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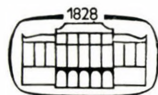

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FOETAL ACTIVE IMMUNIZATION OF CALVES FOLLOWING INOCULATION OF THE DAM WITH A BOVINE VIRAL DIARRHOEA VACCINE (VÉDÉVAC)

By

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(Received March 16, 1972)

The observation that the calves born to cows inoculated with the live bovine viral diarrhoea (BVD) vaccine Védévac (SIMONYI et al., 1967) during the second semester of pregnancy possessed specific serum antibodies already prior to the intake of colostrum, was first described in a preliminary report in 1969 (BOGNÁR, 1969). Since then, succesful protection of pregnant cows and their offsprings by Védévac has been substantiated under field conditions by the clinical and epizootological observations of KOÓS and BERECZ (1971) and SZABÓ (1971) in a herd exposed to BVD. This phenomenon of "foetal active immunization", which was initially demonstrated in the calves of a few cows only, is therefore now postulated to be general in occurrence.

The immunity evoked in the calves concerned must have arisen in the foetus, because according to the still valid assertion of SCHNEIDER and SZATHMÁRY (1939), no antibodies can cross the chorio-epithelial placenta from the maternal into the foetal circulation.

The stimulation of a foetal antibody response by BVD virus has been confirmed independently by three American groups. WARD et al. (1969) infected 11 serologically negative pregnant cows with virulent virus. Directly after parturition, three of the newborn calves showed symptoms of the disease, whereas four had developed serological antibodies to the agent and were not affected. CLASSICK et al. (1970) infected a susceptible 4-year-old cow, previously checked serologically twice every year, with virulent BVD virus and removed her foetus 33 days later by Caesarean operation. No virus could be isolated from the organs and lymph nodes of the foetus, but its serum contained specific anti-BVD antibodies. Finally, KAHRS et al. (1970) have described an outbreak of BVD in a cattle herd during which eight of 29 pregnant cows aborted and two calves developed cerebellar hypoplasia. Of 14 calves examined serologically, eight had serum antibodies prior to the intake of the colostrum

These data support my earlier conclusion that BVD virus can gain access to the bovine foetus and there, depending on its nature, either stimulate antibody formation or produce changes characteristic of the disease.

While these other authors studied the effect of virulent BVD virus on the calf foetus, the experiments reported here were carried out with the aim of improving control of the disease, particularly with regard to the protection of calves which are exposed to virulent virus from birth onwards.

* Technical assistance: J. MARJAI.

Material and methods

Vaccine. The preparation "Védévac" (PHYLAXIA, Budapest) was employed for immunization of the cows. This vaccine contains BVD virus of the Oregon $C_{24}V$ strain, modified by serial transfers in embryonic calf kidney epithelial cell culture and alternate passages in animals (SIMONYI et al., 1967).

Tissue culture. The examinations were carried out on monolayer cultures of trypsinized calf testicle cells, using a growth medium of 0.5% lactalbumin hydrolysate in Hanks' solution containing 15—20% calf serum.

Experimental animals. The investigations were conducted in a previously infected herd of the "Gyöngyvirág" Agricultural Cooperative, Egerág (Hungary).

Vaccination. A total of 102 cows were vaccinated with 2 ml Védévac intramuscularly, during the second semester of pregnancy. Of these, 31 were given a single injection 12—113 days before parturition, while 71 were vaccinated once 33—127 days before calving and a second time 25—35 days later. Blood samples were taken from all cows on the day of the first vaccination and also from 72, on the day of parturition, along with colostral samples. Whenever possible, serum samples were taken from the calves prior to the first sucking and again at 3—10 days of age. A third blood sample was collected from 20 of the calves between 37 and 215 days of age.

Virus neutralization test. Blood serum and colostral samples were assayed for antibody content by virus neutralization test in calf tecticle monolayer cell cultures. For this purpose, serial four-fold dilutions of each sample, incubated for 30 minutes at 56 °C, were added to 100—300 $TCID_{50}$ of the virus. The mixtures were incubated for 1 hour at 37 °C then 0.2 ml was transferred to each of three tube cultures, either immediately after this or after further incubation for 1 hour at 4 °C. The tube cultures were observed for 7 days and the 50% virus neutralizing dose of the serum or colostrum was assessed from the degree of cell destruction.

Quantitative determinations of the virus dose used in the neutralization test were carried out in five replica tube cultures for each dilution step. In every case, the same anti-BVD hyperimmune serum preparation (PHYLAXIA, Budapest) was used as serum control.

Samples taken at different times from each cow and her calf were examined simultaneously. All samples were stored at —20 °C until use.

Results

Immunological status of the herd. BVD was first observed among rearing stock on the farm in 1967, when 15% of the calves born in that year died (HELLNER et al., 1969). From then up to the time of the present study, the

management had arranged for regular vaccination of all calves with Védévac at about 10 days of age and again one month later.

This and other strictly observed control measures had the result that no symptoms of BVD occurred subsequent to 1967, and no calf died from the disease. Non-immunized cows had shown no symptoms of BVD at the time of the outbreak, although the cow houses were sited alongside the rearing premises. Nevertheless, the virus had probably been introduced into the cow houses as well, because 37 of the 102 cows examined exhibited specific serum antibodies. The antibody titres assessed in serum samples from 24 of these cows ranged from 1 : 16 to 1 : 640, with a mean titre of 1 : 280. The distribution of serum antibodies in the cows according to age is shown in Table I.

Table I

Age distribution of cows with and without serum antibodies

Age (years)	3	4	5	6	7	8	9	10	11	12	13
Number	3	11	19	11	25	12	9	3	6	2	1
Serum antibodies absent	2	6	18	7	15	4	8	1	4	—	—
Serum antibodies present	1	5	1	4	10	8	1	2	2	2	1

The data clearly point to a high susceptibility of the 5-year-old age group. At the time of the outbreak, cows of this age group had been kept in the heifer rearing premises, where no disease had occurred.

*Titration of serum samples collected on the day of parturition**

The serum antibody titre of the dams rose measurably when at least 14 days had elapsed between vaccination and parturition. The extent of vaccine-induced antibody formation differed according to whether antibodies were already present or absent in the cow at the time of vaccination. Serum samples taken on the day of parturition from 10 originally susceptible animals which had been vaccinated on a single occasion had a mean neutralizing antibody titre of 1 : 869, compared with 1 : 1426 for samples from 37 such cows vaccinated on two occasions.

The average pre-vaccination titre of three once-vaccinated cows which possessed antibodies prior to immunization was 1 : 126 while at parturition

* Mean titres were calculated solely from the data of those animals which calved, or were born, at least 14 days after vaccination.

the same animals displayed an average serum titre of 1 : 236. For 20 twice-vaccinated cows, also possessing antibodies prior to immunization, the average pre-vaccination titre was 1 : 320 and the titre at parturition 1 : 1190 (see Table II).

Table II

Distribution of cows according to serological and colostral antibody levels

No. of vaccinations	Pre-vaccination status	Test material	Neutralization titre					Total no. of samples	Mean titre
			Negative	cc–40	54–410	640–3480	>6553		
Single dose	Negative	serum	0	1	3	6	0	10	869
		colostrum	0	0	1	2	7	10	10,067
	Positive	serum	0	1	1	1	0	3	236
		colostrum	0	0	1	2	1	4	2509
Two doses	Negative	serum	0	2	6	27	2	37	1426
		colostrum	0	0	0	8	23	31	9684
	Positive	serum	0	3	9	7	1	20	1190
		colostrum	0	0	1	11	8	20	5565
Unvacc. control	Negative	serum	6	0	0	0	0	6	0
		colostrum	6	0	0	0	0	6	0
	Positive	serum	0	2	3	2	0	7	223
		colostrum	0	1	0	3	3	7	5772

Antibody content of the colostrum

Samples for colostral antibody determination were obtained from 65 of the vaccinated cows. Colostrum from those which had displayed no serum antibodies at vaccination neutralized the virus in the titre range 1 : 2560—1 : 16,384, the average colostral antibody titre of 10 such cows vaccinated on a single occasion was 1 : 10,067, while that of 31 twice-vaccinated cows was 1 : 9684.

As with the serum, the colostrum of cows which had already possessed antibodies at vaccination displayed a lower neutralizing effect compared to colostrum from originally susceptible animals: the average colostral antibody titre of once-vaccinated cows was 1 : 2509 (range: 1 : 54—1 : 16,384) and that of the twice-vaccinated cows 1 : 5565 (range: 1 : 640—1 : 16,384) (see Table II).

Pre-sucking virus neutralizing serum titres of calves from cows vaccinated during pregnancy

Of the 72 vaccinated cows concerned, 48 did not possess serological antibodies on the day of vaccination. Calves of 34 cows from the latter group had antibodies in serum samples taken prior to the first sucking. Calves dropped by once-vaccinated cows and possessing antibody prior to the intake of colostrum showed a mean serum antibody titre of 1 : 1231 (range: 1 : 10—1 : 3480), compared with 1 : 4046 (range: 1 : 160—1 : 10,240) for those born to twice-vaccinated dams.

The calves of the 24 cows which already had antibodies at vaccination, produced no antibodies themselves, regardless of whether the dam was vaccinated once or twice (see Table III).

Table III

Antibody levels of calves born to the examined cows

No. of vaccinations	Pre-vaccination status of cow	Number	Titre	Number	Titre
		of calf sera examined			
		before 1st sucking		at 3—10 days of age	
Single dose	Negative	6	1231	3	5631
	Positive	4	0	3	115
Two doses	Negative	28	4046	25	5623
	Positive	20	0	15	265
Unvaccinated control	Negative	6	0	6	0
	Positive	7	0	4	38

Serum antibody titres of calves at 3—10 days of age

The data have to be divided into two groups for evaluation, according to whether the calves already possessed antibodies at birth, or only acquired them passively through intake of colostrum.

Twenty-eight calves had a mean pre-colostral serum antibody titre of 1 : 5630 (range: 1 : 218—1 : 10,240). Antibody levels acquired only through sucking the colostrum, on the other hand, did not exceed 1 : 115—1 : 265. Similar titre values were found in those cases in which the cow had originally been susceptible, but her calf did not display antibodies before the first sucking (see Table III).

Serum antibody titres of calves between 37—215 days of age

The serum of 12 calves which had possesed pre-colostral antibodies was examined again 44—215 days after parturition. The mean pre-colostral serum titre of these animals was 1 : 2174, but 1 1/2—7 months after birth this had declined to 1 : 1490 (range: 1 : 218—1 : 2560) (see Table IV).

Table IV

Serum antibody levels of calves at 31—215 days of age

Designation of calf	Serum antibody titre		Age at 2nd blood sampling	Serum antibody titre at 2nd sampling
	before 1st sucking	3—10 days of age		
393/1	160	—	44	218
233/1	10,240	—	59	2560
290/1	2560	6553	67	1638
378/1	3480	3480	107	640
135/1	640	2560	126	2560
60/1	160	2560	170	2560
258/1	10,240	10,240	170	410
243/1	870	6553	177	640
197/1	870	3480	209	218
325/1	218	3480	210	1638
369/1	4096	4096	211	2560
236/1	2560	2560	215	2660
319/1	neg.	—	37	160
250/1	neg.	—	42	25
157/1	neg.	160	52	25
350/1	neg.	—	56	54
190/1	neg.	410	73	40
377/1	neg.	1638	100	25
201/1	neg.	25	114	neg.
152/1	neg.	160	131	4

The serum titres of calves acquiring the antibodies passively through the colostrum fell gradually with age and in no case attained the levels of actively formed antibodies. At 37—131 days of age, the average serum titre was 1 : 41 (range: 0—1 : 160).

Serological and colostral antibody contents of unvaccinated control cows

Serum samples and colostrum collected from 13 unvaccinated cows on the day of parturition as well as the pre-sucking serum samples of their calves were examined.

In six of the cows no antibodies were detected either in serum, or colostrum, while the other seven had a mean serum titre of 1 : 223 and their colostrum neutralized the virus up to an average dilution of 1 : 5772. The colostral antibody levels of the naturally infected cows fluctuated considerably between individuals.

The calves of those cows which possessed neither serological, nor colostral antibodies at parturition, did not acquire antibodies from the dam. Tests on the serum of the calves of four from the seven cows which possessed antibodies demonstrated that the serum antibody titre of the young animals at 3—10 days of age depended on the antibody content of the colostrum, but was on average only 1 : 38 (Tables III and IV).

Passive immunity conferred by anti-BVD hyperimmune serum

At birth calves of three unvaccinated cows not possessing antibody on the day of parturition, were treated intravenously with 100 ml hyperimmune serum. The serum neutralized 7350 $TCID_{50}$ virus at 1 : 1536 dilution. Three days after treatment the calves displayed serum antibody levels of 1 : 40—1 : 54, and this declined to 1 : 14 by the 21st day.

Discussion

The investigations demonstrate that the BVD vaccine Védévac successfully immunizes pregnant cows. The vaccine is innocuous for the cows and for the foetus throughout intrauterine life. The main indication of the vaccine is in herds in which the disease occurs regularly among the calves and/or cows, or in which exposure cannot be prevented.

Vaccination of the cows during pregnancy presents two advantages. One is that it boosts the degree of protection bestowed by the colostrum a rule to which there are only few individual exceptions; a high colostral antibody level can be ensured for the newborn calf by picking the optimal time for vaccination of the dam. The other advantage is that if the dam is susceptible at vaccination, the vaccine virus may gain access to the foetus and there stimulate antibody production, so that the calf is already at birth provided with a significant degree of active protection.

The colostral antibody level cannot be determined before parturition, but the pre-parturition serum titres provide some guide to the expectable protection. It may be asserted, with certain reservations, that serum antibody titres reflect the colostral antibody content: high pre-parturition serum antibody levels almost invariably signified a high degree of subsequent colostral protection. The parallel studies of serum and colostrum samples from 65 cows showed that the antibody levels in colostrum were always higher than in serum. The control tests on 13 unvaccinated cows underline the point that if humoral antibodies are absent, it is likely that no specific antibodies will appear in the colostrum either.

The serum antibody level attained depended to some extent on whether vaccination was carried out once or twice. It seems important to note, however that while in half the cows already possessing antibodies at vaccination the serum antibody titre did not rise above the average level evoked in response to natural infection (1 : 300), among the cows which were susceptible before the first vaccination, only 13% had comparably low levels at parturition. On the other hand, the mean colostral virus neutralizing titre was 1 : 2500 in originally unsusceptible, once-vaccinated cows but 1 : 5500 in twice-vaccinated animals. Although these titres were assessed at relatively low dilutions, the colostral antibody contents of the animals in question were sufficient to confer a useful passive immunity.

Specific antibodies also appeared in the serum and colostrum of naturally infected cows. At the onset of — or even during — an outbreak, however, only part of the herd may come into contact with the infectious material: in the cow herd studied, 65 of 102 cows did not possess serological antibodies. Cows which become infected with virulent virus in an advanced state of pregnancy may become severely affected by the disease and even may not secrete colostral antibodies, if the time between infection and parturition is too short for the development of an immune response. The colostral antibody levels vary greatly with the individual because they depend on the time of infection, individual susceptibility, virulence of the infectious agent, etc. Individual cases of passive immunization can be regarded as an established fact, because it is often observed that some of the rearing calves from an infected herd develop neither symptoms nor oral lesions during the first two months of life, even when the rearing premises are overcrowded. Calves whose dam either catch the disease or come into contact with the agent, acquire adequate passive protection through the colostrum to prevent infection for a certain time.

Of the 102 cows vaccinated, 37 already possessed serum antibodies prior to treatment. Serum from 24 cows of this group and pre-sucking serum samples of their calves were examined. None of the calves born to dams already immune at vaccination possessed virus neutralizing serum antibodies prior to the intake

of colostrum, indicating that the virus could not have had access to the foetal organism. This rule appeared to be unrelated to the actual serum antibody level of the cow, even if the pre-vaccination maternal antibody level was only measurable in undiluted serum. Apparently, even a low maternal serum antibody content was sufficient to neutralize the virus content of the vaccine *in vivo*. The same process probably takes place when the calf acquires maternal antibodies from the colostrum: such passive antibody seems able to inhibit virus multiplication to the degree that no symptoms appear. The same applies to the protection of the calf by inoculation with hyperimmune serum.

The high serum and colostral antibody levels stimulated by the vaccination of susceptible cows suggest that the BVD vaccine virus can persist in the host organism for a long time, all the while exerting an additional antigenic stimulus. If, however, serum antibodies are already present prior to vaccination, they largely impede this function of the virus, and the effect of the vaccine is reduced to that of a single antigen booster, to which the vaccine responds according to its individual sensitivity. In the interests of promoting antigenic action to the fullest possible extent we recommend a two-dose immunization schedule for pregnant cows, because it can not generally be ascertained in practice whether any given animal of a herd is still susceptible or already possesses antibodies. Even if the latter should be the case, treatment with two immunizing doses will produce an average colostral antibody level of 1 : 5000, which is sufficient to provide a good passive protection of the newborn calves.

The serum of 3—10 days old calves born to originally immune, twice-vaccinated cows displayed an average virus neutralizing titre of 1 : 265 — roughly one-twentieth of the maternal colostral antibody content.

The occurrence of foetal active immunization can be regarded as firm evidence that the calf possesses immunologically competent cells capable of specific antibody production already during intrauterine life. Among the calves originating from 48 cows not possessing antibodies prior to vaccination, 34 had serological antibodies already before first sucking. Reference has been made in this context to the statement of SCHNEIDER and SZATHMÁRY, that no maternal antibodies can reach the bovine foetus in intrauterine life. The finding that 24 calves had higher pre-sucking serum antibody levels than their dams is further proof of the reality of foetal active immunization.

The experimental data fail to explain why only 70% of the calves born to dams found susceptible at the first vaccination had active antibodies at birth. As yet only a tentative answer can be advanced. Possibly the applied method was not sensitive enough to detect minute quantities of antibody. It is known that humoral antibodies disappear from the blood sooner or later following convalescence, whereas the cell-bound protective mechanism persists and is immediately reactivated on renewed exposure to the antigen (infectious material, vaccine). The wide range of antibody levels assessed on the day of

vaccination in this experiment suggests that the involvement of a cellular immune mechanism indeed cannot be excluded.

The theoretical aspects of the problem and the identification of the foetal organ responsible for antibody production remain the topics for further study.

The occurrence of active antibody production during foetal life is further affirmed by the measurements of serum antibody levels in several months old calves. The active antibody level acquired during foetal life, rises after the intake of the colostrum then tends to decline gradually: but it is still fairly high at 6—7 months of age, usually corresponding with the initial, pre-sucking value. Against this, the level of antibodies acquired through the colostrum falls slowly, but continuously. The colostral protection bestowed on the calf depends on the original colostral antibody level, but irrespective of this by 3—4 months it has either vanished completely or remains only in traces. The fact that calves with active and passive immunity were kept together on the farm has to be counted as an experimental error, because the animals may have picked up antigen by contact, and this natural exposure may have reinforced the immunogenic stimulus. Nevertheless exposure would presumably have been uniform, and in any case, this does not alter the conclusion that immunity acquired in foetal life is firm and durable.

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SUMMARY

Field studies in a cattle herd previously infected with bovine viral diarrhoea (BVD) confirmed an earlier observation that the majority of the calves of susceptible cows vaccinated against the disease in the second semester of pregnancy develop an active immunity to the agent during foetal life, whereas the offsprings of cows already possessing specific antibodies at the time of vaccination do not possess any detectable active immunity at birth. It was found that a two-dose vaccination schedule (with inoculation of the pregnant cows at 2 and 1 months, respectively, before the expected term) ensures a firm active immunity for the calves of originally susceptible cows and a high degree of passive colostral protection for the calves born to dams which already possessed antibodies at the time of the first vaccination. The preparation Védévac (PHYLAXIA, Budapest) proved innocuous for the pregnant cow and the foetus.

Additional protection of the calves by vaccination later in life is, nevertheless, important, because screening of the young animals for active or passive immunity to BVD virus is impracticable under field conditions. Two doses of vaccine, given at the age of 1—2 months and 30 days later, stimulate an active immunity by the time the colostral protection declines.

Vaccination of pregnant cows and calves is strongly recommended in problem herds in which BVD occurs regularly, as well as in cases where exposure of the young animals cannot be prevented.

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IMMUNOLOGICAL STUDY OF RABBIT CYSTICERCOSIS

VI. ISOLATION OF SPECIFIC ANTIBODIES FROM SERUM OF RABBITS INFECTED WITH THE LARVAL FORM OF *TAENIA PISIFORMIS* (BLOCH, 1780) GMELIN, 1790

By

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In the serum of rabbits infected with oncosphaeres of *Taenia pisiformis* (Bloch, 1780) Gmelin, 1790 (Cestoda: Taeniidae) antibodies are formed to antigenic components of the adult tapeworm and its larval stage. Serum antibodies to the parasite have been demonstrated by several tests, such as ring precipitation (CAMPBELL, 1938; SHUL'TS and ISMAGILOVA, 1965), *in vitro* precipitation of artificially hatched and activated oncosphaeres (SILVERMAN, 1955; 1956), agar gel diffusion precipitation (NÉMETH, 1965), complement fixation (SHUL'TS and ISMAGILOVA, 1965) and indirect haemagglutination (SHUL'TS and ISMAGILOVA, 1965; NÉMETH, 1965).

It has been shown that the humoral immune response of rabbits to experimental infection with *T. pisiformis* oncosphaeres is heterogeneous. Although the bulk of the antibodies formed belong to the IgG class, high-titre serum collected from rabbits infected on two occasions also contained antibodies of the IgM type (NÉMETH, 1972 b). Antibodies to the antigenic component(s) of the parasite have not as yet been isolated from the serum.

This paper reports the isolation of pure antibodies from the serum of rabbits experimentally infected with *T. pisiformis* oncosphaeres.

Materials and methods

Antigens

The acid-soluble fraction of a crude extract of infective larvae of *T. pisiformis* (NÉMETH, 1971) was used as antigen for the preparation of the immunoabsorbent and in the agar gel diffusion precipitation tests.

The antigen for indirect haemagglutination tests was prepared from *T. pisiformis* by the method of LANCEFIELD (1928), as modified by NÉMETH (1965).

Antisera

Precipitating polyspecific goat immune serum to rabbit serum proteins, anti-rabbit IgG + IgM sheep immune serum and monospecific anti-rabbit IgG and anti-rabbit IgM sheep immune sera were prepared as described previously (NÉMETH, 1972 a, b).

Serum of infected rabbits

Twenty New Zealand rabbits aged 5—6 weeks, weighing 700—900 g, were each infected with 500 *T. pisiformis* eggs administered directly into the stomach by means of a rubber tube fitted to a syringe. The eggs were isolated from gravid proglottids spontaneously passed by experimentally infected dogs, as described previously (NÉMETH, 1970). Forty days after the infection, one half of the rabbits was killed by exsanguination through the carotid artery, the other half was reinfected with 10,000 eggs at the 60th day and killed by bleeding 40 days later. Serum samples which reacted positively in the agar gel diffusion precipitation test were selected, those of similar titre in indirect haemagglutination test (IHA) being pooled for further investigation. Four serum pools were thus prepared for antibody isolation: pools I and II (haemagglutinaton [HA] titres of 1 : 640 and 1 : 160, respectively) from samples collected at 40th days after the first infection; and pools III and IV (HA titres of 1 : 1536 and 1 : 4096), from samples collected 40 days after the second infection. The serum pools were stored at —20 °C without preservative.

Preparation of the immunoabsorbent

The working principle was adopted from AVRAMEAS and TERNYNCK (1969) in that the proteins present in the antigen solution were polymerized with glutaraldehyde. Preliminary polymerization experiment carried out in this laboratory at pH 4 and 5 (in 0.2 M acetate buffer), pH 6, 7 and 9 (in 0.1 M phosphate buffer) showed that if 10—20 mg glutaraldehyde* is added for every 100 mg protein-antigen, complete insolubilization of all proteins in the antigen solution (concentration: 15 mg/ml) takes place overnight in the pH range 4—6. Accordingly, polymerization was thereafter carried out at pH 5 as follows: 200 mg bovine serum albumin** was added as carrier protein to an antigen solution containing 100 mg protein, and the mixture was dialysed against several changes of acetate buffer (0.2 M, pH 5.0), for 6 hours under continuous magnetic stirring.

After dialysis the pH was checked and, if necessary, adjusted to precisely 5 with 1.0 M acetate buffer (pH 5.0). Fifteen mg glutaraldehyde in 2.5% aqueous solution was then added for every 100 mg of protein and the mixture was allowed to stand overnight at room temperature. After the addition of glutaraldehyde, the final concentration of proteins was about 20 mg/ml. The further treatment of the gel formed as a result of protein insolubilization corresponded in every respect with the procedure of AVRAMEAS and TERNYNCK (1969).

* Reanal, Budapest, Hungary.
** Phylaxia, Veterinary Biologicals and Feedstufs, Budapest, Hungary.

Absorption and elution of antibodies

Isolation of antibodies from the serum of infected rabbits was carried out according to the batch procedure of AVRAMEAS and TERNYNCK (1969). An appropriate quantity of the immunoabsorbent was added to previously defatted serum in centrifuge tubes, the suspension was slowly stirred for 30 min at room temperature and then centrifuged at 3000 revolutions per minute (r.p.m.) for 15 min at 4 °C. If antibodies were still demonstrable in the supernatant by IHA test, the procedure was repeated with a fresh aliquot of absorbent. The immunoabsorbent-antibody complex was suspended in 100 ml isotonic saline, washed, and centrifuged at 3000 r.p.m. for 15 min. The combined washing and sedimentation procedure was continued until the O.D. of the supernatant at 280 nm measured by Unicam SP-500 spectrophotometer reached about 0.02. The washing fluid was collected, concentrated by ultrafiltration and tested for antibodies by IHA test.

The absorbed proteins were eluted (i) in 0.1 M glycine-HCl buffer (pH 2.8), or (ii) in 2.5 M and then 5.0 M $MgCl_2$ solution in 0.05 M Tris-HCl buffer (pH 7.5). In case (i) the washed immunoabsorbent was suspended in 4 ml eluent, stirred for 10 min at room temperature and centrifuged at 10,000 r.p.m. for 15 min at 4 °C, then the entire procedure was repeated twice over. In case (ii) elution was carried out twice in 20 ml each of 2.5 M $MgCl_2$ solution and once in 20 ml 0.5 M $MgCl_2$ solution, in the same manner as above.

The centrifuged eluates were mixed, passed through an asbestos filter pad, dialysed against frequent changes of isotonic saline, concentrated by ultrafiltration, dialysed against phosphate-buffered saline (PBS: 0.04 M, pH 7.3) containing 0.01% merthiolate, and stored at 4 °C until use.

Demonstration of antibodies

Antibodies in whole serum and antibody preparations were demonstrated by IHA and agar gel diffusion precipitation tests.

Indirect haemagglutination test (IHA) was carried out with tanned erythrocytes as described previously (NÉMETH, 1965). The haemagglutination system was checked before each test with three standard sera with titres of 1 : 256, 1 : 1024, 1 : 4096 and only used if these reacted with the antigen-coated red blood cells according to their titre and the requisite controls were clear. From each test sample serial twofold dilutions were made, beginning at 1 : 2, 1 : 3 and 1 : 5 initial dilutions and the highest dilution still producing complete haemagglutination was regarded as the reaction titre.

Agar gel diffusion precipitation test was performed by the radial double diffusion procedure, as described previously (NÉMETH, 1971).

Immunoelectrophoretic analyses were performed in an 1.5% Difco Noble Agar gel* buffered with veronal sodium-sodium acetate-HCl (pH 8.6, ionic strength 0.05) (NÉMETH, 1972 a).

Ultrafiltration was carried out by negative pressure dialysis at about 50—100 mm Hg, using 8 DC dialysis tubing** (CRAIG, 1968).

Defatting of serum used for antibody isolation was made with chloroform, according to the procedure of WOERNLE (1961).

Determination of antibody content

Determination of the protein content of immunoprecipitates was performed by the quantitative precipitation method of DÉVÉNYI and GERGELY (1963), using Folin-Ciocalteu phenol reagent. In every case, three replica measurements were made on 2 ml samples.

Quantitative determination of IgG and IgM globulins

The amounts of IgG and IgM eluted from immunoadsorbent-antibody complexes were determined by means of monospecific anti-rabbit IgG and anti-rabbit IgM sheep immune serum, according to the procedure of FAHEY and McKELVEY (1965).

Protein determinations were carried out by the method of CHASE and WILLIAMS (1968), using Folin-Ciocalteu phenol reagent.***

Results

After absorption with the insolubilized antigen, no antibodies were demonstrable in the sera either by IHA or by agar gel diffusion precipitation tests. No antibodies were found either in the washing fluid of the immunoabsorbent-antibody complex.

Results of the elution of immunoabsorbent-associated proteins with the two solvent systems are shown in Table I. The total yield of the original serum antibody protein content recovered by elution was 83—96% and 54—64% in glycine-HCl buffer (0.1 M, pH 2.8) and in $MgCl_2$ solutions, respectively.

Analysis of the concentrated eluates (1—4 mg protein/ml) by gel diffusion, using monospecific anti-rabbit IgG and anti-rabbit IgM and polyspecific anti-rabbit immune sera, established that the protein composition of the eluted material differed with the solvent, although fresh immunoabsorbent from one

* Special Agar-Noble, Difco Laboratories, Detroit, Michigan, U.S.A.
** Union Carbide Corp., Chicago, Illinois, U.S.A.
*** Fischer Scientific Co., Fair Lawn, New Yersey, U.S.A.

and the same batch was used throughout. With all serum pools tested, the material eluted with $MgCl_2$ contained exclusively IgG, whereas when the glycine-HCl buffer was used for elution the eluates were found to contain IgM as well in case of serum withdrawn 40 days after reinfection (pools III and IV). The serum samples of rabbits infected on a single occasion (pools I and II) contained IgG, exclusively, never any IgM, irrespective of the eluting solvent. Polyspecific anti-rabbit goat immune serum failed to detect serum proteins other than IgG or IgM in all antibody preparations obtained.

Table I

Yield of proteins eluted after absorption of rabbit serum on the immunoabsorbent

Serum absorbed				Material eluted in			
				glycine-HCl (0.1 M, pH 2.8)		$MgCl_2$ (2.5 M and 5.0 M)	
Serum pool	Antibody protein μg/ml	HA titre	Volume (ml)	Total quantity of eluted protein (mg)	HA titre**	Total quantity of eluted protein (mg)	Ha titre**
I.	480	1 : 640	20	8.0 (83.3%)*	1 : 512	6.2 (64.6%)*	1 : 384
II.	250	1 : 160	20	4.4 (88.0%)	1 : 128	3.2 (64.0%)	1 : 96
III.	820	1 : 1536	20	15.8 (96.3%)	1 : 1280	9.0 (54.9%)	1 : 768
IV.	900	1 : 4096	20	17.2 (90.6%)	1 : 3072	11.0 (61.1%)	1 : 2048

* Figures in brackets indicate the antibody yield calculated from the ratio of the amount of eluted protein to the antibody-protein content of the whole serum.

** Highest dilution of eluted material still giving full haemagglutination when the eluate was concentrated to 20 ml.

Consistent result were obtained by immunoelectrophoretic analysis of the eluates against polyspecific anti-rabbit goat immune serum, anti-rabbit IgG + IgM sheep immune serum and monospecific anti-rabbit IgG and anti-rabbit IgM sheep immune sera. The results of one such analysis are presented in Fig. 1. The precipitation line representing IgG appeared with all antibody preparations, whereas the line corresponding to IgM was only present in materials originating from serum pools III and IV and recovered from the absorbent by elution with glycine-HCl buffer. No other classes of serum proteins could be detected with the polyspecific anti-rabbit goat immune serum, although this antiserum reacted with at least 18—22 serum components of different antigenicity upon immunoelectrophoretic analyses of normal rabbit serum.

The total quantities of immunoglobulins recovered from the different serum pools are shown in Table II. The total amount of IgG + IgM globulins,

as determined by simple radial immunodiffusion, was almost identical with the total amount of antibody protein determined by the Folin-Ciocalteu phenol reagent. The quantity of IgM amounted to 8.1—9.4% of the total immunoglobulin content of the eluates.

