИЦ У ГУСЯ

М. ПОЛЮШИК и Е. КОПЛИК-КОВАЧ

Авторами изучался эффект фузариотоксинов на кладку яиц у племенных гусей Гуси-несушки кормились кормом, содержащим культуру грибков **Fusarium culmorum** и **F. sporotrichioides** на кукурузе, продуцирующих токсины F2 и T2 соответственно.

При кормлении кормом, содержащим 3 мг% токсина T2, кладка яиц в течение 10 дней полностью прекратилась. Животные этой группы, за исключением убитых ради изучения, все погибли. При вскрытии обнаружены сильное похудение, атрофия яичников и яицевода, сильное перерождение сердечной мышцы, некроз и дифтероидные наслоения на слизистой рта, гортани и языка.

Гистологическим исследованием в органах гусей, получавших T2, воспаление обнаружить не удалось, но гибель фолликул в яичниках наступила скоро. В возникновении микроскопических кровеизлияний в сердечной мышце и печени — согласно авторам — не исключена роль стенку сосудов повреждающих трихотекановых токсинов.

Токсичный корм кроме токсина T2 содержал и другие, ближе неидентифицированные трихотекановые токсины.

Скармливание корма, содержащего 100 мг% токсина F2, по существу не отразилось отрицательно на кладке яиц и их вылупляемости.

СЕЗОННАЯ ИЗМЕНЧИВОСТЬ В ИСПОЛЬЗОВАНИИ ЩИТОВИДНОЙ ЖЕЛЕЗОЙ I^{131} И СЕКРЕЦИИ ТИРОКСИНА У БУЙВОЛОВ

П. ПРАКАШ и Д. ШАРМА

У буйволят обоих полов и разных возрастных групп изучалась сезонная изменчивость в использовании щитовидной железой I^{131} и секреции тирокисна. У буйволят-бычков от лета до зимы использование повысилось на 28—40%, а у буйволят-телок — на 25—37%. Максимальные показатели получились за срок 36 часов и 42 часов осенью и весной соответственно.

Средние показатели секреции тироксина в группах буйволят-бычков разного возраста от лета до зимы повысились с 0,29 до 0,60 мг/100 кг живого веса. Подобное повышение секреции пироксина с 0,30 до 0,62 мг/100 кг ж. в., наблюдалось и у буйволят-телок. С возрастом у буйволят обоих полов, независимо от сезона, зарегистрировано снижение показателей секреции тироксина.

ДВЕ РАДИОИММУННЫХ ПРОБЫ С СТОЙКОЙ ФАЗОЙ ДЛЯ ИЗУЧЕНИЯ КОЛИЧЕСТВА АНТИТЕЛ ПРОТИВ Clostvidium botulinum A.

Е. ХАБЕРМАН и Ш. БЕРНАТ

Разработаны две радиоиммунных пробы, базирующиеся на гонке антител, связанных с свободной и стойкой фазами, за меченым изотопом токсином. Связанный со стойкой фазой антиген в первом методе получался путем обволакивания пластмассовых тубиков, во втором-же антитела связывались ковалентной связью с бромацетил-целлюлезой. Если изучаемую пробу с антителом и меченный I^{131} токсин совместно инкубировали со связанным с стойкой фазой антителом, то чувствительность метода при обоих способах равнялась примерно 0,008 МЕ/мл. Если изучаемые антитела и меченный I^{131} токсин предварительно инкубировались совместно, то чувствительность повысилась в два раза.

ОБНАРУЖИВАЕМОСТЬ РЕОВИРУСОВ В ИЗОЛЯТАХ ИЗ ГУСИНЫХ ЗАРОДЫШЕЙ ПРИ СМЕРТЕЛЬНОМ ВИРУСНОМ ЗАБОЛЕВАНИИ ГУСЯТ И В КУРИНЫХ ЗАРОДЫШАХ

Д. ДЕРЖИ, Я. КИШАРИ, Л. М. КОНТРИМАВИЧУС и НАДТОЧЕЙ

В куриных зародышах удалось размножать хлороформ-устойчивые вирусные штаммы, изолированные из гусят при вирусных эндемиях в Советском Союзе, Венгрии и Италии. Подобный вирусный штамм удалось изолировать путем слепого поссажа материала, полученного из незараженного куриного зародыша. Размноженный в курином зародыше вирус на основании электронно-микроскопической картины и некоторых физико-химических свойств оказался реовирусом.

Изменения в погибших от заражения реовирусом куриных зародышах (отечность зародышевых оболочек, отечность кожи, кровеизлияния, изменения в сердечной мышце и печени) были подобны изменениям, обнаруживающимся в погибших от реовируса гусиных зародышах.

Антисыворотки, полученные использованием некоторых гусиных парвовирусов, нейтрализировали размноженный в курином зародыше реовирус, но антисыворотки, полученные использованием последнего, не действовали против штаммов гусиного парвовируса. Это является доказательством того, что некоторые группы гусей естественным путем заражаются реовирусом, после чего уних наступает энергичное образование антител против данного вируса.

Определение патогенности реовирусных штаммов в отношении гусей, изучение их роли в патологической картине и формировании падежей при эндемиях, вызванных парвовирусами, требует дальнейших исследований.

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