The proteins eluted from the immunoabsorbent were assayed for antibody activity by the gel diffusion precipitation and IHA tests. All four original serum pools reacted in the former test, a distinct precipitation arch being formed with all four close by the serum-containing well, while with three pools there also appeared additional, weaker arches, located further away from the serum well, toward the antigen well: one arch with pool I and two arches each with pools III and IV. The antibody preparations likewise precipitated the antigen and produced identity reactions with the precipitation arches of the

Table II

Total quantities of IgG and IgM immunoglobulins eluted from the immunoabsorbent

Serum absorbed		IgG (mg)	IgM (mg)	IgG (mg)	IgM (mg)
		eluted in			
Serum pool	Volume (ml)	glycine-HCl (0.1 M, pH 2.8)		$MgCl_2$ (2.5 and 5.0 M)	
I.	20	8.4	0	6.4	0
II.	20	4.8	0	3.6	0
III.	20	14.8	1.3	9.2	0
IV.	20	15.8	1.64	11.2	0

corresponding serum (Fig. 2). It should be mentioned that the antigen concentrations required for optimal precipitation reactions varied according to the serum pools and the antibody preparations, hence it was difficult to demonstrate all precipitation lines on a single plate.

The results of IHA tests carried out with the same batch of antigen-sensitized erythrocytes on the same day are shown in Table I. The reaction titres of the eluates recovered in glycine-HCl buffer and $MgCl_2$ solution amounted to 75—83% and 50—60% of the original serum titre, respectively, if the eluate was concentrated to the original volume of the serum pool (20 ml). If, however, the concentration of the eluate was so adjusted that its protein content closely approximated to the antibody protein content of the original serum pool, the reaction titres were nearly identical. The minimal antibody protein content still capable of producing full haemagglutination varied between 0.27 and 1.72 μg/ml with the different preparations and was of similar order of magnitude to the minimal antibody protein content required for a full reaction with the original serum (0.17—1.56 μg/ml).

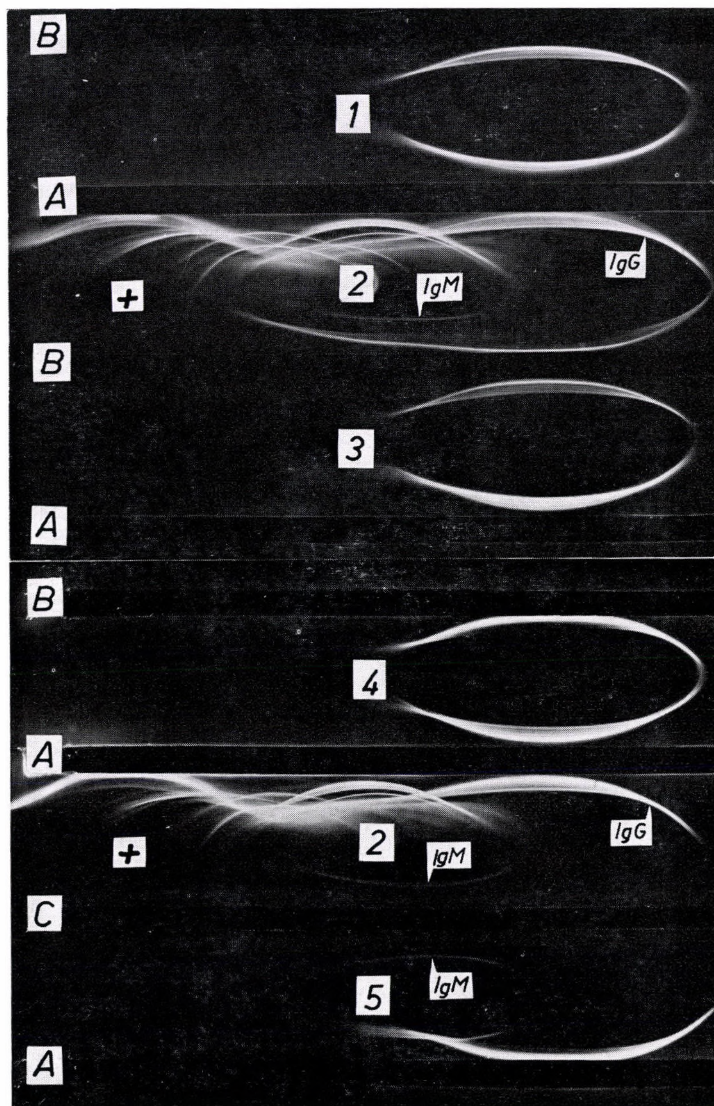

Fig. 1. Immunoelectrophoretic analysis of proteins eluted from the immunoabsorbent. 1, 3, Proteins from serum pools I and II (eluted with 0.1 M, pH 2.8 glycine-HCl buffer); 2, Normal rabbit serum; 4, 5, Proteins from serum pool IV(eluted with $MgCl_2$ solution and 0.1 M, pH 2.8 glycine–HCl buffer, respectively); A, Goat anti-whole rabbit serum; B, Sheep anti-rabbit IgG + IgM immune serum; C, Sheep anti-rabbit IgM immune serum

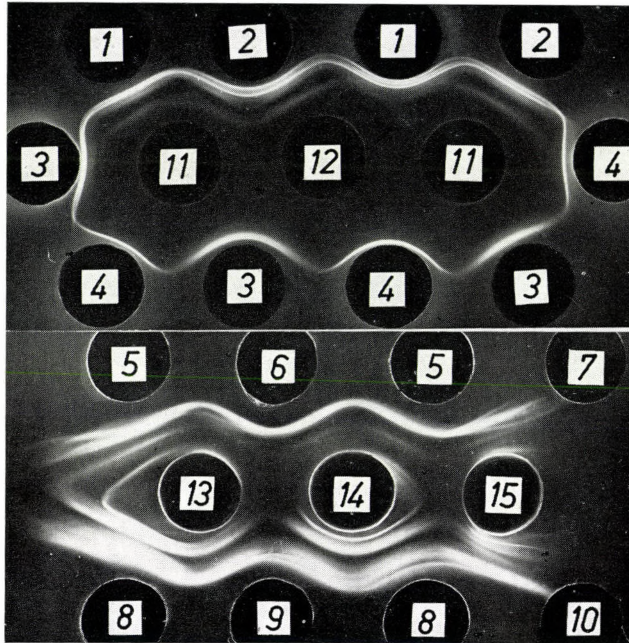

Fig. 2. Gel diffusion analysis of precipitating antibodies in whole serum of infected rabbits and in material eluted from immunoabsorbent. Content of wells: 1, Serum pool I; 2, Material isolated from serum pool I (two different preparations); 3, Serum pool II; 4, Material isolated from serum pool II (three different preparations); 5, Material isolated from serum pool III; 6, Serum pool III; 7, Normal rabbit serum; 8, Material isolated from serum pool IV; 9, Serum pool IV; 10, PBS (0.01 M, pH 7.1); 11, 12, 13, 14 and 15, Antigen solutions containing 4, 8, 12 and 3 mg/ml protein, respectively

Discussion

Avrameas and Ternynck (1969) observed that addition of glutaraldehyde to protein solutions results in the formation of insoluble protein derivatives through the establishment of covalent cross-linkages between the free amino groups of certain proteins. Further examinations with different protein-antigen — antiprotein-antibody systems demonstrated that glutaraldehyde-insolubilized protein-antigens can be utilized as specific and stable immunoabsorbents for the isolation from serum of humoral antibodies in an immunochemically pure state. The first to isolate antibodies to parasites by this method was Pérez-Esandi (1970), who, using polymerized sheep hydatid fluid as immunoabsorbent, was able to isolate pure anti-echinococcus antibodies from the serum of human patients suffering from, or artificially immunized against, hydatidosis.

Earlier studies on the humoral antibody response of rabbits to infection with oncosphaeres of *Taenia pisiformis* have established that in the course

of cysticercosis, the parasitic antigens induce the formation of antibodies of chiefly the IgG class and to a lesser extent the IgM class (Németh, 1972 a, b). This observation is substantiated by the present isolation of both classes of antibodies from the serum of experimentally infected rabbits by means of a glutaraldehyde-immunoabsorbent. Most antibody preparations consisted in fact, of IgG alone; IgM-type antibodies were present only in the serum of twice-infected animals, at concentrations as low as 9—10% of the IgG antibody content. The failure to isolate IgM antibodies from the serum of once-infected rabbits is explicable either by their genuine absence, or by their presence in such low concentrations as to be undetectable by the applied method.

All antibody preparations contained immunochemically pure antibodies, because no serum proteins other than IgG or IgM could be detected with the applied polyspecific anti-rabbit goat immune serum, either by immunoelectrophoresis, or by gel diffusion analysis.

The quantity of pure antibody protein recovered from the immunoabsorbent varied from 54—96% of the antibody protein content in the original serum, which corresponds with the yield obtainable from other antigen-antibody systems (Avrameas and Ternynck, 1969; Pérez-Esandi, 1970). The yield was much higher if the bound antibodies were eluted from the immunoabsorbent with glycine-HCl buffer (0.1 M, pH 2.8) than with 2.5 and 5.0 M $MgCl_2$ solutions. Apart from the difference in the mechanisms of action of the two solvents, two further circumstances may be involved here. On the one hand, the insolubilized antigen sedimented very slowly in the concentrated $MgCl_2$ solution, so that part of the immunoabsorbent together with a corresponding amount of still uneluted antibody may have been lost at each centrifugation. On the other hand, removal of $MgCl_2$ by dialysis from the solution containing the eluted proteins caused a marked increase of the dialysand volume and subsequent concentration resulted in the formation of a greater or lesser amount of insoluble precipitate. As it is known that of the globulins above all the macroglobulins have a propensity to aggregate, the formation of such a precipitate may be held responsible for the failure to recover IgM antibodies from eluates obtained with $MgCl_2$ solution, despite the fact that the IgM antibodies were evidently absorbed by the insolubilized antigen, to judge from their successful recovery with glycine–HCl eluant.

That the antibody proteins isolated by means of the insolubilized antigen retain their specific antigen combining activity is confirmed by the results of the IHA and agar gel diffusion precipitation tests. The HA titres of the antibody preparations were usually lower than those of the original serum, but this decrease was clearly due to a loss of antibody protein associated with the procedure rather than to a decline of antigen combining capacity. If the protein concentration of the antibody preparation closely approximated the antibody protein content of the original serum the respective HA titres were

almost equal. Also, the lowest quantity of antibody protein still capable of producing a full haemagglutination reaction in the IHA test was within the same order of magnitude for whole serum and antibody preparation.

The acid-soluble fraction used for antibody isolation is a complex antigen, consisting of at least three different components, according to the gel diffusion analyses. The fact that the precipitation arches formed by the various antibody preparations in these tests corresponded in every respect with those formed under similar conditions by the original whole serum indicates that the isolated pure antibodies preserved the reactivity of the original serum to all antigenic components.

Several authors have reported that protective antibodies are formed in rabbits infected by *Taenia pisiformis* oncosphaeres (Kerr, 1935; Sima, 1937; Campbell, 1938; Leonard, 1940; Leonard and Leonard, 1941; Bachmann, 1964). Such antibodies are passively transferable with rabbit serum, and if the latter is applied in an appropriate dose simultaneously with infection, it may confer a firm passive protection against invasion of the parasite. Previous findings in this laboratory are in support of this observation (Németh, 1970). The antigen(s) responsible for the induction of protective antibodies have not as yet been identified and explanations of their nature are still hypothetical rather than based on specific evidence. Since, however, the procedure employed in the present study offers a relatively simple technique for the isolation of antibodies in an immunochemically pure state and in reasonable quantity, it might well be utilized for the study of protective antibodies, independently of other serum factors, in passive immunization experiments. The information emerging from such investigations will probably facilitate the identification of the worm antigen invoking the production of protective antibodies.

Acknowledgement. The author is indebted to Miss I. Halasi for her excellent technical assistance.

SUMMARY

Antibodies detectable by indirect haemagglutination and gel diffusion precipitation test were isolated from the serum of rabbits infected experimentally with *Taenia pisiformis* oncosphaeres, by elution from a specific immunoabsorbent prepared by polymerization with glutaraldehyde of the acid-soluble fraction of crude cysticercus extracts.

Most isolated antibodies belonged to the IgG class, but antibody preparations from the serum of rabbits infected on two occasions contained IgM antibodies as well. No other serum components were detected in any of the antibody preparations by immunoelectrophoresis and immunodiffusion in agar gel using a polyvalent anti-rabbit immune serum.

The antibody yield of the different preparations varied between 54 and 96% of the antibody protein content of the original serum sample. The isolated antibodies retained their full antigen combining activity in agar gel diffusion precipitation and indirect haemagglutination tests.

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FOETAL DISEASE CAUSED BY SWINE ENTEROVIRUSES

(OCCURRENCES OF "SMEDI" DISEASE IN HUNGARY)

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The reproduction disorders of sows cause great problems in large-scale swine farming. Part of these disorders are unrelated to infection and can simply be ascribed to conditions of management deviating so much from the natural living conditions of the domestic swine that they would be bound to result, sooner or later, in disturbances of reproduction biology. Feeding not satisfying fully the nutritional requirements of high production breeder sow probably also affects reproduction.

The commonest infectious foetopathogenic diseases of swine are brucellosis and leptospirosis, but streptococci, staphylococci, corynebacteria, *Erysipelothrix rhusiopathiae* and fungi have also been shown to damage the swine embryo (Kemenes and Széky, 1970; Szabó, 1969). Of the viral swine disease, Aujeszky's disease (Csontos et al., 1961), swine fever and foot-and-mouth disease always affect the foetus.

Recent studies of outbreaks of a foetopathogenic swine disease not previously observed in Hungary have attracted attention to a further virus infection, which was first described by Dunne et al. (1965). The disease was observed in several herds and the viruses isolated from stillborn foetuses were identified as enteroviruses later found to be serologically identical with certain earlier isolates from swine associated with such a condition (Dunne et al., 1967; Morimoto et al., 1968; Wang and Dunne, 1969). The foetopathogenic effect of the isolates has been affirmed experimentally (Dunne et al., 1965, 1969).

Experimental

The condition was first observed in the swine herds of two State Farms situated in the Transdanubian region of Hungary; the outbreaks occurred in January and May of 1971, respectively. Symptoms were the same in both herds: the number of liveborn piglets was markedly reduced, stillborn and mummified foetuses in several litters and conception failures became more frequent than usual.

Herd A

The first herd was a breeder herd consisting of 150 sows, 15% of which were Large White animals imported from the United Kingdom, 20% had been

procured from eastern Hungary at growing age, whole the rest originated from the farm's own stock of predominantly white bacon pigs.

The animals were managed in the traditional way and the herd was free from both leptospirosis and brucellosis. The sows were kept in a single building divided into four compartments, each of which housed a group of females served within the same one-month period. For farrowing, the sows were transferred to separate premises about 150 m away. The ration was a sow feed formulated according to standard prescriptions, and once or twice daily the animals were out to the common pasture of the farm to graze.

The disease was first noticed at the end of December 1970. The number of liverborn piglets was markedly reduced and many of these were non viable

Table I

Data of abnormal farrowings in herd A

Ear-mark of sows	Date of farrowing	Viable	Number of piglets stillborn	Unviable
8	23. 12. 1970	3	—	—
45	6. 1. 1971	3	—	—
50	20. 1. 1971	—	10	—
51	8. 2. 1971	—	10	—
81	6. 2. 1971	—	12	—
87	6. 2. 1971	—	6	—
94	4. 2. 1971	3	—	2
96	18.1. 1971	—	—	6
105	1. 1. 1971	—	—	6
146	7. 1. 1971			

dying within a few hours after birth, while stillborn and mummified foetuses occurred in several litters.

Four stillborn piglets and foetal membranes submitted to the Central Veterinary Institute for bacteriological and pathohistological examination on 7th January, 1971 proved to be free from brucella and leptospira organisms. This findings was confirmed by serological examination of the sows.

Precise records were kept only of the numbers of liveborn piglets, whether viable or not (Table I). Mummified foetuses, 1—5 in number, were observed in the litters of 10—15 sows but, unfortunately, no precise records were made.

One of the pregnant sows was transferred to this Department for observation during farrowing. This lasted 5 hours and eight live piglets were born of which one animal, weighing about 600 g, died after 2 hours; in addition

three mummified foetuses, 8—15 cm long, were expelled. The virus strain "B5" was isolated from the placenta of the sow.

Before and after the outbreak litter numbers were the normal for the breed (Fig. 1), but in the period between December 1970 and March 1971 a substantial part of the sows farrowed less than five piglets (Fig. 2).

Two gilts (Nos 137 and 124), which had been served for the first time on 16th and 10th October, 1970, respectively did not conceive for a long time;

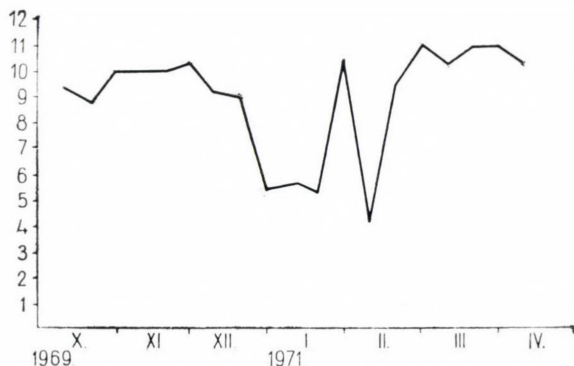

Fig. 1. Average number of litters of sows farrowing between Oct. 1969—April. 1970. In average 15 sows farrowed in a month

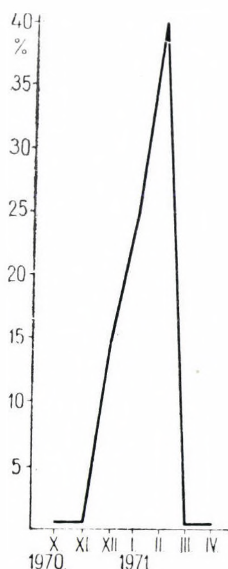

Fig. 2. Proportion of sows farrowing less than 5 living piglets. In average 15 sows farrowed in a month

Animal No. 124 was emergency slaughtered in January, while gilt No. 137 still showed no heat in May. Sows Nos 107, 75 and 116 returned to oestrus five, four and three times, respectively before conceiving.

Herd B

The average number of sows in the second herd was 1200. The animals were kept under a closed, intensive system in four buildings each capable of accomodating 400 animals. Each house was divided into stalls for groups of 12 sows and was equipped with a central manure disposal system. The sows were transferred for farrowing to separate premises. The herd was free from brucellosis but was infected with leptospirosis from the beginning.

The sows were Large Swedish × Large White crosses. The sow houses were stocked by the end of 1969, and except for four pregnant Belgian Lowland sows and a boar procured from Belgium in June 1970 together with five other boars bought in from various Hungarian herds, no further animals were introduced into the herd all replacements being made from the sows' own progeny. Initially, growing gilts were returned from the fattening piggery for this purpose, but later breeder gilts reared in a separate unit on the farm, the sow house were used. The sows were fertilized by artificial insemination from the beginning. The sow feed was prepared at the farm and rationed according to the stage of pregnancy. Vaccinations against leptospirosis were performed from the beginning up to 15. 12. 1970 and after a six-month interruption, resumed from 3. 6. 1971 vaccinations against Aujeszky's disease were administered from 15. 4. 1971. Outbreaks of severe diarrhoea occurred in both the fattener and sow herds in December 1970 and January 1971; the cause was not be identified, but transmissible gastroenteritis (TGE) could be excluded.

Sporadic occurrence of reproduction abnormalities was observed from August 1970 and continued until January 1971; losses became economically important between January and May, and thereafter fluctuated before ceasing altogether in September.

Initially the birth of dead foetuses was the most conspicuous sign. Many sow farrowed fewer piglets than previously and several produced mummified foetuses, while repeated failures of conception caused a great problem. During the peak of the outbreak, however, about 30% of the sows produced 1—10 dead or mummified foetuses. The weight of many liveborn piglets was less than the average, and several of them died shortly after birth. Some 20% of the sows farrowing in this period gave birth to less than five live piglets. Return to heat still caused further problems.

Examinations on the spot were possible from May 1971. From then on incidence of the disease was periodic, affecting chiefly the newly established

breeder sow groups. In alternate periods all farrowings were normal, then scarcely any normal litter was encountered for some days. Some of the sows farrowed very few piglets, others farrowed both normally developed piglets and dead or mummified foetuses varying in size from that of a cherry to mature embryonic dimensions. In some litters no live piglet was found at all (Figs 3 and 4; Table II). Severely runted piglets (weighing less than 800 g at birth) occurred in several litters alongside normally developed littermates (greater than 1200 g body weight at birth). In the latter stages of the outbreak

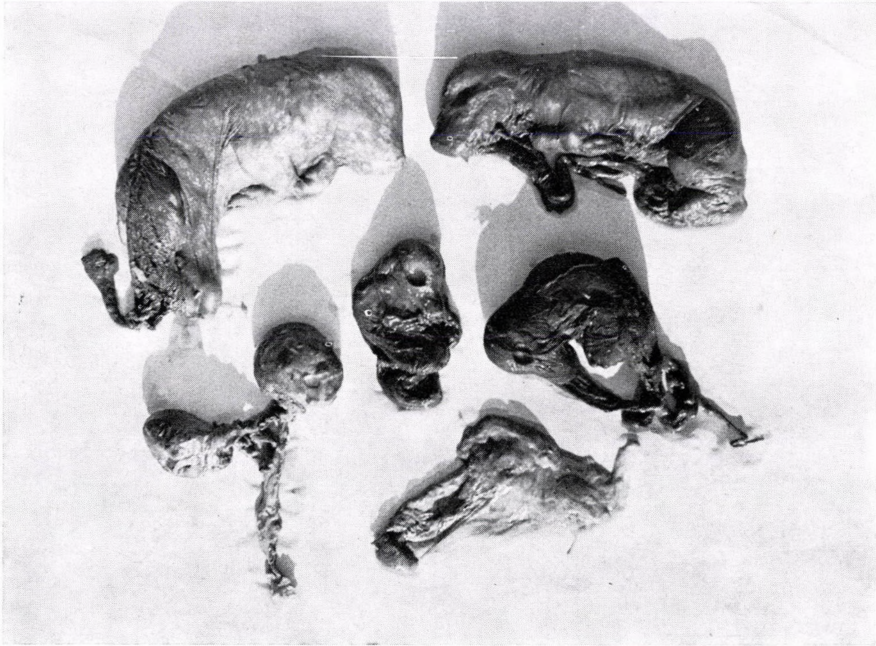

Fig. 3. Mummified litter from "B" herd. A nut-size foetus can be seen at the bottom of the picture

the majority of the sows farrowed normally, but conception failure caused problems to the end.

Positive isolations of virus were made from the inner organs of one stillborn animal (SzI) and from the kidneys of an unviable piglet (2 AV), which on birth weighed only 422 g.

The greater part of losses occurred between January and May of 1971. Indirect losses arose from cullings, increased neonatal mortality and retarded weight gain at weaning.

Losses were high throughout the outbreak. Livebirths numbered on average 7.2—7.8, stillbirths 1—1.7 per litter. The proportion of sows farrow-

Table II

Data of some abnormal farrowings in herd A

Earmark of sows	Viable piglets	Number of stillborn piglets	Mummified foetuses
2336	—	—	10
162	4	5	2
2065	3	1	12
2205	1	—	7
2521	—	—	9
1530	—	3	6
2289	2	1	5
1140	—	4	—
1826	—	4	1
2120	1	—	—
2150	1	—	—
1851	3	2	4
052	1	—	7
1018	—	3	5
1044	2	1	6

Fig. 4. Mummified litter from "B" herd

Table III

Service data of some sows with prolonged anoestrus (herd B)

Earmark of sow	First insemination	Second insemination	Number of days between oestruses
375	19. 4. 1971	6. 10. 1971	170
847	12. 12. 1970	20. 4. 1971	128
500	2. 3. 1971	2. 6. 1971	71
1101	22. 2. 1971	13. 7. 1971	141
1181	31. 3. 1971	4. 8. 1971	95
1238	9. 1. 1971	7. 4. 1971	88
1345	9. 1. 1971	2. 5. 1971	113
1481	18. 1. 1971	12. 4. 1971	84
1848	2. 1. 1971	2. 4. 1971	92
1912	20. 12. 1970	8. 3. 1971	78
1945	25. 1. 1971	9. 4. 1971	74
2019	27. 11. 1970	29. 3. 1971	122
2032	29. 12. 1970	8. 3. 1971	69
2069	19. 12. 1970	3. 3. 1971	74
2071	13. 1. 1971	16. 5. 1971	123
2158	18. 12. 1970	6. 3. 1971	78

ing less than five live piglets varied between 11—24% of the total number of sows farrowing within a given month (Fig. 5).

The number of sows displaying recurrent oestrus was also conspicuously high. A total of 108 sows failed to conceive within three or more oestruses.

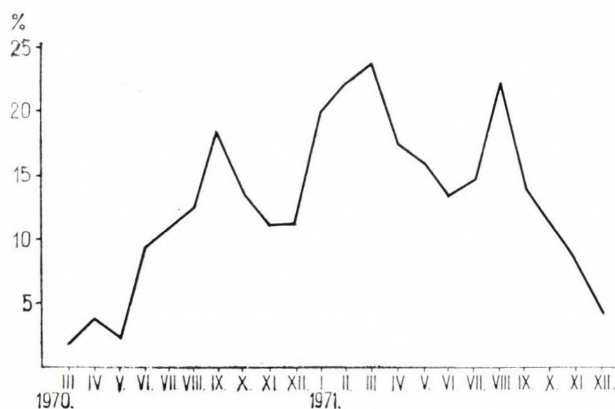

Fig. 5. Proportion of sows farrowing less than 5 living piglets. In average 205 sows farrowed in a month

Many animals did not come into oestrus at all for long periods and did not conceive either. Thirty-six sows displayed no oestrus for as long as 170 days; data relating to some of them are presented in Table III.

Virus isolation

Cell cultures were prepared from trypsinized kidney epithelial cell suspensions from healthy pigs 4—8 weeks old. Hanks' solution containing 15% calf serum and 0.5% lactalbumin hydrolysate was used as growth medium and bovine amniotic fluid as maintenance medium.

The materials examined were organ homogenates from stillborn foetuses and unviable piglets that died shortly after birth. The homogenates were diluted 1 : 10 in PBS solution and centrifuged for 30 minutes at 6000 r.p.m. The tissue cultures were each inoculated with 0.1 ml supernatant. Adsorption was allowed to take place for 60 minutes at 37 °C, then maintenance medium was added and the cultures were incubated at 37 °C. If no cytopathic change occurred in the first two passages, the material was submitted to two additional blind passages. The results were read either directly by microscope or, occasionally, after fixation and staining with haematoxylin and eosin.

Virus neutralization test were carried out by diluting serially the harvested virus suspensions in tenfold logarithmic steps down three successive series, the highest dilution still giving a positive reaction in the first series being used as the starting material for the second series, etc. 100 $TCID_{50}$ ml virus was added to serial twofold dilutions of each serum samples, the serum-virus mixture incubated for 60 minutes at 37 °C, then 0.2 ml was inoculated into each culture.

From herd A, four mummified foetuses and the foetal membranes from one sow were available for virus isolation. Cell cultures inoculated with the homogenate of the foetal membrane showed signs of virus growth from the second passage (strain B5).

More materials were available from Herd B. On the first occasion, mixed homogenates prepared brain, liver, kidney from two dead foetuses as well as the related foetal membranes and a homogenate prepared from mummified foetus were inoculated into tissue cultures. Three days later cytopathic changes indicative of virus growth appeared in the cultures inoculated with the homogenate from the dead embryos. On another occasion, organ homogenates from several stillborn and unviable piglets were examined. In this case, positive isolation was made from the kidney of a liveborn, but very weak animal (422 g in weight). All subsequent attempts at isolation failed.

All three isolates showed the same cytopathic effect; spherically rounded, refringent cells with sharp contours were seen on microscopic examination of the unstained cultures. In stained preparations, the cells showed the characteristic rounded shape and stained uniformly.

On the basis of cytomorphological and complementary examinations, including serological typing, the isolates were identified as type I enteroviruses (Szent-Iványi, 1963, 1969). The two strains isolated from herd B were serologically identical, the single isolate from herd A belonged to another serotype.

All serum samples taken from the sows of the two herds contained neutralizing antibodies to the homologous virus at titres of 1 : 64 or higher.

Discussion

It is known from virus isolation experiments and serological tests that all pigs become naturally infected with enteroviruses at some time in life. Of the many known types of enteroviruses only a few have been established as pathogenic, viz. the agents of Teschen-Talfan disease, meningoencephalitis (type I/2) and certain mild diarrhoeal conditions (Szent-Iványi, 1963; Szent-Iványi and Széky, 1967a, b). The condition reported here is a new enterovirus disease, the agents of which have been named SMEDI viruses.

In both herds affected by the disease the virus was pathogenic for the foetuses only. The time of introduction of the virus into the herds would be difficult to determine, as it may have been present earlier without causing noticeable changes. Experiences from the two outbreaks suggest, however that early pregnancy is the period of maximal foetal susceptibility. The same information emerged from infection experiments by Dunne et al. (1969).

It appears that if the embryo becomes infected before the 30th day of foetal life and dies, it may be resorbed and the sow may return to oestrus. In many cases, however, only some of the foetuses are killed, while the rest develop normally until term; unusually low litter numbers viz. less than five probably have such an explanation history. In several cases "buttons" were found on the placenta which presumably were the remains of resorbed foetuses.

If on the other hand the virus affects the embryo after 30 days of foetal life, the outcome may still be fatal but instead of being resorbed the dead embryo becomes ossified. In such circumstances the sow farrows mummified or dead foetuses, depending on the time of infection. In the majority of cases, the dead foetuses are of different ages, ranging from scarcely visible mummified structures to stillborn piglets. Foetuses that died at roughly the same age were seldom found, and those observed clearly originated either from the early stage of pregnancy or from the period shortly before parturition. The virus did not normally affect all embryos: usually strong, viable piglets were farrowed along with the dead foetuses and there were often also liveborn, but unviable animals of low birth weight, most of which died shortly after farrowing. No virus could be detected in mummified foetuses, but positive isolations

were made from those died shortly before birth and from unviable piglets. The isolation and serological typing of the agent as well as the clinical picture of the condition clearly indicate that it is the SMEDI disease described by DUNNE et al. in 1965.

Foetuses originating from abnormal farrowings in herd B were regularly sent to this Department for bacteriological investigation over the period 1969—71. Results were negative until April 1971 when leptospires were found in a foetal kidney. The some organisms were found again in another foetus submitted for examination on 23rd August 1971. Serological examinations of sows conducted in July and August of that year revealed high antibody titers to *Leptospira pomona* and *L. hyos* in several animals. Nevertheless leptospirosis could not satisfactorily account for the foetal damages we observed. Abortion which a typical accompaniment of leptospiral infection was relatively rare in both herds, and in any case the greater part of the foetuses died before the time required by leptospires to develop a pathogenic effect. During the summer of 1971, towards the end of the outbreak, however, it seems clear from the laboratory findings that leptospires were contributing, but least in part, to foetal losses.

It follows from our observations that viral embryonic disease may occur in swine herds free from both brucellosis and leptospirosis. As this disease differs both aetiologically and clinically from all previously known foetopathogenic conditions of swine, it seems important to distinguish it as such terminologically too. Until a more apt name is found, though the designation "SMEDI disease" can be accepted as characterizing not only the aetiology, but also the nature of the disease.

This report presents only the basic information on the outbreaks and their background. The results of epizootological studies, virological examinations and infection experiments now in progress will be published later.

Acknowledgements. We would like thank Mrs. Á. KOJNOK and Mr. L. BELOPOTOCZKY for technical assistance.

SUMMARY

Outbreaks of a disease affecting the reproduction of sows and producing foetal death occurred over a 2-year period in the breeder hers of two farms managed under traditional and intensive closed systems. The first herd was free from both brucellosis and leptospirosis, the second herd was infected with leptospires, but the symptoms of the disease indicated a condition other than leptospirosis. In both herds, recurrent oestrus and conception failures became frequent among the sows. Many of these that farrowed produced mummified and/or stillborn foetuses along with liveborn piglets and a high proportion of the latter proved unviable, resulting in a marked reduction of litter numbers. Foetal susceptibility to the agent appeared to be greatest during the first 30 days of intrauterine life, though foetal damage culd occur at any stage of pregnancy, its nature depending the time of infection. Positive isolations of a virus, later identified as a swine enterovirus, were made from embryos which died shortly before birth, from foetal membrans, and from the organs of unviable newborn

piglets. On the basis of the clinical picture and virological examinations, the condition was regarded as identical with the "Stillbirth, Mummification, Embryonic Death and Infertility" (SMEDI) disease entirely first described by DUNNE and his colleagues in the United States in 1965.

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ELECTRON MICROSCOPIC STUDY OF THE SARCOLEMMA STRUCTURE IN BEEF IN RIGOR MORTIS

By

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Earlier light microscopic studies of rigor muscle of cattle have revealed that the muscle fibres are surrounded by fibrous coils running in grooves on their surface. In native preparations these coils are thicker than the myofibrils and appear highly refractive, and they have been shown both by chemical and digestion techniques, as well as in histological studies to consist of collagen fibres.

It is now known that whereas in relaxed muscle these fibres are evenly distributed on the surface of the muscle fibres, connected at certain sites by fine cross-linkages, in rigor they become arranged into stout bundles that are directed into the grooves on the muscle fibres (LŐRINCZ and BIRÓ, 1961, 1963; BIRÓ, 1963). The amount of this connective tissue surrounding the muscle fibres is thought to have a substantial influence on the quality of beef, the gathering of the fibrous material into bundles being responsible for a noticeable increase in toughness (BIRÓ, 1969).

As the sarcolemma lies below the resolution of the light microscope, it is reasonable to suppose that the formation of the fibrous bundles around rigor muscle fibres must involve a component from the endomysium. In fact, BENNETT and PORTER (1953), HODGE, HUXLEY and SPIRO (1954) and RUSKA (1954) have all reported observing two darker lines at the margin of muscle fibres, and these two together they regarded as making up the sarcolemma. In frog muscle MAURO and ADAMS (1961) succeeded in demonstrating three different sarcolemmal layers. At the boundary of the sarcoplasm there was a thin plasma membrane and outside this a layer of similar thickness which they called the "amorphous" layer — earlier known as the "cuticular layer" (PORTER, 1954), "basement membrane" (ROBERTSON, 1956) or the "basic substance" (FAWCETT, 1958). The outermost layer of the sarcolemma was fibrous and following REED and RUDALL (1948), DRAPER and HODGE (1949) and ROBERTSON (1956), MAURO and ADAMS described it as consisting of two kinds of fibres. The thicker fibres they considered to be collagen, as DRAPER and HODGE (1949) and RÓZSA, SZENT-GYÖRGYI and WYCKOFF (1950) had concluded in the basis of their submicroscopic cross-striation.

In addition to this morphological evidence of the collagenous nature of the fibrous sarcolemmal substance, there is also some biochemical evidence from the investigations of KONO and KOLOWICK (1961), who carried out amino-acid analysis of the material and established that it is digested by collagenase, although less readily than pure collagen.

The double outline of the sarcolemma — plasma membrane and basement membrane — was demonstrated electron microscopically by LŐRINCZ and LOSONCZY (1966). These two workers also observed the presence of dark granules between the membranes. They failed, however, to establish any connection between the sarcolemma and the endomysial connective tissue.

Material and methods

The biceps brachii was excised from cattle 24 hours after slaughter. As seen under the light microscope, the muscle was in a typical state of rigor mortis, closely spaced grooves, within which spiral contractile formations ran, being visible around the muscle fibres.

For electron microscopic examination samples were fixed in 2.5% glutaraldehyde for three hours, post-fixed in 1% osmium tetroxide, then embedded in Durcupan-Fluka ACM araldite. Sectioning was carried out with a LKB "Ultrotome III" ultramicrotome, and sections were viewed with a WF—SEM 3 electron microscope. Micrographs were taken of the edges of muscle fibres, especially at sites where these could be seen under the light microscope to have retracted. Negatives were taken at a magnification of 6000×, the final magnification of the plates was 30,000×.

Results and discussion

The micrographs all show the grooves formed by the myofibrils at the edges of the muscle fibres. Pressing into the centre of these grooves is a body of material, larger than the myofibril diameter, in which it is possible to distinguish layers of different qualities. These together can be regarded forming the sarcolemmal duplex. The undulation of the invagination, and the accumulation of plentiful cellular debris and large number of mitochondria at the margin of the duplex have also been noticed.

On the side of the invagination facing the myofibrils — especially, on account of the undulation, in places on the double layer — there was a dark, coarsely granulated substance. This was observed only in some places on the sarcolemmal duplex, principally at the bottom of the undulations, and it was thought to consist of accumulations of dense cellular debris on the damaged plasma membrane.

Distinct from this dark granular material, there was also a thin, finely granulated substance running undulately down the length of the sarcolemmal duplex. This is the amorphous layer (or basement membrane) of the sarcolemma.

The sarcolemmal duplex could be seen to contain circular formations representing cross-sections of collagen fibres of the outer fibrous layer of the sarcolemma. These appear in Fig. 1, interspersed in a fine, loosely arranged fibrous debris near the base of the endomysial connective tissue.

A similar picture is observed in Fig. 2 except that here the entire sarcolemmal duplex appears thinner than the invagination in Fig. 1 but it is widened out at the base of the invagination by fibres originating from the endomysial connective tissue.

The presence of part of a cell nucleus, near the bottom of the invagination, in Fig. 3 makes it somewhat easier to appreciate the structure of the sarcolemma, as the nucleus is directly overlaid by the sarcolemma, and the layers of the latter are particularly well differentiated. Again, there is a coarse cell debris deposited on the plasma membrane, but in this case it is unformarly

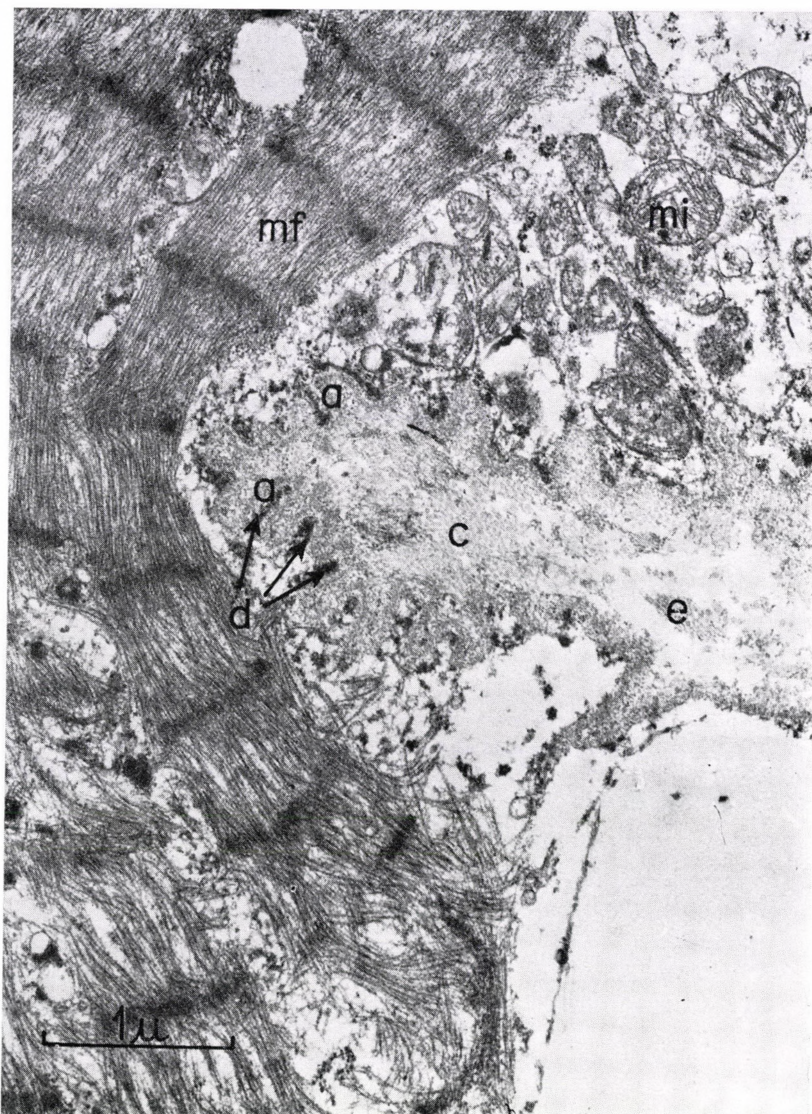

Fig. 1. Electron micrograph of bovine rigor muscle (30,000×). The groove in the myofibril (mf) at the periphery of the fibre is occupied by the sarcolemmal duplex, at the margin of which lies a dense cell debris (d), the amorphous layer (a), and collagen fibres (c) of the outer, fibrous layer of the sarcolemma. Within the sarcolemmal duplex can be seen the fibrous substance of the endomysium (e). Mitochondria are scattered in the sarcoplasm

distributed along the margin of the nucleus. Both the amorphous layer and the circular cross-section of collagen fibres are likewise easily picked out. Penetrating deep into the sarcolemmal duplex, and in loose contact with the collagenous fibrous layer of the sarcolemma, is a connective tissue substance

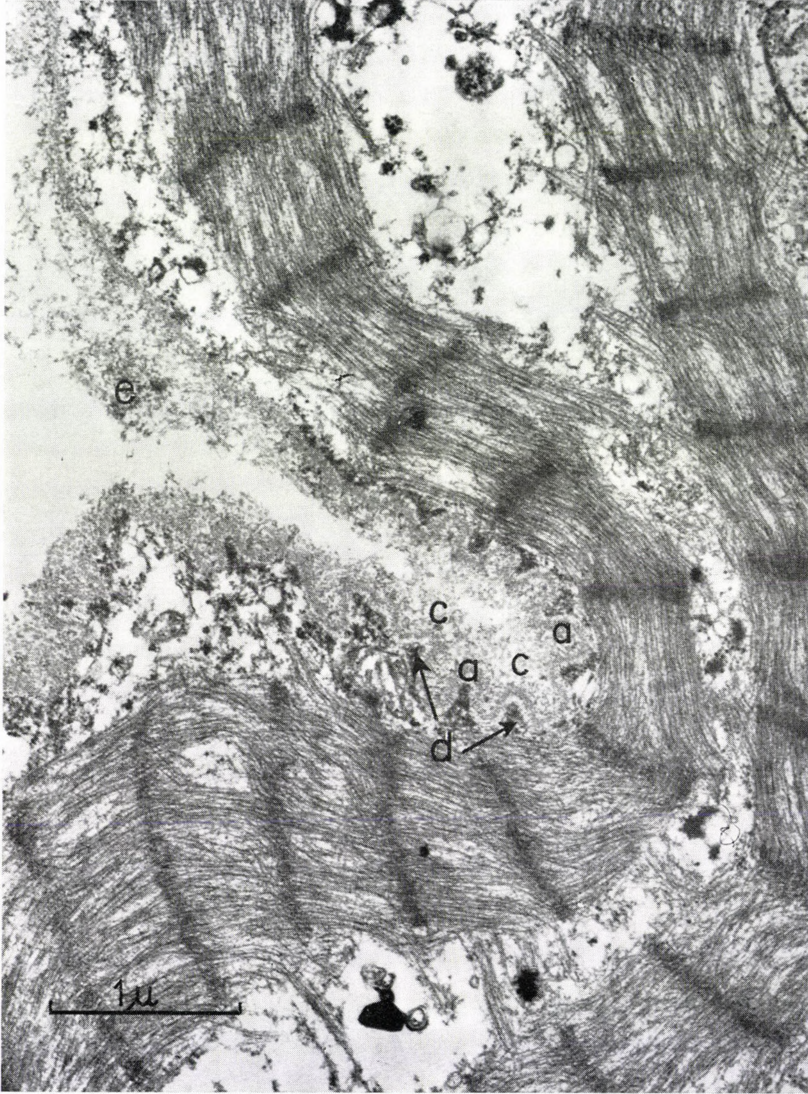

Fig. 2. Within the sarcolemmal invagination, loosely attached to the fibrous layer of the sarcolemma, there is a body of fibrous material originating from the endomysium (e)

the structure of which suggests that it is no longer the part of the sarcolemma but rather belongs to the endomysium.

It can be concluded from the electron micrographs, that the bundles of connective tissue, seen in the grooves of the muscle fibres, are composed of fibrous material derived from both the endomysium and the sarcolemma. In rigor mortis the collagen fibres of both of them become arranged into thick

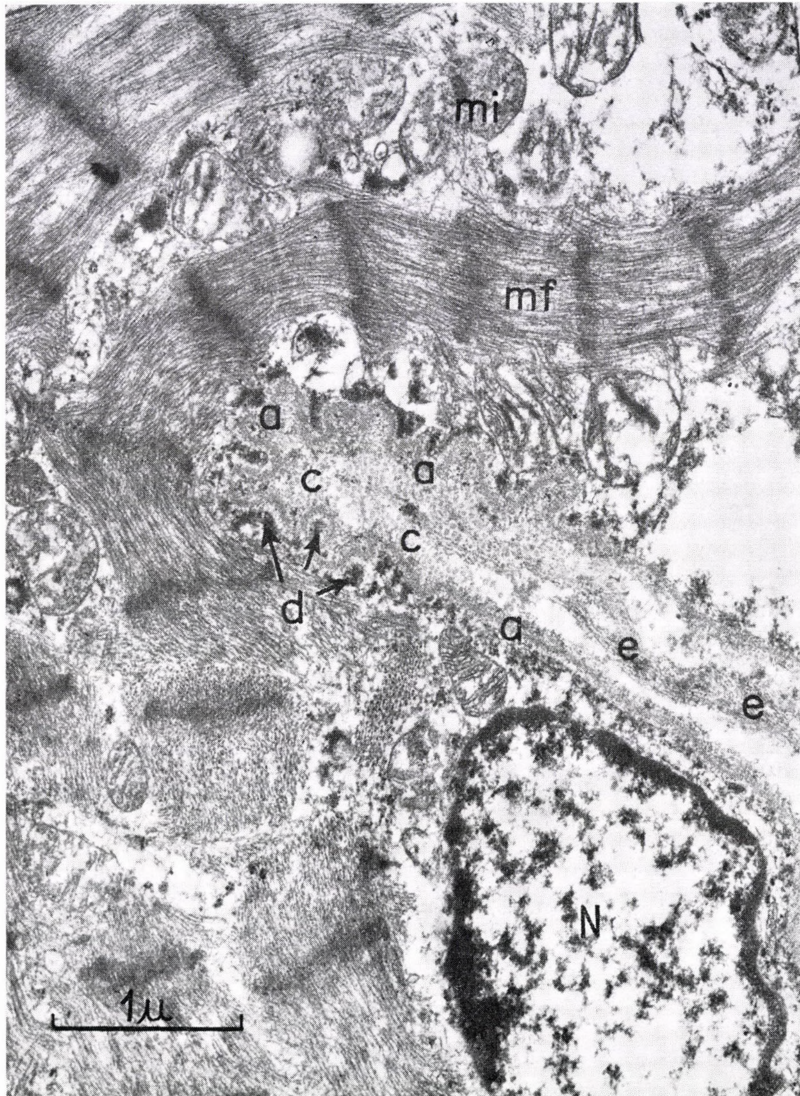

Fig. 3. A section of a cell nucleus (N) is visible next to the sarcolemma. Alongside the outer, fibrous layer of the sarcolemma, which envelopes the nucleus, is a fibrous material derived from the endomysium (e)

bundles, in which the fibres from the sarcolemma are visible in cross-section, apparently surrounding the muscle fibres like an annulus. This supports the light microscopic observations which indicate that in rigor mortis the sarcolemmal and endomysial fibres slip together in the grooves arising on the muscle fibre surface.

It has also been demonstrated that both the sarcolemmal and endomysial connective tissue contribute to the uniform connective tissue layer seen around relaxed muscle fibres under the light microscope, and further evidence has been gained that the re-ordering of this regular network of fibres in rigor mortis plays a part in determining the toughness of beef.

SUMMARY

The annular fibrous bundles observed in earlier light microscopic studies to be lying in grooves around the muscle fibres of beef were examined more closely by electron microscopy. It was established that the double-layered sarcolemmal membrane invaginates into the grooves, and one of that part of the fibrous material composing the duplex is derived from the endomysium. Collagen fibres of the sarcolemma could be seen in cross-section, and their circular shape indicated that they surrounded the muscle fibres in an annulus. The observations proved support for the view that the reordering of the network of connective tissue in rigor mortis is related to the toughness of beef.

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ÜBER DAS KLEBEN VON BLUTGEFÄSSEN MIT DEM GEWEBEKLEBSTOFF HISTOACRYL

Von

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Die erfolgreiche Anwendung der Gewebeklebstoffe zur Wiedervereinigung verschiedener Gewebearten berechtigt zu den schönsten Hoffnungen, eine besondere Bedeutung kommt dem Verfahren aber auf dem Gebiet der Gefäßchirurgie zu. Die Nahttechnik ist nämlich eine langsame, mühsame Arbeit, welche die Operationsdauer wesentlich verlängert, außerdem traumatisieren die Nähte die Gewebe, geschweige denn, daß sich selbst die allerbesten Nähmaterialien mehr oder minder als Fremdkörper verhalten. Zur Eliminierung dieser Nachteile bietet das biologische Kleben eine wesentliche Hilfe. Die Wichtigkeit der Frage widerspiegelt sich auch in der großen Zahl der das biologische Kleben aufarbeitenden Mitteilungen.

Nachdem SELIGMAN und HURWITT die Klebefähigkeit der Cyanoakrylate erkannt haben, bewiesen die ersten Mitteilungen ihrer Forschungsgruppe (CARTON und Mitarb., 1960; NATHAN und Mitarb., 1960) bereits überzeugend, daß sich die lebenden Gewebe gut kleben lassen.

NATHAN und Mitarb. (1960) wandten das Klebeverfahren zuerst zur Versorgung der Wunde der abdominalen Aorta von Hunden an. In 30 der 39 Fälle (4 Verblutungen, 5 mit Aneurysmabildung) war das Ergebnis des Gefäßklebens klinisch befriedigend, obwohl in der Media und Adventitia der Gefäßwände, in der Umgebung des Klebstoffs Koagulationsnekrose und die Anhäufung von Riesenzellen von Fremdkörper-Typ in Erscheinung traten. CARTON und Mitarb. (1961) haben unter Anwendung von Methyl-2-Cyanoacrylat in 85 Fällen eine Fleckplastik vorgenommen; die Ergebnisse waren befriedigend. BRAUNWALD und AWE (1962) sowie AWE und Mitarb. (1963) wandten Eastman-910 (Methyl-2-cyanoacrylat) nach vorangehender erfolgreicher experimenteller Versorgung von Aortenwunden auch beim Menschen zur Stillung der aus der Aorta stammenden Blutung an.

Mehrere Verfasser, so z. B. GARRET und LAW (1961), GULMET und Mitarb. (1964), BRAUNWALD (1966), MATSUMOTO und Mitarb. (1967, 1968) empfahlen die Verwendung von Klebstoff zur äußeren Verdichtung der Gefäßnähte.

HAFNER und Mitarb. (1963) verwendeten bei der Versorgung longitudinaler Inzisionen und der Anlegung von End-zu-End-Anastomosen an der A. femoralis von Hunden ein PVC-Rohr, welches als innere Schiene diente. Bei der Anlegung von End-zu-End-Anastomosen gingen GOTTLOB und BLÜMEL (1965, 1966) folgendermaßen vor: Die äußeren Flächen der vorangehend durch ein Kunststoffring gezogenen Gefäßstümpfe wurden mit Klebstoff bestrichen, zum Ring geklebt und schließlich die zwei ineinander passende Ringe miteinander verschlossen.

Die anläßlich der Anwendung der einzelnen Klebstoffe ermittelten Erfahrungen der verschiedenen Verfasser unterscheiden sich voneinader. Die von INOU und Mitarb. (1965) durchgeführten Untersuchungen, in denen Eastman-910 (Methyl-2-Cyanoacrylat) und Aron Alpha Sanchyo verglichen wurden, ergaben, daß das letzterwähnte Präparat eine geringere Gewebereaktion hervorruft und auch Komplikationen seltener auftreten. WEISBERG und GOETZ (1964) empfahlen die klinische Anwendung von Eastman-910 wegen der beob-

achteten Komplikationen — Arteriennekrose, Thrombusbildung und Stenose — nicht. OTA und Mitarb. (1965) sowie MATSUMOTO und Mitarb. (1967, 1968) erzielten anläßlich der Anwendung von Aron-Alpha sowohl beim Verschluß der longitudinalen Inzisionen von Klein- und Großgefäßen, wie auch bei den End-zu-End-Blutgefäßvereinigungen ausgezeichnete Ergebnisse. Laut GOTTLOB und BLÜMEL (1967) ist das Verhalten von Histoacryl und Vulnocoll mehr gewebefreundlich als das von Eastman-910. Aufgrund ihrer Erfahrungen (1968) scheinen sich die Gewebeklebstoffe zu folgenden gefäßchirurgischen Eingriffen zu eignen: Einhüllen von Aneurysmen, Sicherung der habituellen Nähte, Verschluß von Arteriotomien, direkt oder mittels Fleckplastik, Anlegung von End-zu-End-Anastomosen, eventuell mit anderen Verfahren kombiniert (z. B. mit Nähten) und Versorgung parenchymatöser Organe.

Was die einheimischen Relationen der Frage anbelangt, haben sich mit der Untersuchung der verschiedenen Klebstoffe mehrere Forscher befaßt: BORNEMISZA (1968), BORNEMISZA und GYURKÓ (1969), GYURKÓ (1967, 1968) mit dem japanischen Präparat Aron-Alpha A Sankyo (Äthyl-2-cyanoacrylat), NEMES und SÓTONYI (1967), NEMES (1968, 1970) mit dem amerikanischen Präparat Eastman-910 (Methyl-2-cyanoacrylat), DUBECZ und Mitarb. (1971), LADÁNYI (1969), SOMOGYVÁRI (1971) sowie SZENTGÁLI und Mitarb. (1969, 1971) mit dem westdeutschen Präparat Histoacryl (Butyl-2-cyanoacrylat). RÁNKY (1968) war der erste, der in Ungarn zum Verschluß der Arteriotomie bzw. zur Sicherung der Naht unter klinischen Verhältnissen einen Klebstoff angewendet hat.

Eigene Untersuchungen und Ergebnisse

Unsere Gefäßklebeversuche fanden bei 32 Hunden von unterschiedlichem Alter und Größe statt. Zur Anwendung kam der Butyl-2-cyanoacrylat enthaltende Gewebklebstoff Histoacryl-N-Blau. Die Durchgängigkeit der Gefäße wurde bei den Versuchstieren während der 4monatigen Beobachtungsperiode, vom 1. postoperativen Tag an mit Angiographie regelmäßig kontrolliert, sodann die fraglichen Gefäßabschnitte in verschiedenen Zeitpunkten exzidiert und histologisch aufgearbeitet.

Um eine entsprechende Gewandtheit zu gewinnen, wurden in der ersten Versuchsserie laterale Gefäßverletzungen geklebt, während in der zweiten, die End-zu-End-Vereinigung der quer durchgetrennten Gefäße mit Kleben stattfand.

Die durchgeführten Eingriffe waren, wie folgt:

I. 7 Hunde. Eine etwa 15 mm lange seitliche Inzision der einseitigen A. femoralis wurde mit einem Venenfleck verschlossen. Das Klebeverfahren ist rasch und auch technisch einfach. Sämtliche Eingriffe erwiesen sich als erfolgreich.

II/1. 6 Hunde. In dieser Gruppe wurde das Kleben mit Hilfe der aufgrund der GOTTLOBschen Methode von GERENDÁS hergestellten, sich resorbierenden Bioplastringe versucht. Das Ausranden auf die Ringe war mangels entsprechender Instrumente langwierig, und raumbeengend, die Technik kompliziert und schwierig. Als erfolgreich konnte keiner der Versuche bezeichnet werden.

II/2. 6 Hunde. Die quer durchgetrennten A. und V. femoralis wurden mit Hilfe von Richtungsnähten, mit dem Manschetten-Verfahren (mit einem 4—5 mm langen Venenstückchen) vereinigt. Unmittelbar nach der Operation

waren die Gefäße durchgängig, die Anastomose war aber starr, die Manschette wirkte stets einengend und nach einigen Tagen entwickelte sich eine Thrombose.

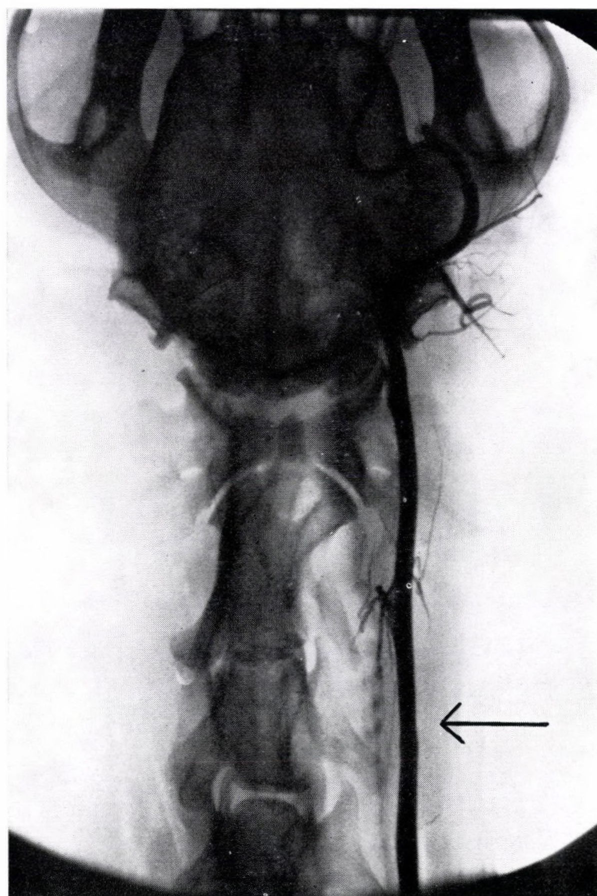

Abb. 1. Arteriographie der A. carotis 120 Tage nach der mit Kleben durchgeführten End-zu-End-Vereinigung

II/3. 13 Hunde. In insgesamt 51 Fällen wurden A. carotis, A. femoralis V. jugularis und V. femoralis quer durchgetrennt, sodann mit Hilfe von Richtungsnähten und Kleben wieder vereinigt. An 6 Gefäßen wurde das zirkuläre Kleben rund um eine durch eine separate Öffnung eingeführte Kunststoffkanüle verfertigt. Zur Vereinigung der spannungsfreien Karotiden genügten 2 Richtungsnähte, in die weiteren Vv. jugulares wurden 4 Nähte eingelegt, in die sich stets spannende A. femoralis 4—5, und in die V. femoralis 5—6. Die den betroffenen Gefäßen entsprechende Verteilung unserer 51 End-zu-End-Gefäßklebungen war, wie folgt: A. carotis 11 Fälle, V. jugularis 11 Fälle,

A. femoralis 15 Fälle und V. femoralis 14 Fälle. Die angiographischen Aufnahmen zeigen stenosefreie Heilungen (Abb. 1—4).

Die Ergebnisse veranschaulicht Abbildung 5. Wie ersichtlich waren

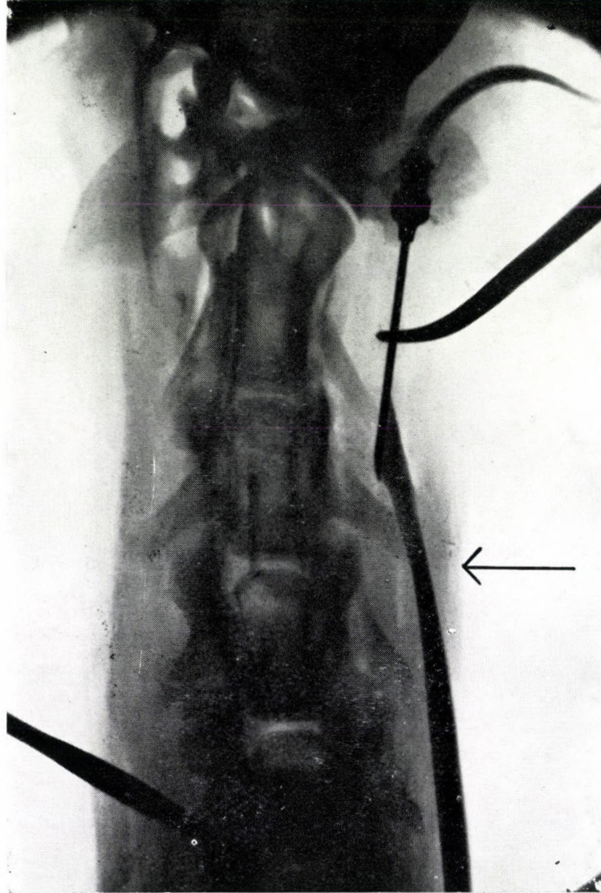

Abb. 2. Phlebographie der V. jugularis 120 Tage nach der mit Kleben durchgeführten End-zu-End-Vereinigung

37 (72,5%) der 51 operierten Gefäße vollkommen durchgängig, 6 heilten mit einer Stenose (11,8%), während 8 Gefäße (15,7%) thrombotisierten.

Histologischer Befund: In den ersten postoperativen Tagen ist der Klebstoff mit rundzelliger Infiltration umgeben. Im Laufe der 1.—2. Woche tritt anstelle der Leukozyten ein an Histiozyten und Fibroblasten reiches Angifibroblastgewebe, welches die Fragmente des sich resorbierenden Klebstoffs umspinnt. Die Resorptionsdauer des Klebstoffs ist — selbst wenn nur eine

dünne Schicht aufgetragen wird — länger als unsere Beobachtungszeit (120 Tage). Die sich in der Umgebung des Klebstoffs entwickelnde Gewebereaktion ist milden Grades (Abb. 6).

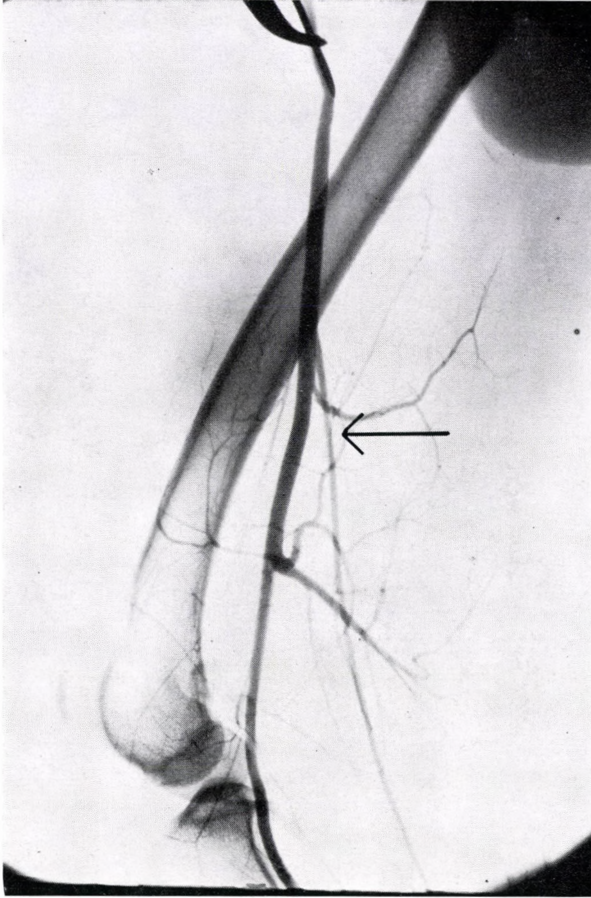

Abb. 3. Arteriographie der A. femoralis 90 Tage nach der mit Kleben durchgeführten End-zu-End-Vereinigung

Besprechung

Wie darauf auch unsere Erfahrungen hingewiesen haben, eignet sich das Klebeverfahren — mit einem Fleck oder auf andere Weise — zur Verschließung der longitudinalen Gefäßinzisionen. Das Ziel unserer Experimente war, die schwierige Technik der End-zu-End-Gefäßvereinigung unter Anwendung des Klebens zu vereinfachen und dadurch die Operationsdauer zu verkürzen.

Die Vereinigung der Gefäßenden mit Kleben ist unseres Erachtens in erster Linie in Kombination mit Richtungsnähten möglich. Die Anzahl der erforderlichen Richtungsnähte hängt von der Spannung der Gefäßstümpfe,

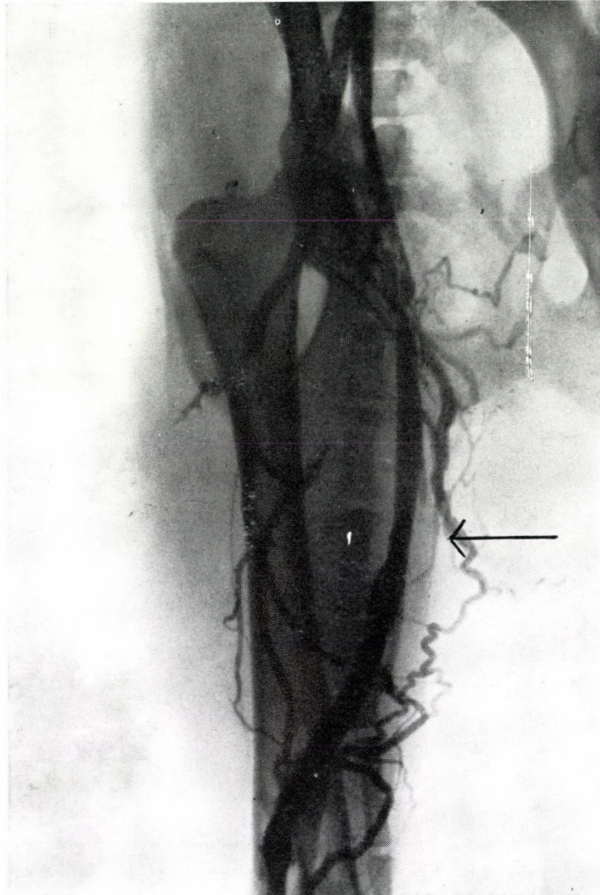

Abb. 4. Phlebographie der V. femoralis 90 Tage nach der mit Kleben durchgeführten End-zu-End-Vereinigung

dem Maß des Gefäßes und davon ab, ob es sich um eine Arterie oder eine Vene handelt. Die Anwendung einer, durch eine separate Öffnung eingeführten inneren Schiene (Polyäthylenrohr, irgendeine Sonde, oder ein geeignetes Instrument) hat sich beim Kleben der Anastomose — hauptsächlich im Falle kleinerer Gefäße und Venen — gut bewährt, da sich auf diese Weise die durch die Stütznähte herbeigeführte Verflachung und die dadurch bedingte Verzerrung des Gefäßes eliminieren läßt.

Die wichtige Anforderung, nämlich die genaue Adaptation der Wund-

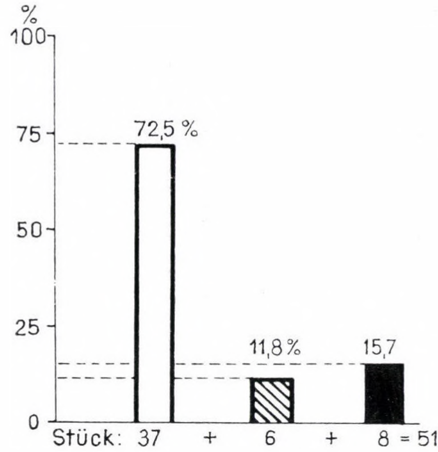

Abb. 5. Durchgängigkeit sämtlicher geklebten Gefäße. □ vollkommene Durchlässigkeit; ▨ Stenose; ■ Thrombose

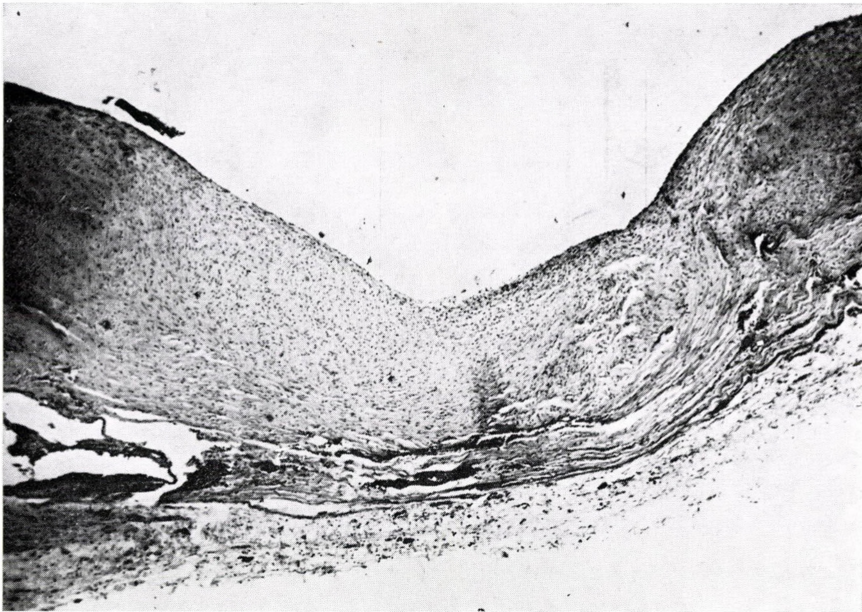

Abb. 6. Das mit Entzündungszellen umsponnene und auch Fremdkörperriesenzellen enthaltende Granulationsgewebe häuft sich in der Umgebung der Richtungsnähte an und befindet sich rings um den Klebstoff nur in kleinen Mengen

ränder, kann durch entsprechendes Auseinanderziehen der Stütznähte und der Adduktion der Gefäßstümpfe erreicht werden.

Es empfiehlt sich, das Kleben in zwei Etappen durchzuführen: zuerst auf der vorderen, sodann auf der hinteren Gefäßfläche. Falls die genaue Ver-

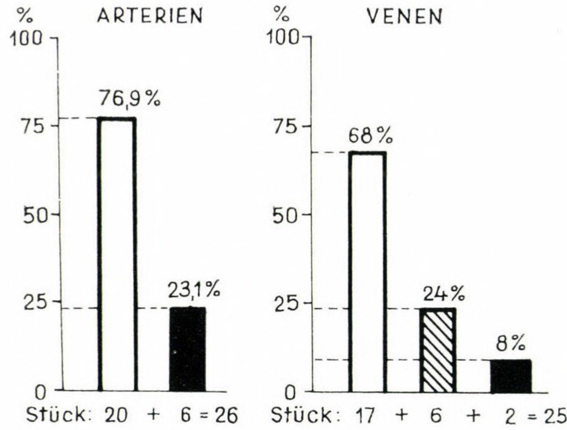

Abb. 7. Durchgängigkeit der Arterien und Venen. □ vollkommene Durchlässigkeit; ▨ Stenose; ■ Thrombose

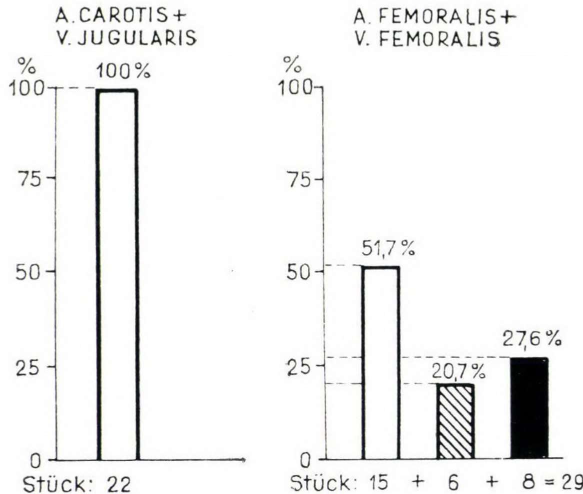

Abb. 8. Durchgängigkeit der Hals- und Schenkelgefäße. □ vollkommene Durchlässigkeit; ▨ Stenose; ■ Thrombose

einigung der Gefäßränder nicht gelingt, kann Klebstoff in das Lumen gelangen und Thrombose verursachen. Die Kontrolle der Adaptation erleichtert der Gebrauch einer Vergrößerungsbrille. Da der Klebstoff den Fäden der Richtungsnähte weniger anhaftet als den Geweben, muß die Umgebung der Stütznähte — um einer Nachblutung vorzubeugen — mit Klebstoff reichlicher bestrichen werden.

Im Laufe der Gefäßoperationen haben wir nur die Gefäßstümpfe mit verdünnter Heparinlösung durchgespült.

Wichtig ist, daß das Kleben an spannungsfreien Gefäßabschnitten

geschehe. In 72,5% unseres 51 Gefäßanastomosen umfassenden Versuchsmaterials konnte eine totale und in 11,8% eine partielle Durchgängigkeit erreicht werden, welche Angaben mit den Ergebnissen anderer Verfasser übereinstimmen (BORNEMISZA). Die weitere Analyse, genauer gesagt die Aufteilung unserer Resultate in eine arterielle und eine venöse Gruppe zeigt, daß 20 der 26 geklebte Arterien durchgängig blieben und 6 thrombotisierten; was die 25 geklebten Venen anbelangt, blieben 17 vollkommen und 6 mit einer Stenose durchgängig, während 2 Venen thrombotisierten (Abb. 7). Wie ersichtlich, liegt die Zahl der thrombotisierten Arterien höher als die der Venen, hinsichtlich der Durchgängigkeit der beiden Gefäßtypen meldet sich jedoch kein nennenswerter Unterschied.

Werden unsere Fälle der Lokalisation entsprechend in zwei Gruppen — Hals- und Schenkelgefäße — eingeteilt, so tritt die Wichtigkeit der spannungsfreien Gefäßvereinigung noch prägnanter in Erscheinung (Abb. 8). In der ersten Gruppe, zu der 22 Halsgefäße gehören, betrug die Prozentzahl der Durchgängigkeit 100% und es kamen weder Stenosen, noch Thrombosen vor, in der zweiten, 29 Aa. und Vv. femorales umfassenden Gruppe dagegen, wo bei der Vereinigung der Stümpfe die Spannung sehr ausgeprägt ist, blieben nur 15 Gefäße vollkommen und 6 partiell durchgängig, während sich in 8 Fällen eine Thrombose entwickelt hat. Unsere Erfahrungen sprechen somit dafür, daß der Erfolg des Gefäßklebens in erster Linie von der spannungsfreien Vereinigung der Gefäßabschnitte abhängt.

Aus den Beobachtungen lassen sich Folgerungen ziehen.

1. Die End-zu-End-Gefäßvereinigung kann am vorteilhaftesten mit der kombinierten Anwendung von Richtungsnähten und Kleben durchgeführt werden. Die Verwendung des Klebstoffs allein ist ungenügend, das Kleben ersetzt die Nähte nicht.

2. Die Anzahl der Richtungsnähte hängt vom Maß der Spannung, von Typ und Weite der Gefäße und von den im Gefäß herrschenden Druckverhältnissen ab.

3. Das Gefäßkleben verspricht auf spannungsfreien Gefäßabschnitten die besten Ergebnisse (z. B. A. carotis und V. jugularis). Im Falle expansiver Gefäßabschnitte (z. B. A. und V. femoralis) sind die Resultate schlechter.

4. Die Vorteile des Klebens gegenüber den herkömmlichen Nähmaterialien lassen sich auch histologisch beweisen: In der Umgebung des dünn aufgetragenen Klebstoffs ist die Fremdkörperreaktion weniger ausgeprägt als in der Nachbarschaft der Nähte.

5. Die strenge Beachtung der Regel der Asepsis ist auch beim Kleben von Wichtigkeit, da im Falle einer Infektion Gefäßthrombose entsteht, die geklebten Flächen sich trennen und eine — eventuell tödliche — Blutung auftritt.

6. Hierzu sei bemerkt, daß die Hunde bereits am ersten postoperativen

Tag aufstehen und sich bewegen, d. h. daß die erforderliche Ruhe nicht gesichert werden kann, trotz allem hat sich aber das Gefäßkleben als erfolgreich erwiesen.

7. Die mit entsprechender technischer Gewandtheit durchgeführte kombinierte (Richtungsnähte und Kleben) Gefäßvereinigung ist rascher und einfacher als die klassischen Verfahren. Dies unterstützen auch die Versuche, in denen bei einem Tier, in einer Sitzung, 8 Großgefäße (2 A. carotis-, 2 V. jugularis-Fälle, 2 Aa. femorales und 2 Vv. femorales) erfolgreich durchgetrennt und wieder vereinigt wurden.

8. Den End-zu-End-Gefäßvereinigungen ist in erster Linie bei den Organtransplantationen und -replantationen eine Bedeutung beizumessen, außerdem ist das Kleben im Falle technischer Schwierigkeiten (Arterien und Venen mit kleinem Durchmesser) oder insofern die rasche Durchführung der Operation entscheidend ist, besonders vorteilhaft.

ZUSAMMENFASSUNG

Die anläßlich der Anwendung des Butyl-2-cyanoacrilat Gewebeklebstoffs gewonnenen Erfahrungen führten zur Feststellung, daß sich das Präparat zur Gefäßvereinigung eignet. Anschließend werden die Operationstechnik beschrieben und die Bedeutung der Methode analysiert.

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COMPARATIVE STUDIES ON THE INTESTINAL LOOP-DILATING EFFECT OF LIVE CULTURE OF *ESCHERICHIA COLI* 0141 : K85, 88 AND ITS ENTEROTOXIN IN MINIATURE AND LARGE WHITE PIGS

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Pig breeders suffer considerable losses from diseases caused by *Escherichia coli*. Following the findings of SOJKA (1965) and SZABÓ (1965) among others, it is now fairly well established that most of the diseases are induced by strains of certain serotypes, but despite the much work on the subject the mechanism through which such strains provoke diarrhoea in pigs is still not elucidated.

According to more recent data (SMITH and HALLS, 1967b and others), the strains involved exert their enteropathogenic effect by producing an exotoxin-like substance, called enterotoxin.

Though the ligated intestinal loop test, as described by DE and CHATTERJE (1953), has been found suitable by many for determining this enteropathogenicity (NAMIOKA et al., 1958; MOON et al., 1966; SMITH and HALLS, 1967a; GYLES and BARNUM, 1967; NIELSEN and SAUTTER, 1968), there are still a few contradictory reports on its value (SMITH and HALLS, 1967b; TRUSZCZYNSKI and PILASZEK, 1969).

It therefore seemed advisable to examine the loop-dilating effect of some given *E. coli* strain and its enterotoxin together with the reactivity of the test animals to the enterotoxin. This study forms part of a larger-scale investigation into the diseases of the porcine digestive apparatus and the ailments of sucking pigs induced by *E. coli* with which this Institute has long been concerned.

Materials and methods

Production of enterotoxin

The enteropathogenic reference strain *E. coli*, serotype 0141:K85,88 was used* throughout the experiments. From a 24-hour broth culture of strain 1 ml was inoculated into the ligated segments. Enterotoxin from the reference strain by a slight modification of the method of SMITH and HALLS (1967b), was prepared employing a nutrient medium containing 0.3% agar and stored

* Our thanks are due to Dr. H. W. SMITH (Animal Health Trust, Stock, Essex, UK.) for putting the strain at our disposal.

at —20 °C. The sterile enterotoxin in doses of 15 ml without adding antibiotic to the segments was administered. As control substances 15 ml physiological saline to the segments or the same amount of material prepared from the nutrient medium in the same way as enterotoxin, but without inoculation was injected into the remaining segments.

Experimental animals and surgical procedure

The animals of our disposal comprised 16 Minnesota Miniature pigs (ten of them 9 to 12 weeks old, weighing 7 to 14 kg, and six 21 to 27 weeks old, weighing 10 to 19 kg) and 17 large White pigs (7 to 15 weeks old, 10 to 20 kg in weight). After fasting them for 24 hours the animals were anaesthetized with "Hibernal 0.5%"* (1 ml/kg) and Intranarcon** (0.2—0.3 ml/kg) and subjected to left-side laparotomy. Except for along a 1-metre stretch of the duodenum distal to the pylorus, the small intestine was tied with silk thread to produce a variable number of segments (usually 15 to 17). Segments were inoculated with live culture, enterotoxin or one of the control substances as a rule separated by an untreated segment. The animals were killed and dissected 24 hours after operation. A reaction was accepted as positive if the volume of fluid recovered from a ligated segment amounted to or exceeded 8 ml. Investigations now in progress have shown, however, that more exact results would have been obtained if the weight of fluid in the segment had been referred to 1 g of intestinal wall (ratio of fluid weight to weight of gut wall).

Results

The untreated segments of 5 Miniature and 2 Large White pigs were found to contain more than 8 ml fluid at the post-mortem examination, and so these animals were excluded from evaluation.

The remaining 26 animals could be grouped under three categories (Table I): (1) animals in which only the live culture provoked appreciable accumulation of fluid, while the enterotoxin had practically no effect; (2) animals displaying marked reactions in which both the live culture and the enterotoxin elicited a considerable accumulation of fluid in all inoculated segment; (3) animals in which live culture invariably gave rise to notable fluid accumulation, whereas reaction to the enterotoxin was positive in some and negative in other segments.

Disregarding the seven animals excluded from evaluation, only ligated intestinal segments treated with live culture or enterotoxin were found to be

* United Works of Pharmaceutical and Dietetic Products (EGYT), Budapest.
** Chinoin Works, Budapest.

dilated (Fig. 1). Neither the administration of saline nor that of the nutrient medium caused dilatation and in fact the entire 15 ml of inoculated fluid was absorbed. Untreated segments of these animals showed no fluid accumulation either: all that was observed was a small quantity of mucus sticking to the intestinal mucous membrane.

Anterior inoculated segments contained more fluid than the lower loops of one and the same animal. In general, loops more caudally situated than the 17th segment failed to respond to enterotoxin, whereas live culture still

Table I

Reactions to *E. coli* broth-culture and enterotoxin in ligated intestinal loops of Minnesota Miniature pigs and Large White pigs

	Minnesota Miniature pig			Large White pig		
	Number of animals	Broth culture	Enterotoxin	Number of animals	Broth culture	Enterotoxin
		Positive/total segments			Positive/total segments	
Marked reaction	6	11/12	11/12	9	20/20	30/30
Variable reaction to enterotoxin	—	—	—	5	17/17	19/36
Weak reaction	5	9/10	3/14	1	1/1	0/4
Not evaluable	5	—	—	2	—	—

dilated them. Ligation caused multiplication in the number of microorganisms in the treated and untreated loops alike, but positive fluid reactions were observed only in loops inoculated with the live culture of *E. coli* 0141:K85,88 or the enterotoxin.

Results with regard to the sensitivity of the two breeds to the live culture and enterotoxin of *E. coli* were as follows.

Miniature Minnesota pigs

About half of the evaluable animals gave weak reactions. Of the total of 14 segments treated with enterotoxin only three responded positively; they contained 21, 14 and 11 ml of fluid, respectively. Live culture dilated only the anterior segments in all cases, while reaction failed to appear in some of the more posterior loops. The average volume of fluid produced by live culture amounted to 11.4 ml in the anterior segments, and 8.8 ml in segments 9 and 11.

Six of the 11 evaluable animals of this category gave marked reactions (Table II): a large volume of fluid accumulated particularly in the segments inoculated with enterotoxin. Two of the older Miniature pigs (21—27 weeks) gave marked and four gave weak reactions, while weak reaction was observed in only a single animal of the younger age group (9—12 weeks).

Table II

Pronounced reaction to live culture and enterotoxin of *E. coli* in ligated intestinal loops of 6 Minnesota Miniature pigs and 9 Large White pigs

Treatment	Reaction: Positive/total segments		Volume of fluid in segment (ml)	
	Minnesota	Large White	Minnesota	Large White
Live culture into 1st segment	6/6	9/9	47.6 ± 9.14**	57.3 ± 11.95**
Live culture into 9th, or 11th and 7th or 13th segment	5/6	11/11	28.5 ± 7.95	45.4 ± 5.25
Enterotoxin into 3rd, 5th, 7th or 11th segment	11/12	—	46.0 ± 6.98	—
Enterotoxin into 3rd, 5th, 7th or 11th; 3rd, 4th, 5th and 9th, 10th, 11th segment	—	30/30	—	60.1 ± 4.57
Control substance	0/2	0/3	—	—
Saline	0/1	0/4	—	—

* 24-hour broth culture of *E. coli* 0141 : K85,88
** mean value and standard deviation

All animals of this group displaying unevaluable reactions were 9 to 12 weeks old. None of the Miniature pigs showed variable sensitivity to enterotoxin.

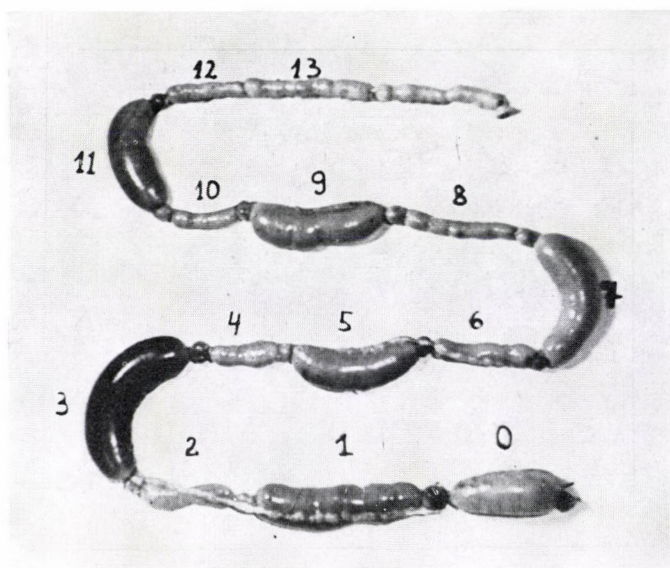

Fig. 1. Effect of *E. coli* broth culture and enterotoxin on ligated segments of the small intestine of pig. 1, 11, live culture; 3, 5, 7, 9, enterotoxin; 2, 4, 6, 8, 10, 12, untreated segments; 13, control substance; 0, duodenum tied up at dissection without treatment

Large White pigs

Only one of the 15 evaluable animals of this group gave a weak reaction. The volumes of fluid which accumulated in the differently treated segments of the nine strongly reacting animals are listed in Table II. It can be seen that all intestinal loops inoculated with live culture or enterotoxin contained a notable volume of fluid. Both the broth culture and enterotoxin provoked more fluid than in the comparably treated loops of Minnesota pigs, though the difference was not significant ($p > 0.05$).

The ligated intestinal segments of five animals displayed varying sensitivity to enterotoxin (Table III). Live culture invariably produced notable

Table III

Responses to enterotoxin of ligated intestinal loops in Large White pigs displaying variable sensitivity

Animal	Broth culture		Enterotoxin	
	Serial No. of segment	Reaction	Serial No. of segment	Reaction
44	1, 9,	+ +	3, 5, 7	+ — +
	17, 25	+ +	11, 13, 15	+ + +
			19, 21, 23	+ + +
45	1, 9	+ +	3, 5, 7	+ — +
	17, 25	+ +	11, 13, 15	+ — —
			19, 21, 23	— — —
52	1	+	3, 4, 5	+ + +
	7	+	9, 10, 11	— — —
	13	+		
53	1	+	3, 4, 5	— — —
	7	+	9, 10, 11	— + +
	13	+		
54	1	+	3, 4, 5	+ — —
	7	+	9, 10, 11	+ + —
	13	+		

+, positive reaction; —, negative reaction

accumulation of fluid range: 16—63 ml, mean value: 29.4 ml), whereas enterotoxin had the same effect in only 19 of the 36 ligated segments, the other 17 loops being found empty. The proportion and arrangement of positive and negative segments varied from animal to animal.

Discussion

One-third of the Miniature pigs were unfit for evaluation, and about half of the evaluable animals failed to respond to enterotoxin. The majority of animals reacting weakly to enterotoxin belonged to the older age group

(21—27 weeks). Employing the loop test, MOON and WHIPP (1970) demonstrated that, starting from the age of six weeks, pigs can develop resistance to *E. coli* with advancing age — but only to certain strains. Although numerous tests were carried out in the present experiments, no evidence was found for the existence of age-determined resistance to the reference strain used. We do not, therefore, regard the lack of sensitivity to enterotoxin observed in our Miniature pigs as a phenomenon related to age. It seems rather that breed and other more or less unknown factors play a certain role in reactions to the administration of enterotoxin.

Though uninoculated segments may dilate in inadequately starved animals carrying *E. coli*, this phenomenon still fails to explain the large proportion of unevaluable Minnesota pigs.

The proportion of weakly reacting Large White pigs was consistent with findings reported by SMITH and HALLS (1967b) and MOON and WHIPP (1970). However, in five cases the same enterotoxin elicited in the same animal a positive reaction in some segments and a negative one in others. No correlation was found between the position and the sensitivity of the segments (Table III); so that the variability of enterotoxin sensitivity cannot be due to the caudally diminishing reactivity of the small intestine. SMITH and HALLS (1967b) do not mention any differences in sensitivity between the various segments, though their results seem to indicate that they must have observed this phenomenon. On the other hand, MOON et al. (1966) affirm that "the response of one loop to an enteropathogenic strain may be positive, whereas the response of another loop (located approximately 30 cm anterior or posterior in the intestine of the same pig) to the same challenge may be negative". We failed to see this effect in segments inoculated with live culture; variable sensitivity was found only in respect of enterotoxin. Though we are not in a position to explain the phenomenon, it is clear that variable sensitivity of the segments must be reckoned with when examining the enteropathogenicity of *E. coli* strains and their enterotoxins.

SMITH and HALLS (1967a, b) found that in ligated intestinal loop of Large White pigs the volume of fluid provoked by live cultures or enterotoxin may fluctuate considerably from animal to animal. Notwithstanding this, no more than 8 of 66 pigs had to be disregarded on account of weak reaction. As in the present experiments, the live culture always produced positive reactions in these weak reactors, but the volume of fluid in the segments was less than usual. This and the present findings are in direct contrast to the conclusions of TRUSZCZYNSKI and PILASZEK (1969) who stated that the same enterotoxin introduced into the ligated small intestinal segments of different Large White pigs does not induce fluid accumulation regularly, and further, that the number of pigs unresponsive to enterotoxin normally exceeds that reported by SMITH and HALLS. Therefore TRUSZCZYNSKI and PILASZEK (1969)

regard the loop test as being of limited value for the demonstration of the enteropathogenic nature of *E. coli* strains and the examination of the potency of their enterotoxins. As regards Large White pigs, our results do not support this opinion.

Investigations of the bacterial flora of ligated segments proved that it is not the increase in bacterial count elicited by ligation but only the enteropathogenic *E. coli* strain or its enterotoxin that is responsible from the dilatation. Provided the nutrient medium is adequate, enterotoxin is obtainable from any enteropathogenic strain of *E. coli* which causes fluid to accumulate in ligated intestinal loops. The processes by which fluid accumulates in ligated intestinal loops treated with enterotoxin are presumably similar to those involved in cases of natural diarrhoea provoked by *E. coli*, which are characterized by a considerable outflow of fluid into the small intestine.

The degree of reaction in gut loops inoculated with live culture or enterotoxin depends on the susceptibility of the animal, which may be *complete* (animals giving marked reaction), *partial* (animals with varying sensitivity) or *strongly reduced* (animals with weak reaction). Enterotoxin, if administered to animals with varying sensitivity, provokes either large volumes of fluid or has no effect whatever. Animals of this kind are of limited value suited for studying the effect of enterotoxin. Those animals showing weak reaction are unsuitable for the study of enterotoxin effect and only questionably suitable for the loop-dilating action of live cultures. There were numerous enterotoxin-resistant Minnesota pigs in our material, so that only the Large White pigs seem to us to be apt for the ligated intestinal loop test. Errors due to varying enterotoxin sensitivity can be prevented by using the live culture and enterotoxin of a standard enteropathogenic *E. coli* strain as positive controls and evaluating the results by comparison with the volume of fluid provoked by the control substances. It follows that the loop-dilating effect of both a given *E. coli* strain and its enterotoxin have to be studied in at least two segments each of two pigs. This requirement makes the intestinal loop test more expensive and laborious, but at present no more reliable method is available. Demonstration of enterotoxin by other biological tests such as mouse lethality, pyrogenic effect on rabbits (Truszczynski and Pilaszek, 1970) have failed to prove specific in our experiments.

SUMMARY

The intestinal-loop dilating effect of broth culture and the enterotoxin of the strain *E. coli* 0141 : K85,88 has been studied in 16 Minnesota Miniature pigs and 17 Large White pigs. Reactions obtained from five Minnesota and two Large White pigs were unsuitable for evaluation. The remaining 26 animals formed three groups: (1) animals displaying weak reaction in which only the live culture provoked appreciable fluid accumulation; (2) animals giving pronounced reaction in which all treated segments were notably dilated by both enterotoxin and live culture: (3) animals displaying varying sensitivity to enterotoxin in which the response to live culture was always positive, while that to enterotoxin was positive in some and negative in other segments.

Only six of the Miniature pigs were sensitive, the other five gave weak reactions, whereas only a single one of the fifteen Large White pigs developed weak reaction, nine showed pronounced sensitivity, and five displayed varying sensitivity.

Evaluating the reliability of the intestinal loop test with regard to the varying sensitivity of the pigs to enterotoxin, it is concluded that the test, carried out under the experimental conditions as described in the paper, is suitable for demonstrating in the Large White pig the *E. coli* strains and their enterotoxins involved in their enteral diseases but is unsuitable for this purpose in respect of the Minnesota Miniature pig.

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ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN ÜBER DIE MIKROGAMETENENTWICKLUNG DES MÄUSECOCCIDS *EIMEIRA FALCIFORMIS*

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Das Mäusecoccid *Eimeria falciformis* halten wir unter den zahlreichen Eimeria-Arten für ein geeignetes Modellcoccid, das alle Bedingungen erfüllt, die an ein solches Objekt gestellt werden. Die Labormaus erweist sich dabei als Modellwirt. Da Parasit und Wirt ständig im Labor gehalten werden können, sind experimentelle Infektionen relativ leicht durchzuführen. Voraussetzung für weitergehende Untersuchungen ist allerdings die eingehende Kenntnis von der Mikromorphologie und Entwicklung dieses Coccids. Wir berichten daher in der vorliegenden Arbeit über die Entwicklung und Differenzierung der Mikrogameten von *E. falciformis*.

Lichtmikroskopisch wurde das Coccid von HABERKORN (1970) untersucht, während an elektronenoptischen Arbeiten Berichte über die Feinstruktur der Makrogameten, Schizonten und Merozoiten vorliegen (SCHOLTYSECK, MEHLHORN, HABERKORN, 1971; PELLÉRDY, HABERKORN, MEHLHORN, SCHOLTYSECK, 1971; MEHLHORN, SÉNAUD, SCHOLTYSECK, 1972). Von besonderem Interesse erscheinen uns weitere licht- und elektronenmikroskopische Untersuchungen zur Cytochemie und Pathologie beim Modellparasiten und beim Modellwirt.

Material und Methode

Coccidienfreie Mäuse (spf. CF_1 Winkelmann und spf. NMRI-Han.) wurden per os mit 10^5 sporulierten Oocysten von *E. falciformis* infiziert. Am 6.—9. Tag nach der Infektion wurden die Mäuse getötet, kleine Darmstückchen aus dem Epithelzellenbereich entnommen und nach der Methode von SJÖSTRAND (1956) fixiert. Die Entwässerung erfolgte über Aceton und die Einbettung in Vestopal W. Die Dünnschnitte wurden mit einem LKB-Ultrotom angefertigt. Für die elektronenmikroskopischen Untersuchungen wurde ein Zeiss EM 9A verwendet.

* Mit Unterstützung der Deutschen Forschungsgemeinschaft.

Ergebnisse

In der Regel treten Mikrogamonten etwa vom 6. Tag p. i. auf, in seltenen Fällen jedoch können diese Stadien auch schon nach etwa vier Tagen beobachtet werden. Eine Deutung dieses Phänomens steht allerdings noch aus. Die Mikrogamonten von *Eimeria falciformis* sind etwa rundlich bis ovoid und ungefähr 13,1×9,5 μ groß. Bemerkenswert ist noch die Tatsache, daß die Mikrogamontengröße bei längeren Infektionszeiten anscheinend zunimmt (s. Tabelle).

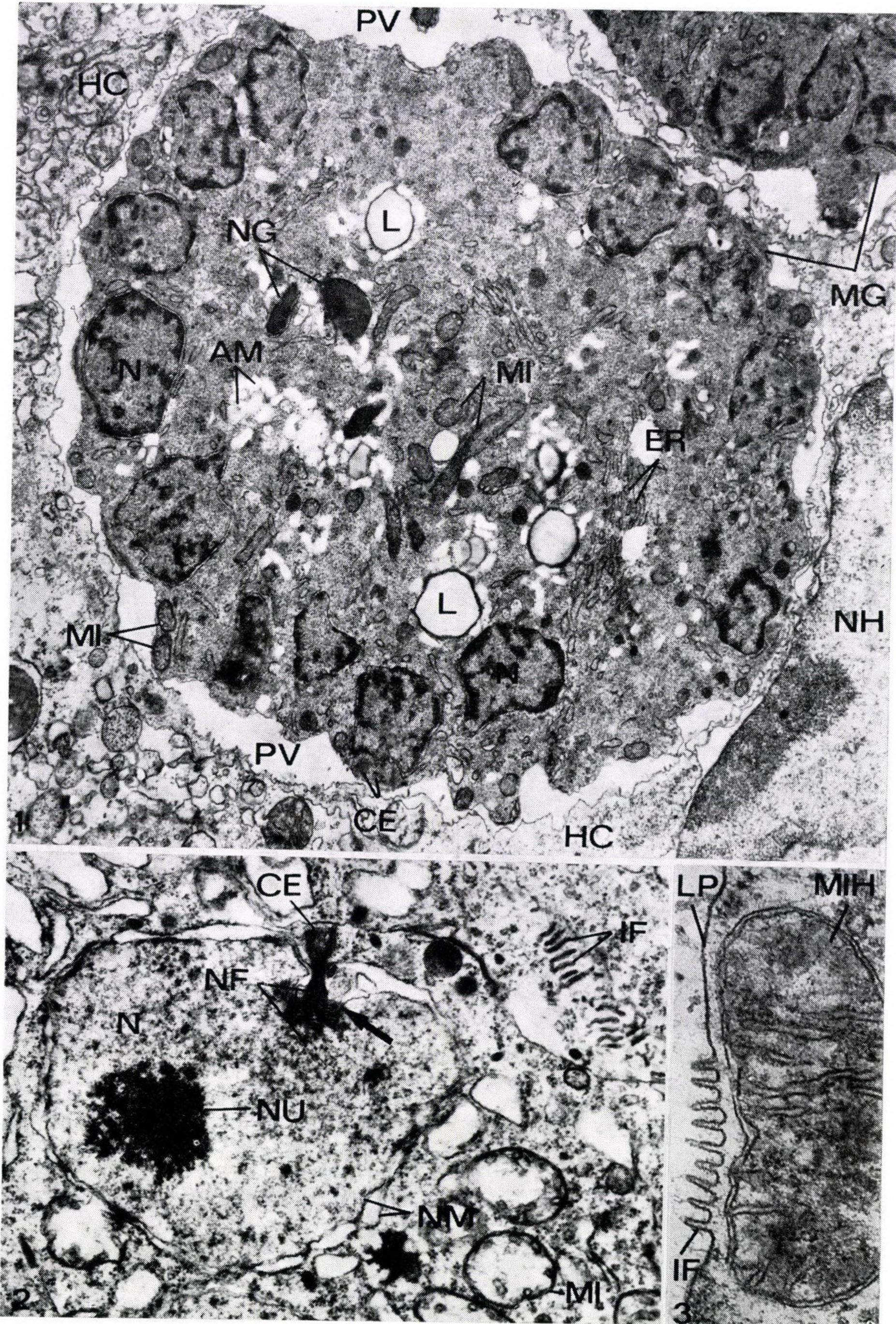
Tage p. i.	Größe der Mikrogamonten	Minimum – Maximum
4,5	9,9×6,1 μ	6,7–16,0×5,3– 8,0 μ
5	—	—
6	11,2×8,4 μ	8,0–20,0×5,3–12,0 μ
7	10,9×8,5 μ	6,7–13,3×5,3–13,3 μ
8	13,1×9,5 μ	6,7–18,7×6,7–14,7 μ

Messungen von Mikrogamonten an histologischen Schnitten. Fixierung: Stieve; Färbung PAS-AO; Schrumpfung im Vergleich zum Frischpräparat 13—20%.

Als Begrenzung des Mikrogamonten, in jungem wie auch ausgewachsenem Zustand, ist eine Elementarmembran ausgebildet, die die äußere Membran der typischen Merozoitenpellikula darstellt. An zahlreichen Stellen treten die typischen Mikroporen auf, die als Ultracytostome im Dienste der Nahrungsaufnahme stehen (Abb. 13; SCHOLTYSECK, MEHLHORN, 1970; SCHOLTYSECK, MEHLHORN, HAMMOND, 1972).

Nach den ersten Mitosen ordnen sich die Kerne an der Peripherie des Mikrogamonten an und führen weitere Teilungen durch. Die Nuklei sind von

Abb. 1—3. Eimeria falciformis. Elektronenmikroskopische Aufnahmen. *1*, Mikrogamont mit fertigen Mikrogameten (NG) und noch in der Teilung befindlichen Kernen (N). ×13 800. *2*, Der Spindelpol (NF) ragt zu dem im Cytoplasma lokalisierten Centriolen (CE). Die Kernmembran bildet an dieser Stelle eine Einsenkung. ×27 600. *3*, Die Wirtszellmitochondrien (MIH) liegen unmittelbar an der Begrenzungsmembran (LP) der parasitophoren Vakuole. Intravakuoläre Falten (IF) lassen einen kammartigen Aspekt entstehen. ×70 000. Abkürzungen: AM, Polysaccharidgrana (Amylopektin); CE, Centriol; F, Geißel (Flagellum); HC, Wirtszelle; IF, Intravakuoläre Falten; L, Lipid; LM, Begrenzungsmembran des Mikrogamonten; LP, Begrenzungsmembran der parasitophoren Vakuole; MG, Mikrogamont; MI, Mitochondrium; MIH, Mitochondrium der Wirtszelle; MP, Mikropore; MT, Mikrotubuli; N, Nukleus; NF, Spindelfibrillen; NG, Kern des Mikrogameten; NH, Kern der Wirtszelle; NM, Kernmembran; NU, Nukleolus; PF, Perforatorium; PV, Parasitophore Vakuole; RF, Rudimentäre Geißel; RN, Restkern; UF, U-förmige Ringfalte; UM, Elementarmembran; WF II, Hüllbildungskörper des zweiten Typs von Makrogameten


PV
HC
NG
L
MG
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AM
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ER
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NH
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PV
CE
HC
1
CE
NF
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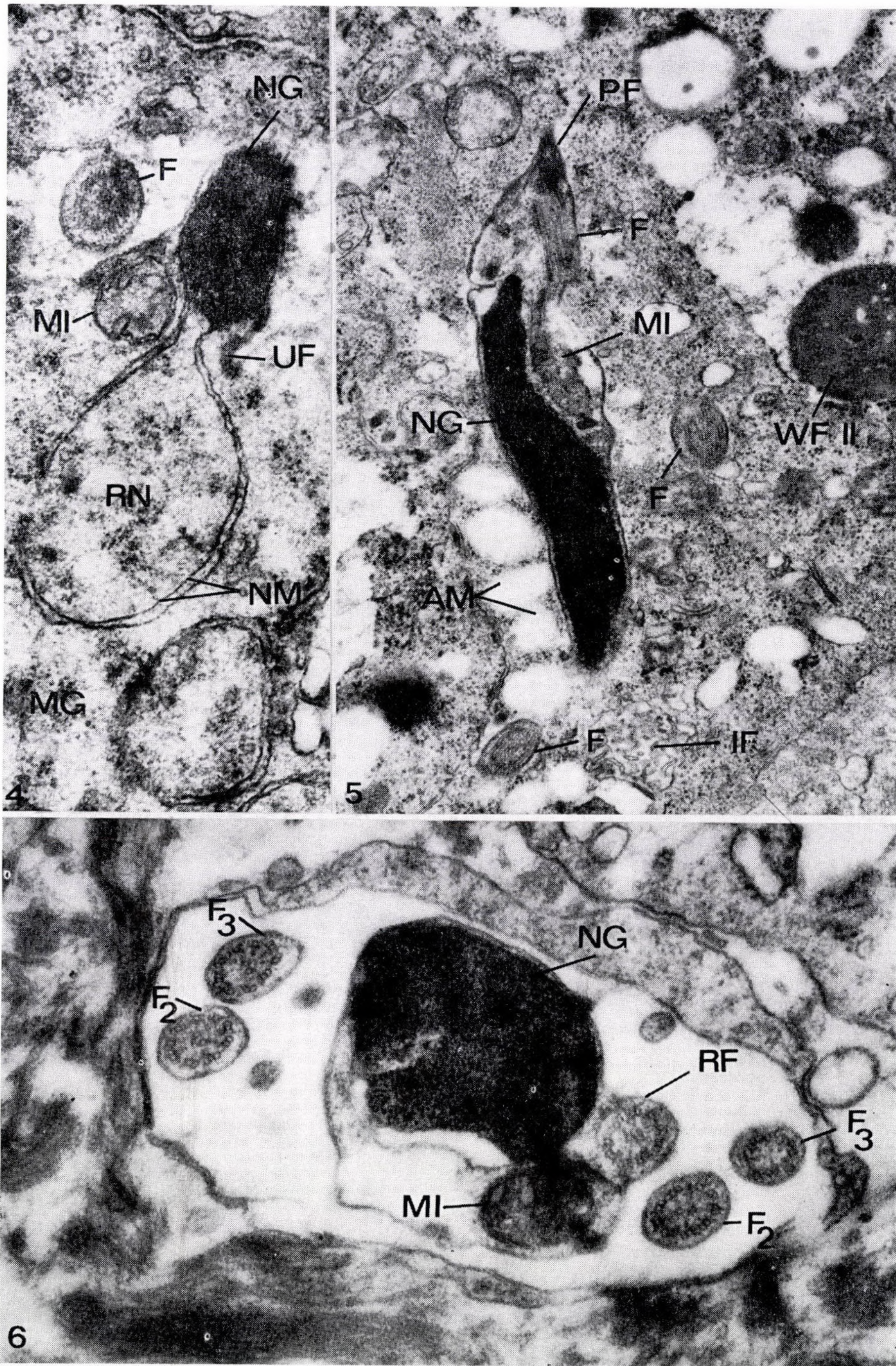
N
NU
NM
MI
2
LP
MIH
IF
3

einer typischen doppelten Kernmembran umgeben, die zahlreiche Kernporen ausspart (Abb. 1, 2, 4, 13). Neben großen Teilen des Karyoplasmas erscheint auch noch ein als Nukleolus anzusprechender Bezirk des Kerninnern als stark osmiophil (Abb. 1, 2). Die Kernteilungen laufen stets in der Form der Zweiteilung ab, wobei die Kernmembranen erhalten bleiben. Zu Beginn der Teilungsvorgänge erscheinen intranukleäre Fibrillen, die zu einem Spindelapparat gehören. In unmittelbarer Nähe jedes Spindelpoles liegen im Cytoplasma zwischen Nukleus und der Gamontenbegrenzung je zwei Zentriolen. In einigen Fällen konnte beobachtet werden, daß der Spindelpol im Cytoplasma liegt, wobei die innere Kernmembran eine grubenartige Einsenkung ausbildet. Die Spindelfibrillen durchziehen dabei unmittelbar die Kernmembran (Abb. 2). Bei den hier auftretenden Zentriolen handelt es sich nicht um die klassische Triplett-Formation, sondern 9 periphere Mikrotubuli umgeben konzentrisch einen zentralen Mikrotubulus. Dieser feinstrukturelle Aufbau der Zentriolen liegt wohl bei den meisten Coccidien vor (SCHOLTYSECK, MEHLHORN, HOPPE, 1972; SCHOLTYSECK, MEHLHORN, HAMMOND, 1972; MEHLHORN, SÉNAUD, SCHOLTYSECK, 1972).

In enger räumlicher Beziehung zu den Nuklei stehen zahlreiche Mitochondrien vom tubulären Typus (Abb. 1, 2, 4, 13). Je eines von ihnen geht dann in den späteren Mikrogameten über, wobei es einen Prozeß der inneren Umstrukturierung durchläuft (Abb. 5—8). Neben den Mitochondrien fallen bei den Mikrogamonten von *E. falciformis* vor allem Anhäufungen des rauhen endoplasmatischen Retikulums (ER) auf (Abb. 1, 13). Diese in der Teilungsphase leicht angeschwollenen Kanalsysteme sind später bei den ausgewachsenen Mikrogamonten verschwunden, was sich im Abklingen der Syntheseprozesse erklären dürfte. Als besonderes Charakteristikum der Mikrogamonten dieses Mäusecoccids sind die zahlreichen, hier glänzend weiß erscheinenden Polysaccharidgrana (Amylopektin) und die große Anzahl von Lipoidtropfen anzusprechen (Abb. 1, 13; AM, L).

Die zweite Stufe der Mikrogamogonie, die Differenzierung der Mikrogameten, setzt bei *E. falciformis* im Gegensatz zu allen bisher untersuchten *Eimeria*-Arten nicht erst nach Abschluß aller Kernteilungen ein. Es lassen sich nämlich am gleichen Mikrogamonten schon differenzierte Mikrogameten und noch in der Teilung befindliche randständige Nuklei beobachten (Abb. 1, N, NG). Diese Tatsache läßt sich nur durch einen sukzessiven Verlauf dieses Prozesses erklären, während sonst ja die Mikrogameten (mit Ausnahme von

Abb. 4—6. Eimeria falciformis. Elektronenmikroskopische Aufnahmen. *4*, Bei der Bildung der Mikrogameten teilt sich der Kern in einen elektronenlichten Restnukleus (RN) und in den osmiophilen Gametenkeren (NG). ×41 400; *5*, Längsschnitt durch einen Mikrogameten, der sich auf dem Weg zu einem weiblichen Gameten befindet. ×27 000; *6*, Querschnitt durch einen Mikrogameten. Eine Geißel erweist sich als Schleppgeißel (RF). Die zweite und die dritte Geißel (F_2, F_3) sind je zweimal getroffen. ×51 000


NG
F
MI
UF
RN
NM
MG
4
PF
F
MI
NG
WF II
F
AM
F
IF
5
F3
NG
F2
RF
F3
MI
F2
6

T. gondii) mehr oder weniger gleichzeitig entstehen (SCHOLTYSECK, MEHLHORN, HAMMOND, 1972; PELSTER, PIEKARSKI, 1971).

Die Ausbildung der Mikrogameten beginnt mit der Streckung der an der Peripherie befindlichen Kerne, und so entstehen bis zu 5 μ lange Vorwölbungen an der Oberfläche des Mikrogamonten. Dabei läuft in dem Teil des Nukleus, der zum Rand des Mikrogamonten hingewendet ist, ein Verdichtungsprozeß ab (Abb. 4). Der elektronenlichte Teil verbleibt schließlich im Restkörper, während sich der osmiophile Teil abschnürt und zum Nukleus des Mikrogameten wird (Abb. 4, 8; RN, NG). An der Abschnürstelle der Mikrogameten findet sich stets eine im Längsschnitt u-förmige Ringfalte. Sie ist in allen Fällen von einer osmiophilen Schicht unterlegt (Abb. 4; UF). Diese Strukturen haben offenbar die Aufgabe, ein zu frühes Abreißen der »Brücke« zu verhindern, indem sie den Teil verstärken, durch den der Verbindungsschlauch vom Restkern zum Mikrogametenkern verläuft. Der eigentliche Trennungsprozeß des Mikrogameten vom Restkörper wird dadurch eingeleitet, daß in dem Verbindungsstück ein Spaltraum entsteht, der zum Cytoplasma hin durch eine Elementarmembran begrenzt ist und durch Vergrößerung schließlich das Abreißen des Mikrogameten herbeiführt.

Wie bereits erwähnt, liegen die Mikrogamonten von *E. falciformis*, die schematisch in Abb. 13 dargestellt sind, wie die übrigen intrazellulären Stadien in einer parasitophoren Vakuole, deren Begrenzungsmembran zur Wirtszelle hin charakteristische Strukturen entstehen läßt. So bildet nämlich diese Membran zahlreiche intravakuoläre Falten aus, die wegen ihrer regelmäßigen Anordnung und ihres stets etwa gleichen Durchmessers einen kammartigen Aspekt vermitteln (Abb. 2, 3, 13). Dicht an der Membran der parasitophoren Vakuole liegen zahlreiche große Mitochondrien der Wirtszelle.

Die Feinstruktur der Mikrogameten

Die Mikrogameten von *E. falciformis* sind langgestreckt und ziemlich schmal. Sie erreichen in der Längenausdehnung etwa 4—5 μ und messen an ihrer breitesten Stelle auf Höhe der Mitochondriummitte etwa 0,75 μ im Durchmesser, während sie sich zum Hinterende hin auf einen Durchmesser von etwa 0,3 μ verjüngen (Abb. 5, 6, 7, 8, 9, 10, 12, 14). In Querschnitten

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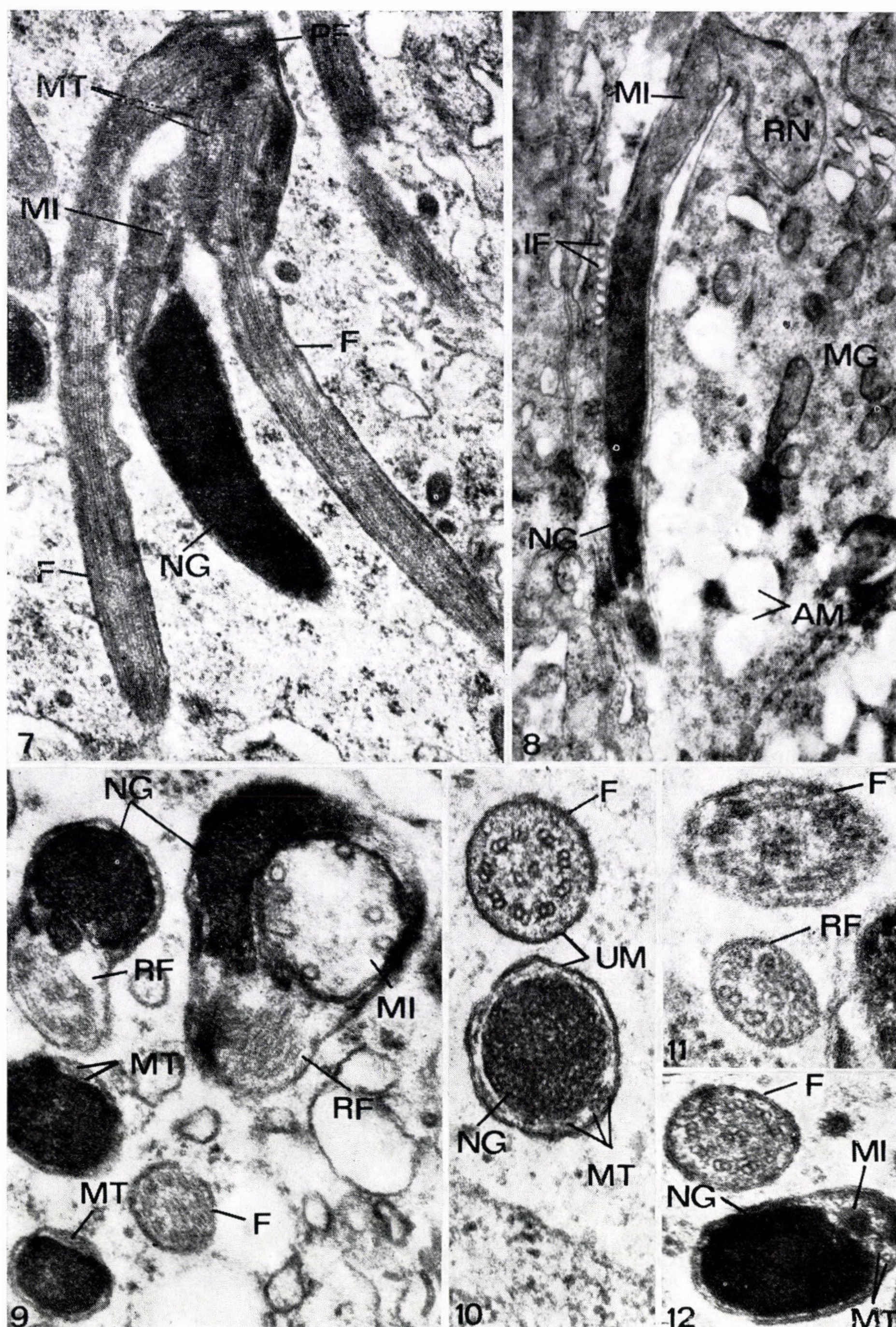
Abb. 7—12. Eimeria falciformis. Elektronenmikroskopische Aufnahmen. *7*, Längsschnitt durch einen differenzierten Mikrogameten. $\times$35 000; *8*, Schnitt durch einen noch mit dem Mikrogamonten verbundenen Mikrogameten. $\times$27 000; *9*, Querschnitt durch einen Mikrogameten in Höhe der Mitochondriummitte (MI) und drei weiteren unterhalb des Mitochondriums. $\times$54 000; *10*, Querschnitt durch den hinteren Zellpol eines Mikrogameten. Drei Mikrotubuli (MT) sind getroffen. $\times$80 000; *11*, Querschnitte durch eine intakte Geißel (F) und eine rudimentäre (RF). $\times$82 000; *12*, Querschnitt durch einen Mikrogameten am hinteren Ende des Mitochondriums (MI). $\times$66 000



MT
MI
F
NG
F
7
MI
RN
IF
MG
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AM
8
NG
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MT
RF
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9
F
UM
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MT
10
F
RF
11
F
MI
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MT
12

erscheinen sie mehr oder weniger drehrund (Abb. 6, 9, 10, 12, 14). Eine Elementarmembran, die sich von der Begrenzungsmembran des Mikrogamonten herleitet, umschließt den aus Nukleus, Mitochondrium und Geißeln mit Basalkörpern bestehenden Mikrogameten, der in Abb. 14 schematisch dargestellt ist.

Der langgestreckte Zellkern verleiht dem Mikrogameten seine Gestalt. Der Nukleus wird durch eine typische doppelte Kernmembran begrenzt; allerdings sind die beiden Membranen sehr dicht aneinandergelegt, so daß kein perinukleärer Raum entsteht (Abb. 6, 9). Das Karyoplasma erscheint fein granuliert und erweist sich als stark osmiophil. Zum Vorderende des Mikrogameten hin verläuft der Kern fingerförmig zugespitzt. Das Mitochondrium liegt in einer Kernfurche, die allerdings am vorderen und hinteren Pol des Mitochondriums nicht mehr deutlich ausgeprägt ist (Abb. 6, 9, 12). In einem Prozeß der inneren Umstrukturierung ist dieses Mitochondrium aus einem charakteristischen Mitochondrium des Mikrogamonten hervorgegangen (Abb. 1, 13). Als Innenstruktur können beim Mitochondrium des Mikrogameten 6 Reihen ellipsoider Tubuli beobachtet werden, die zu je dreien gegenüberliegen (Abb. 5, 7, 8). Das Mitochondrium steht an der Zellspitze in unmittelbarem Kontakt mit den Basalapparaten der Geißeln. Diese Basalapparate, deren Feinstruktur wegen der Osmiophilie leicht verloren geht, formen eine als Perforatorium bezeichnete Spitze, die auf Grund der dadurch bedingten Versteifung das Eindringen des Mikrogameten in das weibliche Stadium erleichtert.

Die Mikrogameten von *E. falciformis* besitzen drei Geißeln, von denen eine ein Stück als Schleppgeißel verläuft und wesentlich kleiner im Querschnitt erscheint (Abb. 6, 9, 11). Die beiden übrigen Geißeln sind schon unmittelbar hinter den Basalapparaten frei beweglich (Abb. 5, 7). Dieses Faktum erklärt die Seltenheit von Schnittbildern, auf denen alle drei Geißeln eindeutig zu verifizieren sind. Außer diesen drei Geißeln konnten zusätzlich noch einzelne Mikrotubuli ermittelt werden, die ihren Ursprung ebenfalls an der unmittelbaren Zellspitze nehmen (Abb. 10, 12). Drei derartige Röhrchen erwiesen sich als Norm; daneben wurden aber auch nur eins bzw. zwei von solchen Elementen, deren Durchmesser mit 180 Å in den für die meisten Mikrotubuli bekannten Dimensionen liegt, angetroffen. Sie verlaufen entlang des Mitochondriums (MI) und erstrecken sich offenbar bis zum hinteren Zellpol (Abb. 9, 10; MT). Möglicherweise handelt es sich bei diesem Mikrotubulisystem um die rudimentären Reste einer vierten Geißel. Der Gesamtdurchmesser der Geißel beträgt etwa 0,27 μ. Die rudimentäre Geißel verjüngt sich auf etwa 0,21 μ. Beide Werte sind in Anbetracht des geringen Gametendurchmessers von ca. 0,75 μ recht erheblich. In der Längenausdehnung konnte die gesamte Geißellänge wegen der Biegungen im elektronenmikroskopischen Bild nicht gemessen werden. So waren lediglich Abschnitte von maximal 5—6 μ zu beobachten; aus lichtmikroskopischen Analysen geht jedoch hervor, daß die Geißellänge bei etwa 10—14 μ liegt.

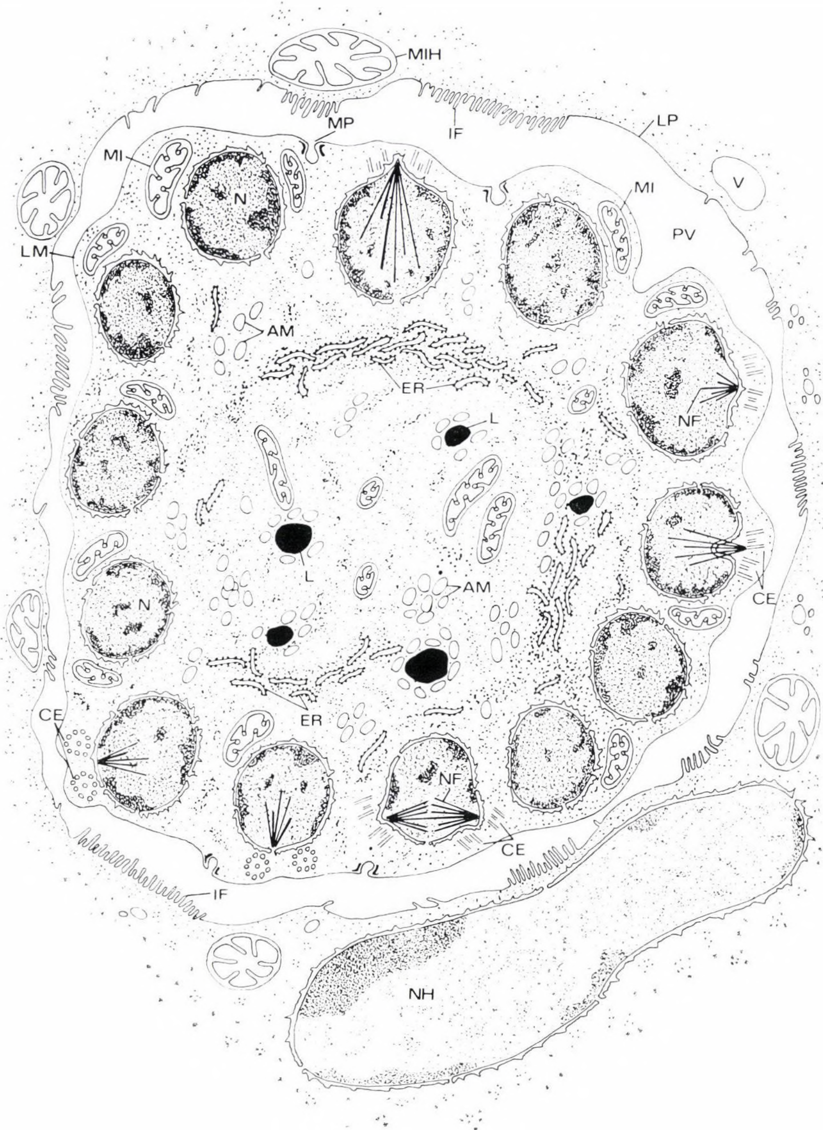

Abb. 13. Eimeria falciformis. Schematische Darstellung eines Mikrogamonten

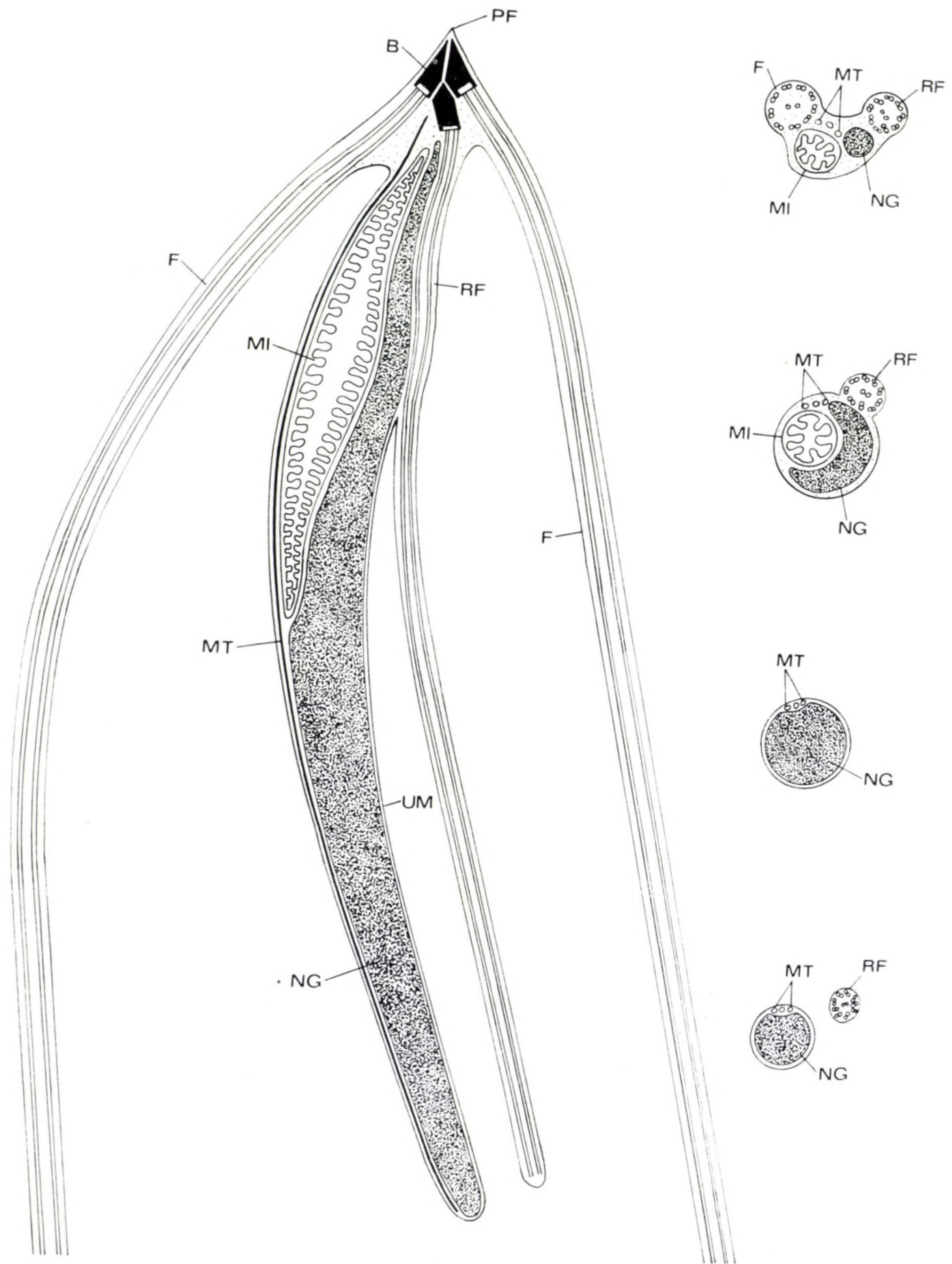

Abb. 14. Eimeria falciformis. Schematische Darstellung eines Mikrogameten in Längs- und Querschnitt

Diskussion

Über den Prozeß der Mikrogametenentwicklung bei Coccidien liegen nur wenige elektronenmikroskopische Untersuchungen vor (SCHOLTYSECK, 1965; GARNHAM, BIRD, BAKER, 1967; BRADBURY, TRAGER, 1968; HAMMOND, SCHOLTYSECK, CHOBOTAR, 1969; SCHOLTYSECK, MEHLHORN, HAMMOND, 1972). Einige weitere Autoren beschäftigten sich mit der Mikromorphologie der männlichen Gameten der Coccidien (CHEISSIN, 1965; BARDELE, 1966; MCLAREN, 1969; SNIGIREVSKAYA, 1969; AIKAWA, HUFF, STROM, 1970; DESSER, 1970; PORCHET-HENNERÉ, 1970; COLLEY, ZAMAN, 1970; PELSTER, PIEKARSKI, 1971).

Die einzelnen Entwicklungsschritte der Mikrogameten eines Mikrogamonten von *E. falciformis* verlaufen nicht simultan, wie es für die bisher untersuchten *Eimeria*-Arten ermittelt werden konnte (SCHOLTYSECK, 1965; SCHOLTYSECK, MEHLHORN, HAMMOND, 1972). Der Differenzierungsprozeß einzelner Mikrogameten eines Gamonten beginnt nämlich hier nicht erst nach Abschluß aller Kernteilungen. So findet man in einem Gamonten neben differenzierten Mikrogameten noch sich teilende Mikrogamontenkerne, eine Beobachtung, die auch schon für die Mikrogametenentwicklung von *Toxoplasma gondii* beschrieben wurde (PELSTER, PIEKARSKI, 1971). Der Ablauf der Mikrogametenentwicklung entspricht jedoch den Verhältnissen, wie sie von den bisher untersuchten *Eimeria*-Arten her bekannt sind, bei denen die Abschnürung eines »Restkerns« ein charakteristisches Phänomen darstellt.

Bei den Kernteilungen der Mikrogamonten von *E. falciformis* läßt sich beobachten, daß die intranukleäre Spindel in den cytoplasmatischen Raum zu den dort lokalisierten Centriolen hinzieht. Diese Tatsache und das gelegentliche Auftreten von centriolenähnlichen Strukturen im Kerninneren sind ein Schritt zur Klärung des Zusammenspiels von Centriolen und Spindel bei den dynamischen Vorgängen während der Kernteilung (MEHLHORN, SÉNAUD, SCHOLTYSECK, 1972). Die Kernteilungen erfolgen wohl unter der steuernden Wirkung der Centriolen, die zu Paaren an den Spindelpolen im Cytoplasma liegen. Während des gesamten Kernteilungsprozesses bleibt die charakteristische Kernbegrenzung erhalten. Bei einigen Hämosporidien und bei den meisten Metazoen dagegen werden die Kernmembranen während des Teilungsprozesses aufgelöst (BRADBURY, TRAGER, 1968).

Besonders zahlreich sind in den Mikrogamonten von *E. falciformis* Polysaccharidgrana, die nach der hier angewendeten Technik leuchtend weiß erscheinen. Die Mikrogameten von *E. falciformis* entsprechen in ihren Ausmaßen und ihrem Feinbau den Mikrogameten der bisher untersuchten *Eimeria*-Arten (SCHOLTYSECK, MEHLHORN, HAMMOND, 1972). Sie besitzen 3 Geißeln, von denen eine ein kurzes Stück als Schleppgeißel verläuft, bald endet und an diesem Ende eine deformierte Struktur im Querschnitt aufweist. Eine derartige Dreigeißeligkeit konnte bisher elektronenmikroskopisch für *E. per-*

forans, E. tenella, E. nieschulzi, E. maxima, E. auburnensis und *Aggregata eberthi* sowie lichtmikroskopisch für *E. contorta* ermittelt werden (SCHOLTYSECK, 1965; COLLEY, 1967; MCLAREN, 1969; HAMMOND, SCHOLTYSECK, CHOBOTAR, 1969; HELLER, 1970; MEHLHORN, 1972). Ein solcher Befund ist nur mit sehr zahlreichen Parallelschnitten eindeutig zu verifizieren, so daß zu erwarten steht, daß auch bei weiteren Arten und bei *Toxoplasma gondii* eine derartige Feinstruktur bestätigt werden kann. Neben diesen Geißeln treten bei *E. falciformis* noch in der Regel 3 einzelne Mikrotubuli in Erscheinung, die rudimentäre Reste einer vierten Geißel darstellen könnten. Sie wurden auch in der Ein- bzw. Zweizahl angetroffen. Vergleichbare Mikrotubuli wurden schon bei *E. auburnensis, E. maxima, E. intestinalis* und *Toxoplasma gondii* beschrieben (HAMMOND, SCHOLTYSECK, CHOBOTAR, 1969; CHEISSIN, 1965; PELSTER, PIEKARSKI, 1971; MEHLHORN, 1972).

Das Mitochondrium der Mikrogameten von *E. falciformis* liegt in einer Kerngrube, ein Befund, der bisher lediglich bei *E. perforans* anzutreffen war (SCHOLTYSECK, 1965). Als Innenstruktur ließen sich 6 Reihen ellipsoider Tubuli ermitteln. Hierin bestehen offenbar Artunterschiede innerhalb der Gattung *Eimeria* (SCHOLTYSECK, MEHLHORN, HAMMOND, 1972).

Eine besonders wichtige Veränderung der Wirtszelle wird durch die vom Parasiten induzierte Bildung der parasitophoren Vakuole vollzogen. Besonderes Charakteristikum sind hier zahlreiche intravakuoläre Falten, die wegen ihrer gleichen Länge, ihrer großen Anzahl und ihres gleichen Durchmessers einen kammartigen Aspekt vermitteln. Sie stehen offenbar ebenso wie die zahlreichen Wirtszellmitochondrien, die unmittelbar an der Begrenzungsmembran der parasitophoren Vakuole lokalisiert sind, in engem Zusammenhang mit dem Stofftransport zwischen Parasit und Wirtszelle. Das Schicksal der Wirtszelle, also die Pathologie dieses intrazellulären Parasiten, soll einer späteren Publikation vorbehalten bleiben.

ZUSAMMENFASSUNG

Die Feinstruktur der Mikrogamonten und Mikrogameten des Mäusecoccids *Eimeria falciformis* aus dem Dickdarmepithel künstlich infizierter Labormäuse wurde untersucht, beschrieben und dargestellt. Dabei ließ sich auch der Ablauf der Mikrogametendifferenzierung in allen Phasen rekonstruieren.

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IMMUNOLOGICAL INVESTIGATIONS INTO THE CAUSES OF ASPECIFIC ABR-REACTIONS

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The ABR test is now commonly applied in Hungary (as elsewhere) as a routine procedure for the screening of cattle herds for brucellosis. In the course of screening it is not unusual to obtain positive reactions when brucellosis can be excluded with certainty on the basis of the herd's clinical history, epizootological status and multiple serological examinations.

Such aspecific, false-positive reactions in the ABR test have been the subject of a number of investigations. Hajdu (1964) who was the first to examine the phenomenon attributed the reactions to an increase of macromolecular substances in the milk or to loss of stability of the antigen suspension. Guthy et al. (1969) implicated physical and chemical changes associated with the increase of globulin. Earlier, Gergely et al. (1966) had observed that serum proteins and above all immunoglobulins have a strong binding affinity to erythrocytes and claimed later (1967, 1969) that exclusively the papaine-resistant IgG molecules are capable of aspecific combination with these cells. However, absorption of this kind was demonstrated by Kávai, Csaba and Kesztyüs (1968) to take place not only on the surface of the erythrocyte, but on any kind of cell, cytophilia being a general characteristic of globulins. To prevent aspecific ABR reactions, Hill and Cremers (1967) as well as Holmes (1967) suggested the dilution of milk samples, while Körmendy (1970) recommended that complementary tests should be conducted. According to Scheibner (1967), natural acidification inhibits rather than enhance a positive ABR reaction, whence it can by no means be held responsible for aspecific reactions.

In this laboratory, an immunological approach to the problem was attempted by immunodiffusion and immunoelectrophoretic analysis of the globulin classes present in milk of positive and negative reactors to the ABR test. The methodological and other information required for the performance of the experiments was derived from Feinstein and Hobart (1969), Götz and Heinebrodt (1969), Hammer et al. (1968), Kickhöfen et al. (1968) Nyiredi and Tuboly (1968), Pan et al. (1968), Penhale and Christie (1969), Porter (1969), Scheibner (1967).

Material and methods

The sets of samples examined could be divided into three categories:

a) ABR-negative milk and blood samples from a herd free from brucellosis and mastitis;

b) Milk and blood samples from a brucellosis-infected herd;

c) ABR-positive milk samples from eight herds free from brucellosis (ABR-positivity fluctuated between 2 and 8%).

Apart from the ABR test, all milk and blood samples were subjected to agglutination, complement fixation and Coombs' tests and evaluated the routine score systems. The majority of the milk samples retained an anti-complementary effect after inactivation at 60—64 °C.

Immunoelectrophoresis and immunodiffusion were performed on 2% agar gel spread on plates 9×6 cm or 7.5×2.5 cm in size. The fluid (whey) phase of the milk was collected after coagulation of the sample with rennet powder dissolved in saline. Wells 7 and 4 mm in diameter were punched into the agar layer for the whey samples. Rabbit anti-bovine immunoglobulin serum and anti-bovine IgG serum were employed as immune sera (HUMAN, Budapest). Immunoelectrophoresis runs were carried out in a semi-micro paper electrophoresis apparatus (LMM), using a pH 8.6 veronal buffer solution of 0.025 ionic strength. The running time was 2—2.5 hours.

Results

Radial double immunodiffusion analysis of ABR-negative whey sample from brucellosis-free herds against anti-bovine immunoglobulin serum resulted in most cases in the formation of a single sharp medium-dense, narrow precipitation line between the milk-containing and serum-containing wells; an indistinct second arch of low density also appeared in about half the cases (Fig. 1). Reactions of all these samples in agglutination, Coombs' and complement-fixation tests were negative or could not be evaluated on account of anti-complementary effect.

ABR-positive whey samples from known brucellosis-infected herds produced broad, well-delineated, confluent, high-density precipitation arches close by — some even touching — the antiserum well. In most cases there was also a sharp, narrow high-density second arch (Fig. 2). Nearly all the samples (94.6%) reacted positively in the agglutination, Coombs' and complement fixation tests as well.

ABR-positive milk samples from the eight serologically checked brucellosis-free herds formed sharp, broad, high-density precipitation arches near the antiserum well; the narrow, high-density second arch was often doubled (Fig. 3). All samples reacted negatively in agglutination, complement fixation and Coombs' tests.

The quantitative relationships of immunoglobulins in the three categories of samples can be clearly seen from the immunodiffusion patterns presented in Figs 4, 5 and 6. In each case the upper row of wells contained rabbit

anti-bovine globulin serum, while the lower wells held the undiluted milk and dilutions of 1 : 1, 1 : 2 and 1 : 4. The immunodiffusion pattern of the series clearly shows that the quantity of immunoglobulins was much higher in the

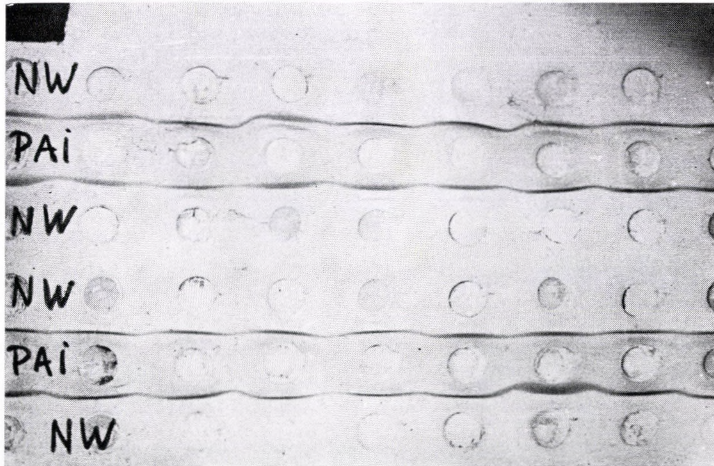


Fig. 1. Immunodiffusion of ABR-negative whey samples versus anti-bovine globulin serum, Symbols used: NW, ABR-negative whey; PW, ABR-positive whey; APW, aspecific ABR-positive whey; PAI, polyspecific anti-immunoglobulin; BS, bovine serum

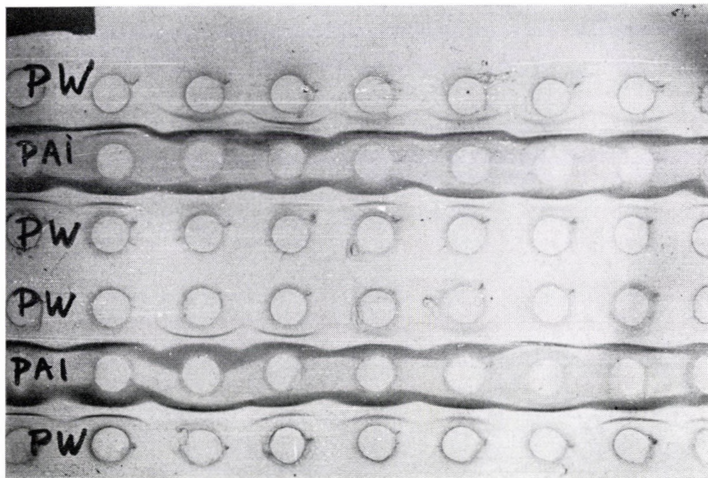


Fig. 2. Immunodiffusion of ABR-positive whey samples versus anti-bovine globulin serum

ABR-positive and especially in the aspecific series, than in the ABR-negative samples.

Comparative runs were performed to determine whether the precipitation lines obtained by radial double diffusion were identical, just related or

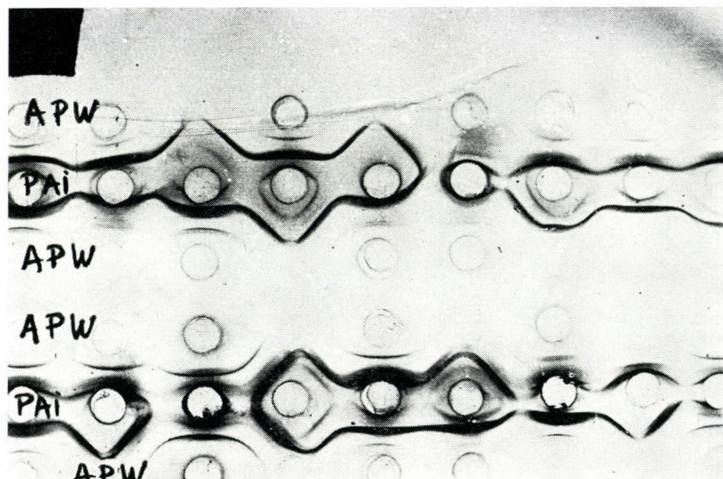


Fig. 3. Immunodiffusion of aspecific ABR-positive whey samples versus anti-bovine globulin serum. One well was left empty between whey samples of different origin

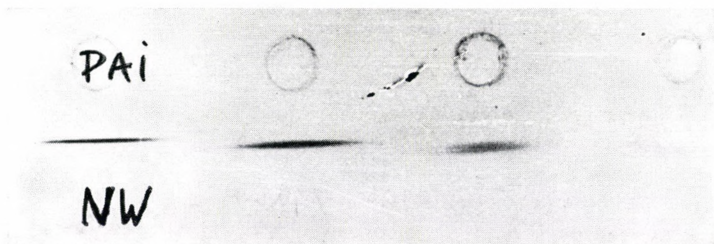


Fig. 4. Immunodiffusion of serial dilutions of ABR-negative whey sample

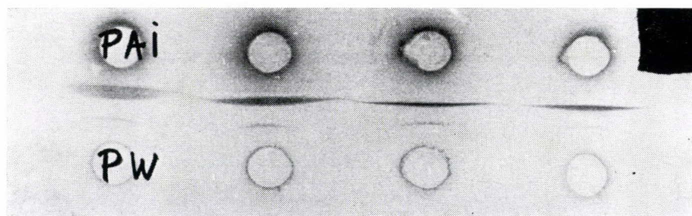


Fig. 5. Immunodiffusion of serial dilutions of ABR-positive whey sample

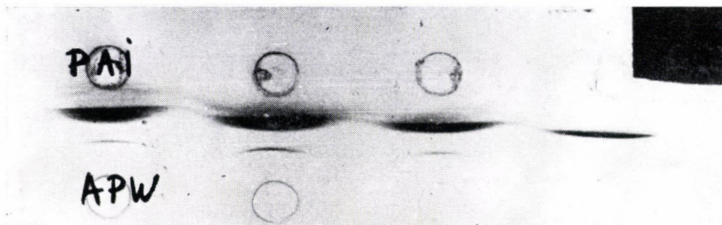
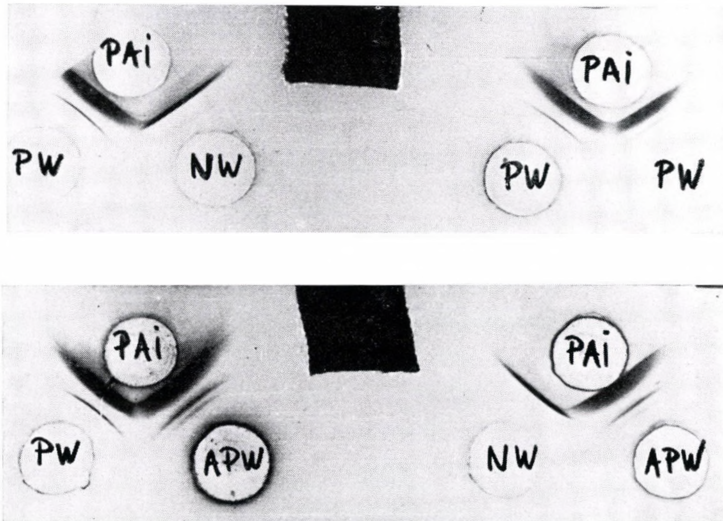


Fig. 6. Immunodiffusion of serial dilutions of an aspecific ABR-positive whey sample versus anti-bovine globulin serum

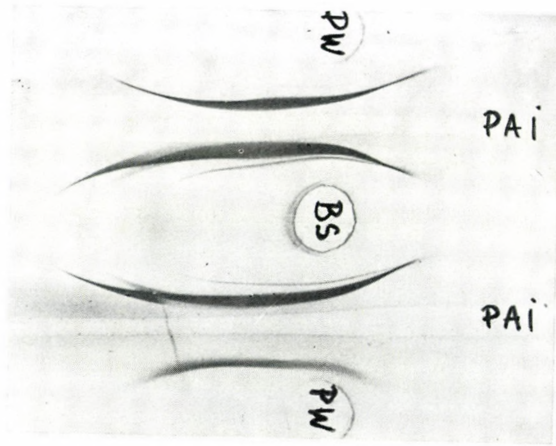
unrelated. Fig. 7 shows the results of such runs with ABR-positive and ABR-negative samples and of two ABR-positive samples, using rabbit anti-bovine immunoglobulin serum. ABR-positive and negative samples were similarly compared with an aspecific ABR-positive sample (Fig. 8). The diffusion patterns demonstrate that both specific and aspecific positive reactions are related to an increase of one and the same class of immunoglobulin. The immunoglobulin component of the ABR-negative milk samples reacted with that of the positive sample as a related globulin.



Figs 7—8. Comparative immunodiffusion analysis of ABR-negative and specific and aspecific ABR-positive whey samples

ABR-negative as well as specific and aspecific ABR-positive milk samples were further analyzed by immunoelectrophoresis. In order to identify the immunoglobulins, pooled bovine serum originating from several animals was compared with ABR-positive (Fig. 9) and ABR-negative (Fig. 10) milk samples. In another series, ABR-positive and -negative milk was run simultaneously with pooled bovine serum (Fig. 11) and finally several aspecific ABR-positive milk samples were compared (Fig. 12).

On the basis of electrophoretic mobility, IgM and IgG_1 as well as an additional IgG immunglobulin — probably IgGs — could be easily identified in the milk samples. Unlike the sharp, very narrow arches given by ABR-negative samples, the broad double arches of specific and aspecific positive samples are characteristically divided into IgG_1 and IgM. Double IgG or IgM arches were not infrequently seen with the aspecific ABR-positive milk samples (Fig. 12).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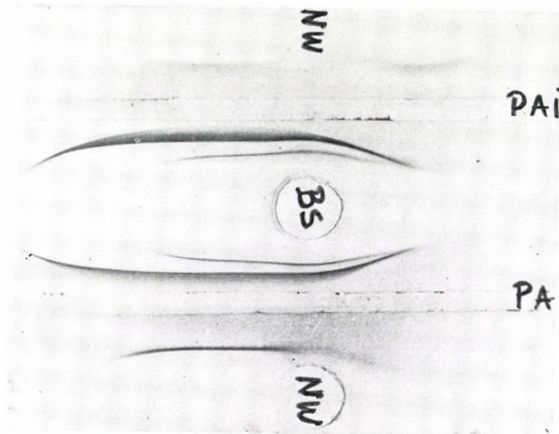
PAi
BS
PAi
PW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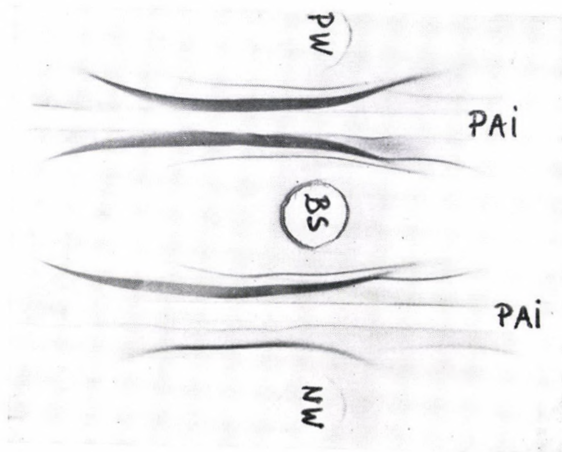
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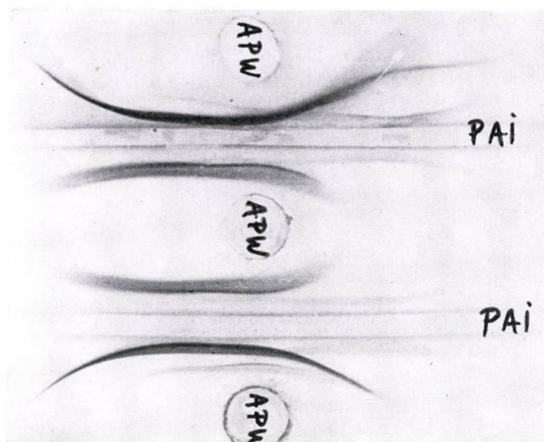

Figs 9—12. Comparative immunoelectrophoretic analysis of bovine serum with ABR-negative and ABR-positive (specific and aspecific) whey samples

Discussion

The quantitative relations of immunoglobulins in ABR-negative and specific or aspecific ABR-positive milk samples are readily analysed by immunodiffusion and immunoelectrophoresis. The immunoglobulin content of the negative samples was generally found to be low, consisting chiefly of IgG, though a few samples contained IgM as well. Comparative immunodiffusion indicated that this IgG component is related to, but not identical with IgG_1, whence it has been classified as IgGs. The bulk of the immunoglobulin component of most positive milk samples was also IgG, more precisely IgG_1, and IgM was invariably present. The composition of immunoglobulin from aspecific ABR-positive milk samples was similar, though the quantities of both IgG_1 and IgM were as a rule greater and in some cases the two kinds of immunoglobulin formed a double precipitation arch.

The IgG associated with aspecific ABR-positive reactions of milk is therefore probably not specific itself, and so its combination with the antigen cannot be specific, either. The reaction is most likely due to the adsorption of non-specific immunoglobulin complexes onto the lipid globules of milk. This possibility should also be taken into consideration in those cases in which no abnormality can be detected by organoleptic examination of the milk. Analysis of the lactic immunoglobulins in conjunction with appropriate serological tests seems — from these results — to be a suitable method for the detection of aspecific ABR-reactions.

SUMMARY

Immundiffusion and immunoelectrophoretic analysis of the whey of ABR-tested milk samples demonstrated the presence of three kinds of immunoglobulin against rabbit anti-bovine Ig serum. ABR-negative samples contained a relatively small amount of an IgG component qualified as IgGs, and infrequently IgM. Specifically and aspecifically reacting ABR-positive samples contained a much greater quantity of IgGs and often IgG_1 and IgM as well. Thus a positive ABR reaction was associated with a quantitative increase of immunoglobulin, especially if the reaction was aspecific. Antibodies stimulated by the specific antigen form specific bonds with the homologous antigen, but there is reason to suppose that a rise of immunoglobulin level evoked by non-specific stimuli results in a different binding that is responsible for aspecific ABR reactions. Accordingly, in those cases in which positive ABR tests seem doubtful on the basis of clinical herd history and epizootological considerations, the serological tests should be carried out not only with blood, but also with whey samples, to obtain evidence of the brucellosis status.

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РЕЗЮМЕ

НАБЛЮДЕНИЯ ПО ИММУНИЗАЦИИ ПЛОДА В СВЯЗИ С ВАКЦИНИРОВАНИЕМ КОРОВ ПРОТИВ ВИРУСНОГО ПОНОСА КРУПНОГО РОГАТОГО СКОТА ВАКЦИНОЙ ВЕДЕВАК

К. БОГНАР

В хозяйстве, неблагополучном по вирусному поносу крупного рогатого скота, автором вакцинирована живой вирус содержащей вакциной на 5—8-ом месяце беременности 31 корова раз, 71 корова же с промежутком времени в один месяц дважды.

В день отела от 72 коров взяты образцы крови и молозива, от родившихся же телят образцы крови до первого сосания и на 3—10-ом днях жизни с тем, чтобы изучить их в пробе нейтрализации вируса.

У коров после вакцинации отклонения от нормы не наблюдались, абортов не было, их телята родились здоровыми.

В сыворотке 37% коров в день вакцинации обнаруживались антитела.

Сыворотка и молозиво восприимчивых, вакцинированных коров в день отела содержали антитела при титре 1 : 869—1 : 1426 и 1 : 10000 соответственно.

Сыворотка и молозиво коров, содержащие в момент отела антитела, нейтрализировали вирус при разбавлении 1 : 236 и 1 : 2509 соответственно — после разовой и при разбавлении 1 : 1190 и 1 : 15 565 соответственно — после двухкратной прививки матерей.

В сыворотке телят от 48, в момент прививки восприимчивых коров в 34 случаях (70,8%) обнаружены антитела; в сыворотке же 14 телят (29,2%) антитела появились только после сосания молозива. Титр антител в сыворотке телят, родившихся иммунными, в случае разовой и двухкратной прививки их матерей равнялся 1 : 1231 и 1 : 4046 соответственно.

В сыворотке телят, родившихся от 24 коров, обладающих уже в момент прививки антителами, до первого сосания антител не обнаружено; они появились только после сосания молозива и их титр к 3—4-ому дню равнялся 1 : 115—1 : 265.

Уровень активных антител у телят к 7-ому месяцу жизни равнялся 1 : 218—1 : 2560, тогда как поступившие в организм животных антитела с молозивом к 3—4-ому месяцам жизни уже не удавалось выявить или они обнаруживались только в следах (1 : 4).

На основании своих наблюдений автор приходит к заключению, согласно которому вирус вакцины может попасть в плод и вызвать в нем образование антител. Это явление автор называет внутриутробной активной иммунизацией. Если в сыворотке коровы циркулируют антитела, то вирус наверно не попадает в плод. Автор в неблагополучных по данной заразе хозяйствах рекомендует дважды прививать коров на 7-ом и 8-ом месяцах беременности, ибо таким образом можно наилучше профилактировать урон, наносимый ею. Вакцинированные восприимчивые коровы будут рождать телят, большинство которых уже в момент рода обладает иммунитетом. Телята же невосприимчивых привитых коров таким образом будут получать молозиво с высоким уровнем антител.

VI. ИЗОЛИРОВАНИЕ СПЕЦИФИЧЕСКИХ АНТИТЕЛ ИЗ СЫВОРОТКИ ИНВАЗИРОВАННЫХ C. PISIFORMIS КРОЛИКОВ

И. НЕМЕТ

Из сыворотки искусственно инвазированных C. pisiformis кроликов удалось изолировать выявимые антитела в иммунохимически чистом виде. Для этой цели использован иммуноадсорбент, полученный превращением при помощи глутаральдегида антигенов

кислото-растворимой фракции сырого экстракта инвазионных цистицерков в нерастворимые.

Превалируют глобулины типа IgG. Но препараты антител из сыворотки реинвазированных животных кроме этого содержали и иммуноглобулины IgM. Других компонентов поливалентной анти-кролик иммунной сыворотки не удалось выявить ни иммуноэлектрофоретическим, ни иммунодиффузионным анализами.

При разных методах получения антител 54—96% сывороточного иммуноглобулина удалось изолировать. Результаты проб агар-жель-диффузионной преципитации и индиректной гемагглютинации показали, что полученные из сыворотки чистые антитела не потеряли способности специфической связи к антигенам.

ФЕТАЛЬНОЕ ЗАБОЛЕВАНИЕ, ВЫЗВАННОЕ СВИНЫМИ ЭНТЕРОВИРУСАМИ

ОБНАРУЖИВАМОСТЬ БОЛЕЗНИ СМЕДИ В ВЕНГРИИ

Я. БЕНЕДА, Я. МЕСАРОШ и Й. РАЙБЛИНГ

В двух крупнохозяйственных племенных свинопоголовьях при традиционном и интенсивном содержании животных за период двух лет наблюдали за болезнью свиноматок, вызывающей перебои в размножении и гибель плодов.

Первое поголовье было благополучным по бруцеллезу и лептоспирозу, второе было неблагополучным по лептоспирозу, но симптомы заболевания не напоминали лептоспироз. В обоих поголовьях повторяющаяся охота и ненаступление оплодотворения были частым явлением. Среди родившихся здоровыми поросят было очень много нежизнеспособных животных и мумифицированных плодов, что было причиной существенного уменьшения пометов. Чувствительность плодов к заразному началу была наибольшей в период первых 30 дней внутриутробной жизни, хотя плоды могут пострадать от заразы в любую пору внутриутробной жизни, в зависимости от момента заражения. Вирус, позже идентифицированный как свиной аденовирус, удалось изолировать из плодов, погибших непосредственно перед родом, из плодовых оболочек, из органов нежизнеспособных поросят. На основании клиники и вирусологических исследований патологическое состояние было определено идентичным болезни СМЕДИ (**Stillbirth, Mumification, Embryonic Death- and Infertility — SMEDI — disease**), зарегистрированной первый раз в США Дан с сотрудниками в 1965 г.

ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИЗУЧЕНИЕ СТРУКТУРЫ САРКОЛЕММЫ У КРУПНОГО РОГАТОГО СКОТА ПРИ ОКОЧЕНЕНИИ

Г. БИРО и М. ГАРАМВЁЛДИ

Кольцевые соединительнотканные пучки в желобах около мышечных пучков, наблюдавшиеся раньше нормальным микроскопом, подвергались детальному, электронмикроскопическому исследованию. Обнаружено, что двухслойная сарколеммная оболочка впячивается в желобы (инвагинация) и, таким образом, часть соединительнотканного материала, образующего дупликатуру, происходит от эндомизия. Коллагенные пучки сарколеммы на поперечных срезах хорошо видны и их округлая форма указывает на то, что они окружают мышечные пучки в виде кольца. Наблюдение подтверждает то мнение, что причиной жесткости говядины во время окоченения является переорганизация соединительнотканной сети мышц.

О СПАИВАНИИ КРОВЕНОСНЫХ СОСУДОВ ГИСТОАКРИЛЕМ — КЛЕЕМ ДЛЯ ТКАНЕЙ

Й. ЗАЙЕР, Ш. ДУБЕЦ и К. ШОМОДЬВАРИ

Авторы докладывают об опыте по применению Гистоакрилата — клея для тканей, содержащего бутил-2-цианоакрилат. Препарат зарекомендовал себя в спаивании (склеивании) кровеносных сосудов. Описывают операционную технику и анализируют значение метода.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ КИШЕЧНЫЙ ПРОСВЕТ РАСШИРЯЮЩЕГО ЭФФЕКТА ЖИВЫХ КУЛЬТУР ESCHERICHIA COLI 0141 : K85,88 НА СВИНЬЯХ КАРЛИКОВОЙ И КРУПНОЙ БЕЛОЙ ПОРОД

Г. ШЕМЙЕН и Л. ПЕШТИ

Авторами изучен кишечный просвет расширяющий эффект бульонных культур и энтеротоксина Escherichia coli серотипа 0141 : к 85,88 на 16 свиньях миннесотской карликовой и на 17 головах крупной белой пород. Среди миннесотских свиней 5, а среди белых крупных животных 2 дали неясные реакции. На основании поведения кишечной петли, 26 животных можно разделить на три группы: 1. животные со слабой реакцией, у которых только живая культура вызывала оценимое накопление жидкости в кишечной петле; 2. животные с выраженной реакцией, у которых как энтеротоксины, так и бульонная культура расширяли все кишечные сегменты; 3. животное с переменной реакцией, когда от бульонной культуры получена всегда положительная реакция, от энтеротоксина же по отдельным сегментам получена то положительная, то отрицательная реакция.

Среди 11 миннесотских свиней только 6 оказались чувствительными, у 5 животных получена только слабая реакция. Среди 15 животных крупной белой породы 1 свинья показывала слабую реакцию, 9 — выраженную реакцию и 5 животных реагировало с разной чувствительностью.

Учитывая различную чувствительность свиней к энтеротоксину, авторы оценили надежность пробы на кишечной петле и пришли к выводу, согласно которому при рекомендуемой экспериментальной схеме белая крупная порода пригодна для изучения энтеральных поражений, вызванных Escherichia coli и ее токсинами. Миннесотская карликовая свинья для этой цели неудобна.

ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИЗУЧЕНИЕ ОБРАЗОВАНИЯ МИКРОГАМЕТ У МЫШИНОЙ КОКЦИДИИ EIMERIA FALCIFORMIS

Е. ШОЛТИСЕК, Л. ПЕЛЛЕРДИ, Г. МЕЛГОРН и А. ХАБЕРКОРН

Изучалась тонкая структура микрогамонтов и микрогамет мышиной кокцидии Eimeria falciformis в эпителиальных клетках тонкого кишечника при искусственной инвазии. Удалось реконструировать все фазы течения дифференциации микрогамет.

ИЗУЧЕНИЕ ПРИЧИН НЕСПЕЦИФИЧЕСКИХ АГГЛЮТИНАЦИОННЫХ БРУЦЕЛЛЕЗНЫХ КОЛЬЦЕВЫХ ПРОБ

З. КИШШ

Иммунодиффузионный и иммуноэлектрофоретический анализы образцов молочной сыворотки, изученных агглютинационной бруцеллезной кольцевой пробой (АБКП), указали на наличие трех видов иммуноглобулина против антибычьей Ig сыворотки. АБКП-отрицательные пробы содержали относительно малое количество одного компонента IgG, обозначенного IgG_5 и редко IgM. Специфически и неспецифически реагирующие АБКП-положительные образцы сыворотки содержали в большем количестве IgG_3 и чаще IgG_1 и IgM. Таким образом положительная АБКП была связана с повышением количества иммуноглобулина, особенно, если реакция была неспецифической. Стимулированные специфическим антигеном антитела образуют специфические связи с гомологическим антигеном, но есть основание предполагать, что повышение уровня иммуноглобулина, вызванное неспецифическими стимулами, является причиной связей, реализирующихся в неспецифических АБКП. Соответственно, если на основании истории клиники и эпизоотологических соображений положительные АБКП сомнительны, серологические пробы нужно проводить не только с кровью, но и с молочной сывороткой. Только такими дополнительными исследованиями можно с достоверностью выявить бруцеллезное положение в хозяйстве.

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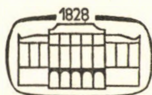

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DIE WIRKUNG EINES ANTIBIOTIKUMFREIEN FUTTERERGÄNZUNGSPRÄPARATES AUF DIE DURCH *ESCHERICHIA COLI* VERURSACHTEN ERKRANKUNGEN UND DIE ENTWICKLUNG VON KÄLBERN UND FERKELN

Von

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(Eingegangen am 12. März 1972)

In letzter Zeit wurde in der tierärztlichen Praxis häufiger festgestellt, daß sich die Heilwirkung einiger Antibiotika bedeutend verringert hat. Der Grund dieser Tatsache ist in der Resistenz und Kreuzresistenz zu suchen, die sich in den Bakterien entwickelt haben. Dies bezieht sich insbesondere auf die bei Magen- und Darmentzündungen per os angewandten Antibiotika, die wegen ihrer nutritiven Wirkung ins Futter gemischt werden. Sie fördern in subtherapeutischen Dosen Entwicklung, Gewichtszunahme und Leistung der Tiere sowie die Futterverwertung.

Bekannt sind die Anregungen in Europa, zu denen es in England, der Bundesrepublik Deutschland und der Schweiz nach dem Erscheinen des Swann- und Kewitz- (Zerle, 1969) bzw. des Jouy-en-Josas-Symposium (Sojka, 1971) Berichte gekommen ist. Aufgrund dieser Berichte wurde in England, mit verschiedenen gesundheitlichen und rechtlichen Argumenten die Verwendung von Antibiotika in Mischfuttern untersagt. Wahrscheinlich werden in Zukunft nur jene Antibiotika zugelassen, die aus dem Verdauungskanal nicht resorbiert und außerdem weder in der Human- noch in der Veterinärmedizin verwendet werden. England ist bei dieser Anregung führend, sein Beispiel ist ermutigend.

Nach den erwähnten Arbeiten sind in der ganzen Welt Forschungen angestellt worden, um Stoffe, Verbindungen und Präparate herzustellen, die dem Futter beigemischt, eine ähnliche oder noch bessere nutritive Wirkung entfalten als die Antibiotika, die außerdem zur Vorbeugung und Heilung von Magen- und Darmentzündungen infektiösen oder diätetischen Ursprungs eventuell geeignet wären und in gewisser Hinsicht die Antibiotika ersetzen könnten. (Brisson, 1963; Davis und Mitarb., 1968; Jacksick, 1963; Kockmann, 1968; Nozaki und Mitarb., 1969).

Die intensive Kälberaufzucht und -mast in Großbetrieben in Verbindung mit der gesteigerten Verfütterung von Konzentraten an Hochleistungskühe und Mastrinder verursacht häufig Verdauungsstörungen in den Vormägen. Diese Verdauungskrankheiten treten zumeist in Form von mangelnder Freßlust, Pansenathonie, rezidivierender Tympanie usw. auf und können mit dem durch die dänische Firma BIOFAC A/S (Kopenhagen) in den Handel gebrachten Präparat Testmix 232 (Stimulex) erfolgreich behandelt werden. Testmix 232 enthält keine Heilstoffe. Es besteht aus Stärke, Dextrose, Hefe, verschiedenen Eiweißen, Peptiden, Aminosäuren usw., enthält ein auf spezielle Art hergestelltes und pulverisiertes steriles Pansensaftextrakt, ist also eigentlich ein das Wachstum einzelner in Verdauungstrakt lebender Bakterien förderndes gutes Nährbodenkonzentrat.

Auffallend war die folgende Beobachtung: Das Präparat wurde einige Tage hindurch einem an Durchfall leidenden jungen Kalb verabreicht. Die mikrobiologische Diagnose hatte *coli*-Dysenterie festgestellt. Die Krankheit, die auf Antibiotika schlecht ansprach, heilte in drei Tagen aus (Kunffy und Tangl, 1967).

Aufgrund dieser Beobachtung wurde versucht, mit Testmix 232 die bei Kälbern und Ferkeln vorkommenden und auf verschiedene Ursachen zurückzuführenden Krankheiten der Verdauungsorgane zu behandeln. Sowohl die an Kälbern als auch die an Ferkeln durchgeführten Versuche fanden immer in demselben Gutsbetrieb statt, die an den Versuchs- und Kontrollgruppen parallel unternommenen Versuche wurden immer gleichzeitig durchgeführt.

Methode und Ergebnisse

Versuche mit Kälbern

Testmix 232 wurde zur Behandlung von neugeborenen Kälbern verwendet, die an von *Escherichia coli* Stämmen verursachte Dysenterie erkrankt waren. Die Krankheit wurde mittels mikrobiologischer Untersuchungen der Fäzes kontrolliert. Die versuchsergebnisse sind in Tabelle I zusammengefaßt.

Tabelle I

Behandlung von neugeborenen Kälbern
(*E. coli*-Dysenterie)

Anzahl der Tiere	Behandlung		Erkrankte Tiere		Verendete Tiere		Durchschnittliches Lebendgewicht in kg am 90. Tag
	prophylaktisch	therapeutisch	Anzahl	%	Anzahl	%	
1383	—	Neo-Te-Sol*	521	38	124	23	104,3
1226	—	Testmix 232	496	40	26	5	108,4
2609			1017	39			(+4,1)
40	Neo-Te-Sol	Neo-Te-Sol	13	32	5	38	105,2
40	Testmix 232	Testmix 232	—	—	—	—	110,6
							(+5,4)

Von 2609 Kälbern erkrankten 1017 (39%). Von den kranken Tieren wurden 521 mit Neo-Te-Sol,* 496 mit Testmix 232 behandelt. Von den mit dem Antibiotikum behandelten Tieren verendeten 124 (23%), von den mit Testmix 232 behandelten lediglich 26 (5%). Nach der prophylaktischen und therapeutischen Anwendung von Testmix 232 traten keine Erkrankungen auf. Die Tiere, denen Testmix 232 verabreicht worden war, wiesen bis zum 90. Lebenstag eine um 4.1—5.4 kg größere Gewichtszunahme auf als die Kontrolltiere.

Therapeutisch erhielten die Kälber 30 g Testmix 232 pro Tier und Tag, 1—3 Tage hindurch, prophylaktisch 10—20 g Testmix 232 pro Tag und Tier

* Gemisch von Neomycin und Oxytetracyclin

10 Tage hindurch (JUHÁSZ und Mitarb., 1969 und 1970). Die besten Ergebnisse erhielten wir, wenn die Behandlung schon am ersten oder zweiten Tag begonnen worden war. Dabei darf natürlich die Behebung der Mängel in der Tränkungs- und Fütterungstechnologie sowie der Haltungshygiene keinesfalls vergessen werden.

Testmix 232 enthält — wie bereits erwähnt — steriles Pansensaftextrakt. In unserer unlängst durchgeführten Versuchsreihe haben wir — obige Ergebnisse in Betracht ziehend — an Kälbern mit Testmix 232 und mit sterilem Pansenextrakt allein eingehendere Untersuchungen angestellt, deren Verlauf in Tabelle II dargestellt ist.

Tabelle II

Kälberaufzuchtversuch mit Testmix 232 (Gr. 1) und sterilem Pansensaftextrakt (Gr. 2)

Gruppe und Anzahl der Tiere	Kontrolle (Gr. K)	Gruppe 1	Gruppe 2
	15	20	20
Zeitdauer	14 — 84 Tage (2. — 13. Wochen)		
Lebendgewicht (Durchschnitt)	Anfang 40,6 kg — Ende 104,0 kg		
Haltung	frei beweglich		
Fütterung	Tränke aus Magermilchpulver + Wirkstoffkonzentrat Kraftfutter ab 3. Woche → max. 1,6 kg Heu ad libitum		
Zusätze			
2. Woche	—	4 Tage lang täglich 20 g Testmix 232	4 Tage lang täglich 200 mg Pansenextrakt
6. Woche	—	Testmix 232	Pansenextrakt
		in Magermilchpulver gemischt	

Das Tiermaterial des Versuches bestand aus 55 Kälbern der Rasse »Ungarisches Fleckvieh«, die für eine spätere Mast vorgesehen waren. Der Versuch dauerte 84 Tage von der 2. bis zur 13. Lebenswoche der Kälber. Die Tiere konnten sich uneingeschränkt bewegen. Die Fütterung bestand wie üblich aus Magermilchpulver, von dem den Kälbern in der 1. Versuchswoche 660 g, in der 2. 770 g und ab der 3. Versuchswoche 880 g in 6—8 l Wasser aufgelöst verabfolgt wurden. Das Magermilchpulver war mit einem Vitamin- und Mineralkonzentrat komplettiert. Hierzu wurden ab der 3. Lebenswoche Heu ad libitum und Kraftfutter bis zu einer maximalen Menge von 1.6 kg je Tier und Tag gegeben. Das Kraftfutter bestand aus 50% Haferschrot, 14% Gerstenschrot, 20% Leinkuchenmehl, 8% Sojaschrot, 6% Trockenschnitzel, 1%

Mineralkonzentrat und 1% Vitaminpremix. Diese Futtermischung enthielt 146 g verdauliches Eiweiß und 632 g Stärke-Einheiten je kg.

Die Kälber wurden in 3 Gruppen eingeteilt, von denen die Tiere der Gruppe 1 (Gr. 1) bzw. der Gruppe 2 (Gr. 2) vom Anfang der 2. und der 6. Lebenswoche an 4 Tage lang täglich 20 g Testmix 232 (Gr. 1), oder 200 mg Pansenextrakt (Gr. 2) in Magermilchpulver gemischt erhielten. Die Ergebnisse des Versuches sind in Tabelle III zusammengefaßt. Bei dieser Fütterung er-

Tabelle III

Durchschnittliche Gewichtsentwicklung und Futterverwertung im Kälberversuch zur Prüfung der Wirksamkeit von Testmix 232 (Gr. 1) und sterilem Pansenextrakt (Gr. 2) (2.—13. Woche)

Gruppe	Anfangsgewicht kg	Endgewicht kg	Gesamtzunahme		Tageszunahme g	Magermilchpulver je kg Zuwachs	
			kg	%		kg	%
Gr. K	41,6	103,3	61,7	100,0	702	1,610	109,3
Gr. 1	40,2	106,8	66,6(+4,9)	109,2	748	1,468	100,0
Gr. 2	40,5	105,0	64,5(+2,8)	104,4	723	1,534	104,5

reichte die Kontrollgruppe (Gr. K) nach 13 Wochen ein durchschnittliches Gewicht von 103,3 kg, die Gr. 1 106,8 kg und die Gr. 2 105,0 kg. Nach 12 Wochen war gegenüber der Gr. K das Wachstum von Gr. 1 um 9,2% und das von Gr. 2 um 4,4% besser. Im Verlaufe des Versuches wurde in der Gr. K eine mittlere tägliche Zunahme von 702 g, in der Gr. 1 von 748 g und in der Gr. 2 von 723 g erreicht. Die Unterschiede in der Gewichtsentwicklung zwischen den drei Gruppen sind nicht groß. Die Differenzen sind statistisch nicht gesichert. Die Zunahmen waren in der Testmix 232 Gruppe (Gr. 1) am Größten, während sie in der Pansenextrakt Gruppe (Gr. 2) geringer waren.

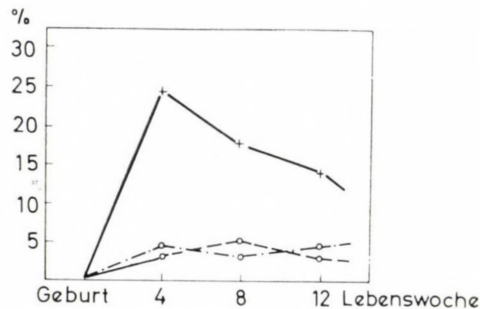

Abb. 1. Durchfallhäufigkeit bei Kälbern in % des Versuchsdauer. —○— Stimulex; —△— Pansenextract; —×— Kontrolle

Auffälligere Differenzen traten bei der Futterverwertung auf, wobei die Gr. 1 auch hier an der Spitze steht. In der Gr. K war die Futterverwertung um 9,3%, in der Gr. 2 um 4,5% schlechter. Beim Verbrauch an verdaulichem Eiweiß und Stärkeeinheiten je kg Zuwachs zeigt sich eine leichte Überlegenheit der Tiere, die Testmix 232 und Pansenextrakt erhalten hatten. So betrug der Verbrauch an Nettoenergie für 1 kg Zuwachs in Gr. 1 1423 und in Gr. 2 1335 Stärkeeinheiten. Diese Differenz ist mit einer Irrtumswahrscheinlichkeit von P = 10% gesichert.

Abbildung 1 zeigt, daß Testmix 232 (Stimulex) und Pansenextrakt auch die Durchfallhäufigkeit herabsetzt. Während bei den Kontrolltieren ohne Zusatz die Zahl der Durchfälle, auf die Zahl der Futtertage bezogen, bis um 24% stark zunimmt, kann durch Stimulex- oder Pansenextrakt-Behandlung — also bei Gr. 1 und 2 — die Durchfallhäufigkeit auf konstantem Niveau von 3—5% gehalten werden.

Versuche mit Ferkeln

Aufgrund dieser Ergebnisse wurde Testmix 232 auch zur Behandlung der *E. coli*-Dysenterie und der Ödemkrankheit bei Ferkeln verwendet (Juhász, Kunffy und Bóné, 1969, 1971). Zweifellos verursacht *E. coli* in unseren Großbetrieben bei Ferkeln die meisten Erkrankungen an Magen- und Darmentzündungen bakteriellen Ursprungs. Diese Erkrankungen haben außerordentliche Verluste zur Folge. *E. coli* spielt bei der allgemeinen achtwöchigen Absetzungstechnologie der Ferkel eine bedeutende Rolle, die in drei Gruppen unterteilt ist, und zwar im Alter von einer Woche (*E. coli*-Dysenterie), im Alter von etwa drei Wochen (Weiße Ruhr) und zur Zeit des Absetzens (Ödemkrankheit). Die im Alter von etwa drei Wochen auftretende weiße Ruhr kommt allerdings bei uns in Ungarn meist nur vereinzelt vor und ist von viel geringerer Bedeutung als die Krankheitsbilder der Neugeborenen und Abgesetzten (Szent-Iványi, 1970).

Die Untersuchungen haben bewiesen, daß in der Pathogenese der durch *E. coli* verursachten Erkrankungen bestimmte, sogenannte enteropathogene (hämolysierende) *E. coli*-Typen eine bedeutende Rolle spielen. Untersuchungen, die die quantitativen und qualitativen Änderungen der Darmmikroflora des gesunden Schweines feststellten, gaben klare Antwort auf die Frage, inwieweit die mit dem Alter zyklisch zusammenhängenden Veränderungen der normalen Darmflora die Disposition der Tiere für den durch E. coli verursachten Erkrankungen beeinflussen. Die Untersuchungen zeigten, daß im Gleichgewicht der eine große Stabilität aufweisenden Darmflora, für das ein Übergewicht der Laktobazillen und Enterokokken charakteristisch ist, eine relative Vermehrung der Kolibakterien in den ersten Tagen nach der Geburt und

dem Absetzen zu beobachten war. In Anwesenheit enteropathogener *E. coli*-Typen sind Ferkel, insbesondere wenn auch noch haltungstechnologische und hygienische Mängel bestehen, für durch *E. coli*-Bakterien verursachte Erkrankungen außerordentlich empfänglich.

Tabelle IV

Behandlung von neugeborenen Ferkeln
(*E. coli*-Dysenterie)

Anzahl der Tiere	Behandlung		Erkrankte Tiere		Verendete Tiere		Durchschnittsgewicht in kg am 80. Lebenstag
	prophylaktisch	therapeutisch	Anzahl	%	Anzahl	%	
60	Kontrollgruppe		26	43	12	20	18,6
7123	—	Neo-Te-Sol	2067	29	780	11	21,5
7790	—	Testmix 232	2051	26	103	1,3	24,6
1260	Testmix 232	Testmix 232	221	17	24	1,8	25,8

Tabelle IV zeigt die nach Behandlung der *E. coli*-Dysenterie bei neugeborenen Ferkeln ermittelten Daten. In Betrieben mit an *E. coli*-Dysenterie erkrankten Ferkeln, bei denen eine Behandlung überhaupt nicht (Kontrollgruppe) oder nur unzulänglich durchgeführt wurde, kann mit einer 43%igen Erkrankung und einem 20%igen Ausfall gerechnet werden. Das Durchschnittsgewicht der Ferkel dieser Kontrollgruppe betrug im Alter von 70 Tagen nur 18.6 kg. In den beiden Gruppen von 7123 bzw. 7790 Ferkeln, die therapeutisch behandelt wurden, wiesen 29% bzw. 26% der Tiere Erkrankungen auf, wobei die Behandlung mit Neo-Te-Sol einen 11%-igen Ausfall sowie im Alter von 70 Tagen ein durchschnittliches Lebendgewicht von 21,5 kg/Tier, die Behandlung mit Testmix 232 hingegen nur einen Ausfall von 1,3% sowie in demselben Alter ein durchschnittliches Lebendgewicht von 24,6 kg/Tier .

Bei prophylaktischer Verabreichung von Testmix 232 und anschließender therapeutischer Behandlung der erkrankten Tiere registrierten wir sogar nur in 17% Erkrankungen und einen Ausfall von 1,8%. Das durchschnittliche Lebendgewicht im Alter von 70 Tagen betrug immerhin 25,8 kg.

Die prophylaktische und therapeutische Behandlung der neugeborenen Ferkel wurde folgendermaßen durchgeführt: Bei der therapeutischen Behandlung wurden pro Ferkel 2 g Testmix 232 mit etwa 3 g Milchpulver vermischt in 5—10 ml lauwarmen Wasser suspendiert und nasal verabreicht. Die Behandlungsdauer von 1—3 Tagen ist bei nicht schwer erkrankten Tieren ausreichend. Prophylaktisch genügt dagegen eine einmalige Behandlung. Bewährt haben sich auch folgende Verabreichungsformen: Das Präparat wird mit etwa 6 g Marmelade oder Honig vermischt auf die Zunge der Tiere gestrichen oder die unvermischte Menge von 2 g von Hand eingegeben und vom Tier abgesaugt.

Tabelle V zeigt die Ergebnisse der therapeutischen und prophylaktischen Behandlung der während der Absetzenszeit auftretenden Ödemkrankheit bei Ferkeln. In den untersuchten Betrieben trat diese Krankheit durchschnittlich bei 65% der Tiere auf; die Mortalität machte 20—30% aus. Mit hohen Antibiotikum-dosen konnte die Mortalität auf 11%, mit Testmix 232 sogar auf 1,6% verringert werden. Ähnliche Ergebnisse erreichten wir bei prophylaktischer und therapeutischer Behandlung mit Testmix 232, aber die Zahl der

Tabelle V

Behandlung von Ferkeln beim Absetzen
(Ödemkrankheit)

Anzahl der Tiere	Behandlung		Erkrankte Tiere		Verendete Tiere		Durchschnittsgewicht in kg am 70. Lebenstag
	prophylaktisch	therapeutisch	Anzahl	%	Anzahl	%	
64	Kontrollgruppe		51	79	18	28	—
135	—	Neo-Te-Sol	105	77	15	11	—
187	—	Testmix 232	81	43	3	1,6	—
324	Neo-Te-Sol	Neo-Te-Sol	165	50	36	11	—
247	Testmix 232	Testmix 232	65	26	7	2,8	—
197	Kontrollgruppe		97	49	40	20	19,5
273	Neo-Te-Sol	—	116	42	15	6	24,6
265	Testmix 232	—	58	22	12	4,5	27,8

Erkrankungen (65 Tiere, 26%) und der Verendeten (7 Tiere, 2,8%) war geringer. Bei allgemeinen prophylaktischen Behandlung verringerte sich die Zahl der Erkrankungen bedeutend (22%) und der Ausfall betrug nur 4,5%. Das durchschnittliche Lebendgewicht im Alter von 70 Tagen war um 8,3 kg höher als das der Kontrolltiere.

Die Behandlung der Ödemkrankheit zur Zeit des Absetzens der Ferkel wurde folgendermaßen durchgeführt: In das Tagesfutter wurden 5—6 g Testmix 232 pro Tier eingemischt und den Ferkeln zuerst verabreicht, um sicher zu sein, daß das Präparat von allen Tieren aufgenommen wird. Bei der prophylaktischen Behandlung wurde ähnlich gehandelt. In diesem Fall gaben wir den Ferkeln pro Tier und Tag 3—4 g Testmix 232 mit dem Futter vermischt 3 Tage vor bis 6 Tage nach dem Absetzen.

In weiteren Versuchen wurde untersucht, ob Testmix 232 enthaltendes Ferkelstarterfutter die Gewichtszunahme und Futteraufnahme der Ferkel beeinflußt. Das Futter war pelletiertes Starterfutter von bester Qualität. Es ist anzunehmen, daß diesem Futter von Vornherein schon Kupfer zugesetzt war, um den Wachstumseffekt zu fördern.

Die Ferkeln wurden in drei Gruppen eingeteilt. Jede Gruppe bestand aus etwa 150 Ferkeln. Die Kontrollgruppe (Gr. K) erhielt Starterfutter ohne Testmix 232. Die Gr. 1 erhielt Futter mit 1.25%, die Gr. 2 mit 2,50% Testmix 232. In allen Gruppen waren Gesundheitszustand, Stallhygiene usw. während der ganzen Versuchsperiode gut. Es wurde Trockenfutter ohne Antibiotika verabreicht, als die Ferkel 2 Wochen alt geworden sind.

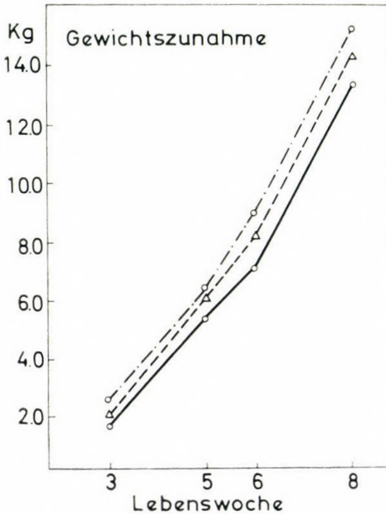

Abb. 2. Gewichtszunahme während der Versuchsperiode. —●— Kontrolle —△— Stimulex 1,25%; —○— Stimulex 2,50%

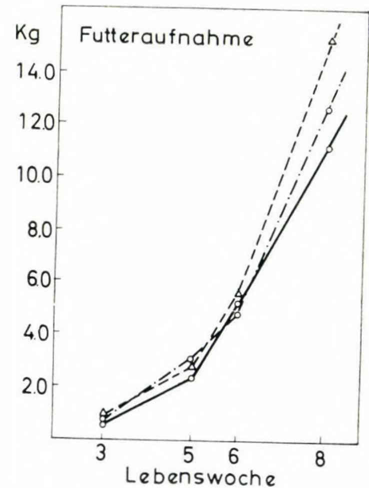

Abb. 3. Durchschnittwerte der Futteraufnahme während der Versuchsperiode. —●— Kontrolle; —△— Stimulex 1,25%; —○— Stimulex 2,50%

Die Ergebnisse sind in Tabelle VI zusammengefaßt. Aus diesem Material gehen Gewichtszunahme und Futteraufnahme hervor, angeführt in absoluten Zahlen und Prozentzahlen angegeben für die Tiere im Alter von 3, 5, 6 und 8 Wochen. Aus der Tabelle ist ersichtlich, daß die Ferkel der Gr. 1 während des Versuches im Durchschnitt 0.68 kg, also um 10% mehr zugenommen haben als die Tiere der Gr. K, und die Tiere der Gr. 2 1,08 kg, also um 15% mehr als die Kontrollgruppe. Demzufolge zeigte Gr. 1 durchschnittlich einen um 25% höheren, und die Ferkel der Gr. 2 einen um 12% höheren Futterverbrauch als die Gr. K.

Abbildung 2 veranschaulicht die Gewichtszunahme in der Versuchsperiode (2—8. Woche). Wie ersichtlich, zeigt Gr. 2 (2,5% Testmix 232) das beste Ergebnis. Den Durchschnittswert der Futteraufnahme während des Versuchs zeigt Abbildung 3. In der 3., 6. und 8. Woche war der Futterverbrauch bei Gr. 1 (1,25% Stimulex) am höchsten.

Abbildung 4 zeigt den Einfluß von Testmix 232 auf die durchschnittliche Gewichtszunahme von Ferkeln im Alter von 2—8 Wochen. Während Gr.

Tabelle VI

Einfluß von Testmix 232 (Stimulex) auf die Ferkel (im Starterfutter gemischt)
(Jede Gruppe: 150 Ferkel)

Alter (Wochen) (W.)	Kontrolle (Gr. K)		Gruppe 1 (1,25% T*)				Gruppe 2 (2,50% T*)			
	Gewichtszunahme	Futteraufnahme	Gewichtszunahme		Futteraufnahme		Gewichtszunahme		Futteraufnahme	
	kg	kg	kg	%	kg	%	kg	%	kg	%
3	1,81	0,53	2,02 +0,20	10,0	0,81 +0,28	33,0**	2,22 +0,41	18,3	0,56 +0,03	5,6
5	5,43	2,25	6,06 +0,63	10,0	2,83 +0,58	20,3***	6,21 +0,77	12,4	2,86 +0,61	21,3
6	7,24	4,48	8,07 +0,83	10,6	5,52 +1,04	18,7	8,87 +1,63	18,4	4,92 +0,44	8,9
8	13,08	11,11	14,16 +1,08	7,7	15,18 +4,06	26,8***	14,59 +1,54	10,6	12,66 +1,55	12,2
Durchschnitt (D.)	6,89	4,59	7,57 +0,68	9,6	6,09 +1,50	24,7***	7,97 +1,08	14,9	5,25 +0,66	12,0
Gewichtszunahme D. kg (2.–8. W.)	2,09		2,36/W. +0,27 (11,5%)				2,42/W. +0,33 (14,0%)			
Futteraufnahme D. kg (1.–8. W.)	1,59/W.		2,18/W. (+0,59; 26,8%)				1,82/W. (+0,23; 11,7%)			

T* Testmix 232; ** signifikant (P=0,05); *** signifikant (P=0,10)

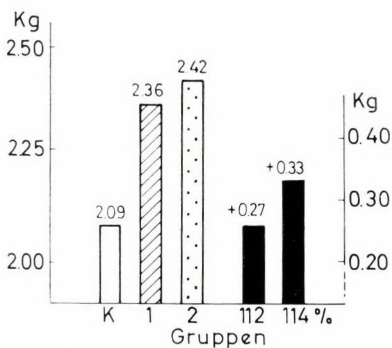

Abb. 4. Einfluß von Stimulex auf die durchschnittliche Gewichtszunahme von Ferkeln zwischen 2–8. Wochenalter. K, Kontrolle; 1, Stimulex 1,25%; 2, Stimulex 2,50%; + Verbesserung

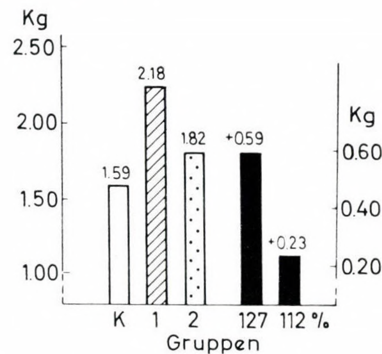

Abb. 5. Einfluß von Stimulex auf die durchschnittliche Futteraufnahme von Ferkeln zwischen 2–8. Wochenalter. K, Kontrolle; 1, Stimulex 1,25%; 2, Stimulex 2,50%; + Verbesserung

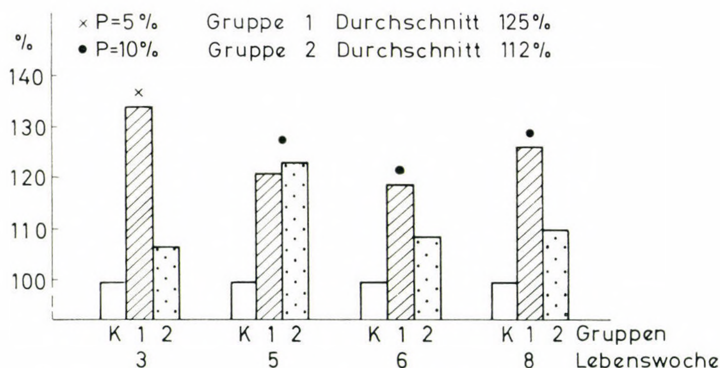

Abb. 6. Verlauf der wöchentlichen Futteraufnahme (in %) zwischen 2—8. Wochenalter. K, Kontrolle; 1, Stimulex 1,25%; 2, Stimulex 2,50%

K im Durchschnitt eine wöchentliche Gewichtszunahme von 2,09 kg aufwies, betrug diese bei Gr. 1 2,36 kg, und bei Gr. 2 2,42 kg, was einen durchschnittlichen Gewichtszuwachs von 0.27 kg (112%) bzw. 0,33 kg (114%) bedeutet. Interessant gestaltete sich der Wert der wöchentlichen durchschnittlichen Futteraufnahme (Abb. 5). Während Gr. K 1,59 kg verbrauchte, betrug der Futterverbrauch bei Gr. 1 2,18 kg, bei Gr. 2 aber nur 1,82 kg. Gr. 1 verzehrte also um 0,59 kg (127%; P = 10%) und Gr. 2 um 0,23 kg (112%) mehr Futter.

Abbildung 6 zeigt die von allen drei Gruppen in der 3., 5., 6. und 8. Woche verbrauchte Futtermenge im Verhältnis zueinander in Prozenten. Es kann festgestellt werden, daß die Ferkel der Gr. 1 im Alter von 3 Wochen um 33% mehr Futter (P=5%) gefressen haben als die Kontrolltiere. Die anderen Ergebnisse sind auch signifikant.

Besprechung

Augrund der bisherigen Versuche sind wir der Meinung, daß das Präparat Testmix 232 von BIOFAC zur Vorbeugung und Heilung der *E. coli*-Dysenterie bei Kälbern und neugeborenen Ferkeln sowie der zur Zeit des Absetzens auftretenden Ödemkrankheit bei Ferkeln mit gutem Erfolg angewendet werden kann. Durch die Vermischung einer entsprechenden Menge dieses Präparates mit dem Ferkelfutter kann die Krankheit nicht nur verhütet werden, sondern die behandelten Tiere erreichen im Alter von 70 Tagen auch ein bedeutend höheres Gewicht als die unbehandelten. Es wurde festgestellt, daß Testmix 232 bei der Behandlung der durch enteropathogene *E. coli*-Stämme verursachten Magen- und Darmentzündungen bessere Ergebnisse zeigt als die Antibiotika.

Die Resultate dieser und anderer Versuche sprechen dafür, daß die Mischung von 1.25% Testmix 232 im Ferkel-Starterfutter (2—8. Woche) eine

gute Gewichtszunahme zur Folge hat (10%) und daß die Verwendung von Testmix 232 die Futteraufnahme fördert (25)%.

Die bisher durchgeführten Versuche sind stets unter Aufsicht und strenger Kontrolle durchgeführt worden. Im Anschluß daran haben wir nach genauer Anleitung, jedoch ohne besonders strenge Kontrollmaßnahmen, die Wirkung des Testmix 232 unter Großbetriebsverhältnissen getestet. Hierfür standen uns insgesamt 192 LPG's mit 70.000 Tieren zur Verfügung, genauer gesagt mit 10.000 Kälbern und 60.000 Ferkeln. Die Resultate waren ebenso gut. Natürlich darf in diesem Zusammenhang keinesfalls die Behebung der Mängel in der Haltungshygiene und Fütterungstechnologie vergessen werden.

Abschließend möchten wir, um Mißverständnissen vorzubeugen, die Tatsache hervorheben, daß Testmix 232 (Stimulex) weder ein spezieller Wirkstoff noch ein Medikament, sondern ein Gemisch, ein Konzentrat diätetisch wirkender Stoffe ist, das in entsprechender Dosierung die im verschiedenen Lebensalter der Ferkel und der Kälber auftretenden Magen- und Darmerkrankungen in bedeutendem Grade vorteilhaft beeinflußt. Die parenterale Verwendung der Antibiotika zur Behandlung der Tiere, oder im Futter kann somit eingestellt werden. Infolgedessen kann auch die Resistenz der *E. coli*-Bazillen gegenüber den verschiedenen Antibiotika nach einer gewissen Zeitspanne eventuell wieder erlöschen.

ZUSAMMENFASSUNG

Testmix 232 (Stimulex) von BIOFAC A/S (Koppenhagen) ist ein Präparat, das keine Antibiotika enthält. Unsere Versuche bewiesen, daß dieses Mittel bei rechtzeitiger Anwendung und in entsprechender Dosierung die durch *E. coli* verursachten Magen- und Darmentzündungen bei neugeborenen Kälbern und Ferkeln sowie die Ödemkrankheit der abgesetzten Ferkel heilt. Das Präparat ist auch zur prophylaktischen Anwendung geeignet. Die Lebendgewichtszunahme und Futteraufnahme der Tiere, die mit Testmix 232 behandelt wurden, oder in das Starterfutter eingemischtes Testmix 232 erhielten, waren besser als die der nicht behandelten Tiere.

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THE VALUE OF THE O_1 AND R SALMONELLA PHAGE TESTS FOR ROUTINE LABORATORY EXAMINATIONS

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The demonstration of *Salmonellae* (referred to hereafter as S.) in foodstuffs of animal origin is one of the most critical tasks of veterinary food control laboratories round the world. Since the detection of S. contamination is equally necessary in food that is imported or exported, several competent bodies, among them the International Organisation for Standardisation (ISO), have made considerable efforts to promote research work on developing uniform procedures of assay with a view to their eventual adoption as standard methods in different countries. With meat and meat products, precisely because of their rapid perishability, the emphasis has been on rapid methods of S. demonstration, with reliability as the other major criterion.

As *Salmonellae* belong to the family *Enterobacteriaceae*, their differentiation from other Gram-negative genera of the family is often difficult, either owing to close antigenic relationship or similar behaviour in biochemical tests. In a typical infection S. are scarcely distinguishable from strains of *Citrobacter* spp. and the *Arizona* group. If such organisms especially (*Escherichia freundii*, Bethesda, Ballerup) occur in the test material, the customary assays for S. may be misleading and therefore sure diagnostic methods have to be utilised to prevent erroneous condemnation of a product.

The value of the O_1 and R phage tests, especially for complementary laboratory examinations of meat, was examined earlier (TAKÁCS, 1964) on a total of 455 model S. strains representing 68 serotypes and belonging to the O-antigen groups of A—T. Of these 437 belonged to the most frequent O-groups A—E_4, having only 18, mostly single strains, to represent the O-groups F—T. Phage O_1 lysed 95.4% of these strains, only 4.6% of them were resistant to it while 1.7% of all examined strains was resistant to both O_1 and R phages.

In certain cases all internationally prescribed S.-tests have to be performed, chiefly because of export regulations. These include not only the ISO Directions but also the prescriptions under the National Formulary XIII and U.S.P. XVIII. The performance of the O_1 phage test for S. detection has been ordered for all meat inspection laboratories under our competence for several years now.

Phage O_1 was isolated and described for the first time in 1943, in connection with examinations of *S. paratyphi B* (FELIX and CALLOW, 1943). According to literary data it lyses the various salmonella species to different degrees (89—99.9%), and produces a minor (0.5—14%) nonspecific lysis of certain *Citrobacter* and *Escherichia* strains (SEIDEL, 1956; THAL, 1957; KIESELWALTER, 1958; COMBIESCO et al., 1958; KUNTER and LEHMANN, 1962; SCHALL, 1962; WESSELINOFF, 1961). Since it lyses the diphasic Arizona strains as well (HERSHEY et al., 1943), the phage plays an important role in the identification of this genus. No lytic activity has ever been observed outside the *Enterobacteriaceae*. SEIDEL (1956) recommended additional testing with a R phage for strains resistant to O_1 (R-phage tests were introduced in East Germany at the Central Phage Typing Laboratory, Wernigerode).

Experimental

Method

Phage O_1 was propagated on cultures of *S. paratyphi* B strain 309 (further on referred to as B 309). An inoculum for propagation containing 1 ml phage and 0.2 ml of a 24-hour broth culture of strain B 309 was added to 9 ml. liquid medium consisting of meat extract + glucose + yeast extract + peptone (TAKÁCS, 1960). The tube cultures were incubated for 6 hours at 37 °C, then stored overnight (18—24 hours) at +4° C and next morning centrifuged at 3000 r.p.m. for 30 minutes. The supernatant was withdrawn with a pipette and heat-treated at 60° C for 30 minutes to kill the surviving B 309 organisms. Survival was checked by streaking on slant agar. The titre of the phage was determined prior to use as the highest phage dilution still producing easily visible plaques on agar plate cultures of strain B 309.

The phage count can also be determined by the pour-plate technique with agar overlay (HERSHEY et al., 1943; PALYUSIK, 1960; 1963). Two per cent agar solution was poured into a Petri dish and after it had solidified the overlay, containing the phage, the strain B 309 and 1% agar, was poured on top. The Petri dishes were then incubated for 16—18 hours at 37 °C. As in this system each phage particle grows in isolation, each individual plaque corresponds to one phage. Others (SEDLÁK and RISCHE, 1961) poured 0,2 ml of a 2-hour broth culture of strain B 309 on the surface of the agar plate and, after drying, placed one drop each from serial tenfold dilutions of the phage (up to 10^{-10}) on a plate and incubated it for 5 hours at 37° C. Phage suspensions used for routine tests should be sufficiently concentrated to produce plaques up to a dilution of 10^{-9} and the critical test dilution (CTD = the highest phage dilution still capable of producing confluent lysis of strain B 309) should be 10^{-3}—10^{-5}.

In the present studies undiluted phage was used. The bacterial strain used for phage production was inoculated into about 6 ml broth and incubated for 16—24 hours at 37° C. One loopful (loop diameter = 4 mm) of the culture was transferred to three sites of an agar plate (meat extract + glucose + yeast

Table I

Behaviour of 4618 *Salmonella* strains towards phages O_1 and R.

	Salmonella species	No. of strains examined	Phage-resistant		Phage-sensitive	
			No.	%	No.	%
More than 100 isolations	*S. cholerae-suis var. Kunzendorf*	1.442	53	3.67	1.389	96.33
	S. anatum	958	861	89.87	97	10.13
	S. derby	490	57	11.63	433	88.27
	S. typhi-murium	309	2	0.67	307	99.33
	S. manhattan	281	27	9.60	254	90.40
	S. abony	135	2	1.48	133	98.52
	S. meleagridis	132	112	91.52	20	8.40
	S. panama	122	9	7.37	113	92.63
10—100 isolations	*S. muenchen*	62	28	45.16	34	54.84
	S. cholerae-suis	57	6	10.52	51	89.48
	S. london	49	28	57.14	21	42.86
	S. gallinarum	45	14	31.11	31	68.89
	S. heidelberg	44	14	31.81	30	68.19
	S. enteritidis	41	25	60.97	16	39.03
	S. bovis-morbificans	32	—	—	32	100.00
	S. bareilly	29	3	10.34	26	89.66
	S. dublin	29	15	51.34	14	48.66
	S. infantis	29	6	20.68	23	79.32
	S. typhi-suis	28	9	32.14	19	67.86
	S. typhi-murium var. koppenhagen	26	1	3.84	25	96.16
	S. typhi-suis var. Voldagsen	24	12	50.00	12	50.00
	S. bredeney	23	—	—	23	100.00
	S. newport	21	1	4.76	20	95.24
	S. minnesota	19	11	57.89	8	42.11
	S. abortus-bovis	12	—	—	12	100.00
	S. kingston var. koppenhagen	12	11	91.66	1	8.34
	S. muenster	11	11	100.00	—	—
	S. worthington	11	6	54.54	5	45.46
3—10 isolations	*S. westhampton*	10	10	100.00	—	—
	S. newington	9	9	100.00	—	—
	S. stanleyville	8	—	—	8	100.00
	S. taksony	8	3	37.50	5	62.50
	S. 4.12 d.—	8	—	—	8	100.00
	S. isangi	7	3	42.85	4	57.15
	S. reading	7	1	14.28	6	85.72
	S. thompson	7	—	—	7	100.00
	S. cholerae-suis var. Amerika	6	—	—	6	100.00
	S. senftenberg var. newcastle	6	6	100.00	—	—
	S. schleissheim	6	6	100.00	—	—
	S. cubana	5	1	20.00	4	80.00
	S. give	5	4	80.00	1	20.00
	S. brandenburg	4	—	—	4	100.00
	S. moscow	4	1	25.00	3	75.00
	S. senftenberg	4	4	100.00	—	—
	S. stanley	4	—	—	4	100.00
	S. abortus-ovis	3	1	33.33	2	60.66
	S. chester	3	—	—	3	100.00
	S. kottbus	3	1	33.33	2	66.66

Table I (cont.)

	Salmonella species	No. of strains examined	Phage-resistant		Phage-sensitive	
			No.	%	No.	%
	S. livingstone	3	—	—	3	100.00
	S. montevideo	3	—	—	3	100.00
	S. saint-paul	3	—	—	3	100.00
2 isolations	*S. binza*	2	2	100.00	—	—
	S. budapest	2	1	50.00	1	50.00
	S. california	2	—	—	2	100.00
	S. paratyphi-B var. java	2	—	—	2	100.00
	S. preston	2	1	50.00	1	50.00
	S. 6.7 fg 1.5	2	2	100.00	—	—
1 isolation	*S. bradford*	1	—	—	1	100.00
	S. blockley	1	1	100.00	—	—
	S. gallinarum-pullorum	1	—	—	1	100.00
	S. kapemba	1	1	100.00	—	—
	S. manila	1	1	100.00	—	—
	S. oranienburg	1	1	100.00	—	—
	group C_1 type unflagellated	1	—	—	1	100.00
Totals	64 *Salmonella* species	4.618	1.384	29.96	3.234	70.03

extract + pepton + agar + gelatine; TAKÁCS, 1960). The three inoculation sites were spaced as far apart as possible, either along a straight line or at the points of an imaginary triangle; in the latter case the bottom left point was reserved for the testing of phage O_1, bottom right for the testing of phage R and the top point for the negative control (broth culture of the strain without phage). Thus first one drop of broth culture was transferred with the loop to each inoculation site, and after drying, one drop each of the O_1 and R phage suspensions was placed on the appropriate sites with a 2 mm loop. Another method is to inoculate densely the agar surface with inocula from the broth culture of the strain; in this case one drop of the phage suspension is transferred to the plate with a Pasteur pipette after the inocula have dried. Either the results are read after incubation for 16—24 hours at 37° C, or two readings are made, the first after 6 hours incubation, the second after 18—24 hours incubation at + 4° C. Readings are performed either with a hand lens (8—10 X) in oblique light or with a colony microscope.

The presence of opaque lysis and plaques of various numbers and sizes is regarded as a positive result.

In urgent cases, rapid diagnosis can be made by transferring S.-like colonies from the selective media to Drigalski agar, either directly with a loop or after suspending in maximum 1 ml broth. One drop of phage is then applied with a Pasteur pipette and phage effect can be read after 6 hours of incubation.

Negative results cannot, however, be accepted unless confirmed by t he usua test procedure.

If there is suspicion of a *S. typhi* infection, phage Vi (I and IV) should also be included in the test, because several *S. typhi* are resistant to phages O_1 and R.

Test procedures for phage R are the same as above, except that it is grown on the propagating strains S. PB U 1233.

Material

Phages O_1 and R have been used for routine S.-diagnosis in this laboratory since 1965 and a total of 4618 S. strains were tested with them up to the end of 1970. All tests were carried out with 16-hour or 24-hour broth cultures as only these were found to be suitable for routine purposes.

Results

The 4618 S. strains tested with the phages belonged to 64 species. Phage effects on each species are summarized in Table I.

As can be seen 1384 strains (30%) were phage resistant and 3234 (70%) were sensitive. The most frequent isolate was *S. cholerae-suis* var. *Kunzendorf* (more than 1000 isolations) and seven other species — *S. anatum*, *S. derby*, *S. typhi-murium*, *S. manhattan*, *S. abony*, *S. meleagridis*, and *S. panama* —

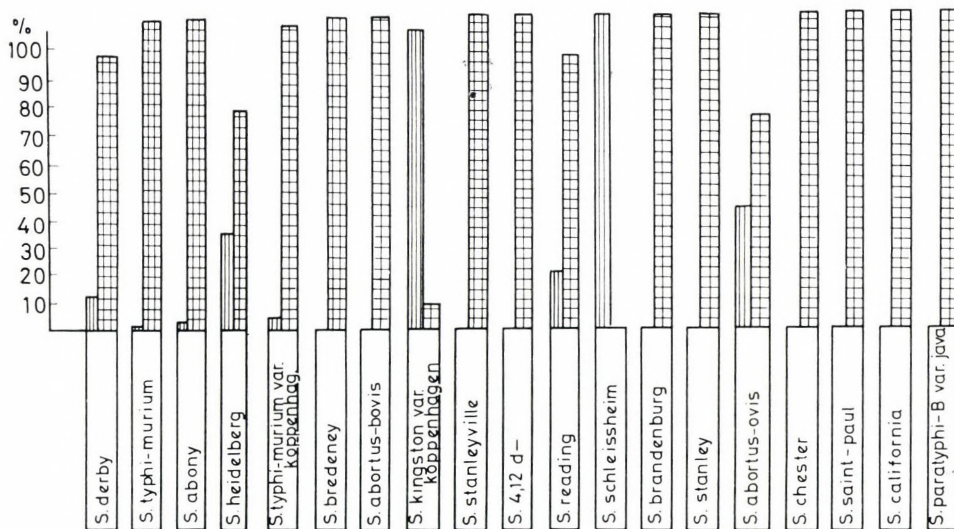

Fig. 1. The result of O_1 phage effect of *Salmonella* strains belonging to the B serological O group. (Appendix of the II table) ⊞ phage-sensitive; ||| phage-resistant

Table II

Phage effect on 4618 *Salmonella* strains of 64 species classed by serotype

Serotype	*Salmonella* species	No of strains examined	Phage-resistant		Phage-sensitive	
			No.	%	No.	%
B	*S. derby*	490	57	11.63	433	88.27
	S. typhi-murium	309	2	0.67	307	99.33
	S. abony	135	2	1.48	133	98.52
	S. heidelberg	44	14	31.81	30	68.19
	S. typhi-murium var. koppenhagen	26	1	3.84	25	96.16
	S. bredeney	23	—	—	23	100.00
	S. abortus-bovis	12	—	—	12	100.00
	S. kingston var. koppenhagen	12	11	96.66	1	8.34
	S. stanleyville	8	—	—	8	100.00
	S. 4.12 d —	8	—	—	8	100.00
	S. reading	7	1	14.28	6	85.72
	S. schleissheim	6	6	100.00	—	—
	S. brandenburg	4	—	—	4	100.00
	S. stanley	4	—	—	4	100.00
	S. abortus-ovis	3	1	33.33	2	66.66
	S. chester	3	—	—	3	100.00
	S. saint-paul	3	—	—	3	100.00
	S. budapest	2	1	50.00	1	50.00
	S. california	2	—	—	2	100.00
	S. paratyphi-B var. java	2	—	—	2	100.00
	S. preston	2	1	50.00	1	50.00
	S. bradford	1	—	—	1	100.00
C_1	*S. cholerae-suis var. Kunzendorf*	1.442	53	3.67	1.389	96.33
	S. cholerae-suis	57	6	10.52	51	89.48
	S. bareilly	29	3	10.34	26	89.66
	S. infantis	29	6	20.68	23	79.32
	S. typhi-suis	28	9	32.14	19	67.86
	S. typhi-suis var. Voldagsen	24	12	50.00	12	50.00
	S. isangi	7	3	42.85	4	57.15
	S. thompson	7	—	—	7	100.00
	S. cholerae-suis var. Amerika	6	—	—	6	100.00
	S. livingstone	3	—	—	3	100.00
	S. montevideo	3	—	—	3	100.00
	S. 6,7 *fg* 1.5	2	2	100.00	—	—
	S. oranienburg	1	1	100.00	—	—
	S. 6.7 *unflagellated*	1	—	—	1	100.00
C_2	*S. manhattan*	281	27	9.60	254	90.40
	S. muenchen	62	28	4.516	34	54.84
	S. bovis-morbificans	32	—	—	32	100.00
	S. newport	21	1	4.76	20	95.24
	S. kottbus	3	1	33.33	2	66.66
	S. blockley	1	1	100.00	—	—
D_1	*S. panama*	122	9	7.37	113	92.63
	S. gallinarum	45	14	31.11	31	68.89
	S. enteritidis	41	25	60.97	16	39.03
	S. dublin	29	15	51.34	14	48.66
	S. moscow	4	1	25.00	3	75.00
	S. gallinarum-pullorum	1	—	—	1	100.00
	S. kapemba	1	1	100.00	—	—

Serotype	*Salmonella* species	No. of strains examined	Phage-resistant		Phage-sensitive	
			No.	%	No.	%
E_1	*S. anatum*	958	861	89.87	97	10.13
	S. meleagridis	132	112	91.52	20	8.48
	S. london	49	28	57.14	21	42.86
	S. muenster	11	11	100.00	—	—
	S. westhampton	10	10	100.00	—	—
	S. give	5	4	80.00	1	20.00
E_2	*S. newington*	9	9	100.00	—	—
	S. binza	2	2	100.00	—	—
	S. manila	1	1	100.00	—	—
E_4	*S. taksony*	8	3	37.50	5	62.50
	S. senftenberg var. newcastle	6	6	100.00	—	—
	S. senftenberg	4	4	100.00	—	—
G_2	*S. worthington*	11	6	54.54	5	45.46
	S. cubana	5	1	20.00	4	80.00
L	*S. minnesota*	19	11	57.89	8	42.11
Totals	64 *Salmonella* species	4.618	1.384	29.96	3.234	70.03

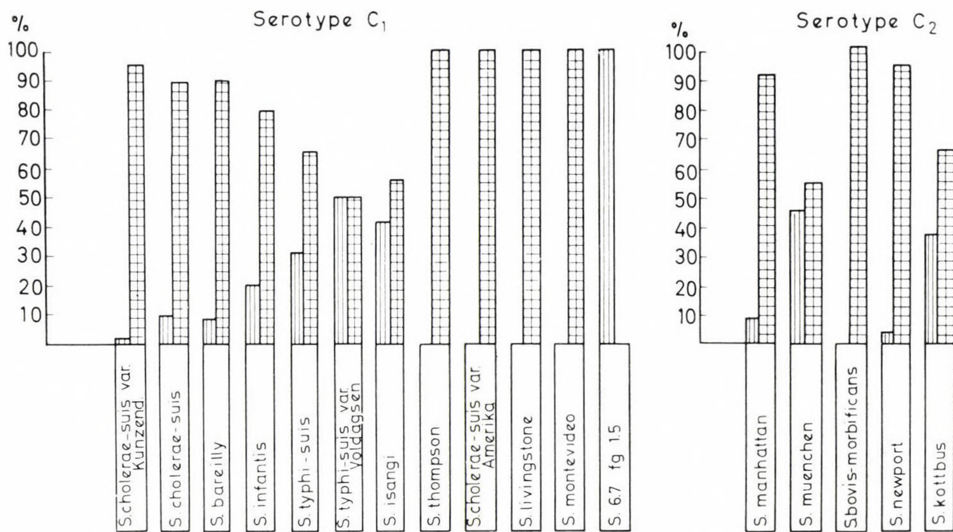

Fig. 2. The result of O_1 phage effect of *Salmonella* strains belonging to the C_1 serological O group. (Appendix of the II table) ⊞ phage-sensitive; ||| phage-resistant

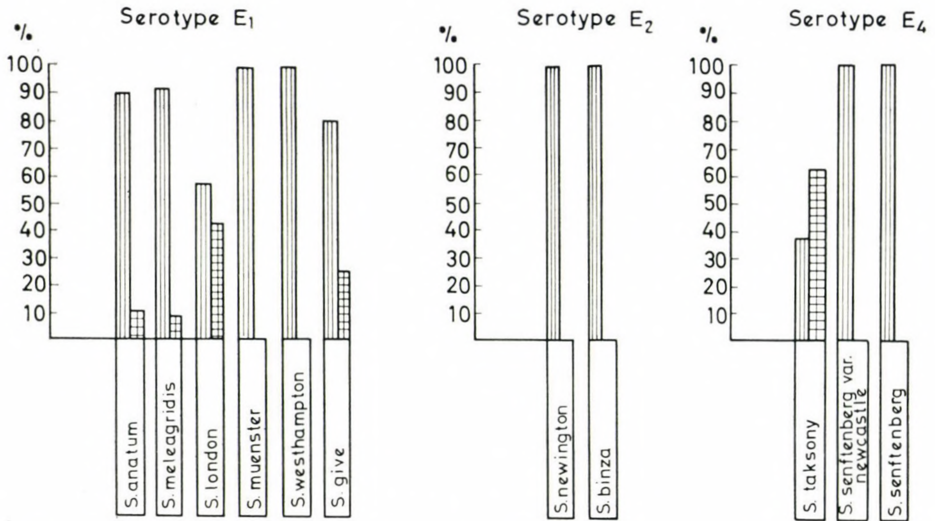

Fig. 3. The result of O_1 phage effect of *Salmonella* strains belonging to the E serological O group. (Appendix of the II table) ⊞ phage-sensitive; ||| phage-resistant

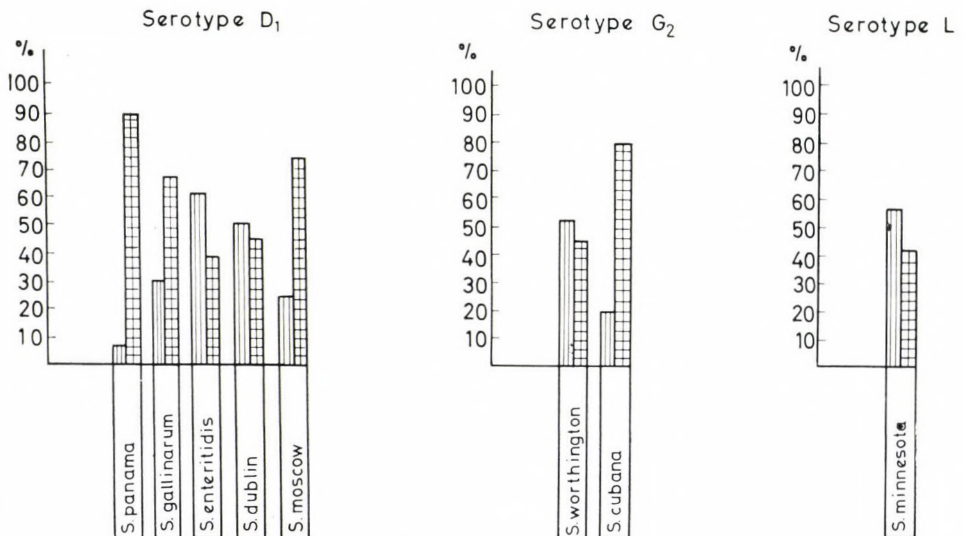

Fig. 4. The result of O_1 phage effect of *Salmonella* strains belonging to the D, G and L serological O groups. (Appendix of the II table) ⊞ phage-sensitive; ||| phage-resistant

were each isolated in more than 100 cases, 10 species in 10—100 cases, 23 species in 3—10 cases, 6 species each in two cases and 7 species each in a single case.

As to O-antigen distribution, 22 species belonged to group B, 14 to C_1, 6 to C_2, 7 to D_1, 6 to E_1, 3 to E_2, 3 to E_4, 2 to G_2 and one to L.

Species grouped according to the frequency of O-serogroups are shown in Table II, along with numerical and percentual data for phage sensitivity and resistance within each group. The proportions of phage-positive and phage-negative strains differed between the O-groups.

To better visualize the distribution of phage-sensitive and -resistant strains within species and O groups, diagrams were drawn for each serogroup (Figs 1, 2, 3, 4). Table II includes the data only of those strains of which more than two isolations were made, or two isolations only, but phage test were unequivocally positive or negative.

Table III and Fig. 5 show the distribution of phage-sensitive and -resistant strains within each O-group. As can be seen, tests with phages O_1 and R were effective with 91.2% of B, 94.2% of C_1, 85.5% of C_2, and 73.3%

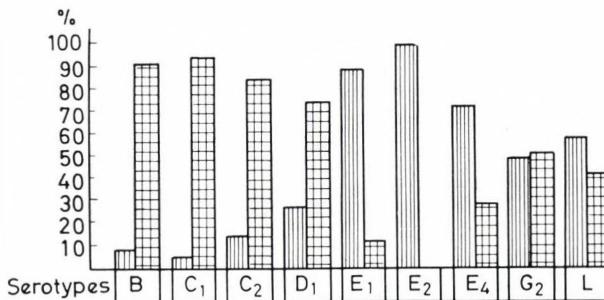

Fig. 5. Graphical presentation of data from Table 3. ⊞ % of phage-sensitive strains; ||| % of phage-resistant strains

Table III

Distribution by serotype of 1384 phage-resistant and 3234 phage-sensitive *Salmonella* strains

Serotype	No of strains examined	Phage-resistant		Phage-sensitive	
		No.	%	No.	%
B	1.106	97	8,78	1.009	91,22
C_1	1.639	95	5,80	1.544	94,20
C_2	400	58	14.90	342	85.50
D_1	243	65	26.75	178	73.25
E_1	1.165	1.026	88.06	139	11.94
E_2	12	12	100.00		
E_4	18	13	72.22	5	27.78
G_2	16	7	49.38	9	50.62
L	19	11	57.89	8	42.11
Totals	4.618	1.384		3.234	

Table IV

Numerical and percentual distribution of 3234 strains according to phages O_1 and R

Serological group O	*Salmonella* species	No. of phage sensitive strains examined	Distribution of phage sensitive strains					
			Sensitive toward phage O_1 Resistant toward phage R		Sensitive toward phage O_1 Sensitive toward phage R		Resistant toward phage O_1 Sensitive toward phage R	
			No.	%	No.	%	No.	%
B	*S. derby*	433	431	99.54	2	0.46	—	—
	S. typhi-murium	307	307	100.00	—	—	—	—
	S. abony	133	133	100.00	—	—	—	—
	S. heidelberg	30	29	96.66	1	3.34	—	—
	S. typhi-murium var. koppenhagen	25	25	100.00	—	—	—	—
	S. bredeney	23	23	100.00	—	—	—	—
	S. abortus-bovis	12	12	100.00	—	—	—	—
	S. kingston var. koppenhagen	1	1	100.00	—	—	—	—
	S. stanleyville	8	8	100.00	—	—	—	—
	S. 4.12 *d* —	8	8	100.00	—	—	—	—
	S. reading	6	6	100.00	—	—	—	—
	S. brandenburg	4	4	100.00	—	—	—	—
	S. stanley	4	4	100.00	—	—	—	—
	S. abortus-ovis	2	2	100.00	—	—	—	—
	S. chester	3	3	100.00	—	—	—	—
	S. saint-paul	3	3	100.00	—	—	—	—
	S. budapest	1	1	100.00	—	—	—	—
	S. california	2	2	100.00	—	—	—	—
	S. paratyphi-B var. java	2	2	100.00	—	—	—	—
	S. preston	1	1	100.00	—	—	—	—
	S. bradford	1	1	100.00	—	—	—	—
C_1	*S. cholerae-suis var. Kunzendorf*	1.389	1.371	98.70	10	0.72	8	0.58
	S. cholerae-suis	51	50	98.03	—	—	1	1.97
	S. bareilly	26	26	100.00	—	—	—	—
	S. infantis	23	22	95.65	1	4.35	—	—
	S. typhi-suis	19	19	100,00	—	—	—	—
	S. typhi-suis var. Voldagsen	12	12	100.00	—	—	—	—
	S. isangi	4	4	100.00	—	—	—	—
	S. thompson	7	7	100.00	—	—	—	—
	S. cholerae-suis var. Amerika	6	6	100.00	—	—	—	—
	S. livingstone	3	3	100.00	—	—	—	—
	S. montevideo	3	3	100.00	—	—	—	—
	S. 6.7 *unflagellated*	1	1	100.00	—	—	—	—
C_2	*S. manhattan*	254	252	99.22	2	0.78	—	—
	S. muenchen	34	32	94.13	—	—	2	5.87
	S. bovis-morbificans	32	32	100.00	—	—	—	—
	S. newport	20	20	100.00	—	—	—	—
	S. kottbus	2	2	100.00	—	—	—	—
E_1	*S. anatum*	97	96	98.97	—	—	1	1.03
	S. meleagridis	20	20	100.00	—	—	—	—

Serological group O	*Salmonella* species	No. of phage sensitive strains examined	Distribution of phage sensitive strains					
			Sensitive toward phage O_1 Resistant toward phage R		Sensitive toward phage O_1 Sensitive toward phage R		Resistant toward phage O_1 Sensitive toward phage R	
			No.	%	No.	%	No.	%
	S. london	21	21	100.00	—	—	—	—
	S. give	1	1	100.00	—	—	—	—
E_4	*S. taksony*	5	5	100.00	—	—	—	—
D_1	*S. panama*	113	113	100.00	—	—	—	—
	S. gallinarum	31	31	100.00	—	—	—	—
	S. enteritidis	16	16	100.00	—	—	—	—
	S. dublin	14	14	100.00	—	—	—	—
	S. moscow	3	3	100.00	—	—	—	—
	S. gallinarum-pullorum	1	1	100.00	—	—	—	—
C_2	*S. worthington*	5	5	100.00	—	—	—	—
	S. cubana	4	4	100.00	—	—	—	—
L	*S. minnesota*	8	8	100.00	—	—	—	—
Totals	52 *Salmonella* species	3.234	3.206	99.13	16	0.49	12	0.37

of D_1 antigens, whereas in O-groups E_1, E_2, E_4, G_2 and L, especially in the first three, many strains were resistant to both phages. In percentual terms, 88.1, 100 and 72.2% of the strains belonging to the groups E_1, E_2 and E_4 were phage-resistant. In the O-groups G_2 and L, the respective percentages were 50.6 and 42.1%.

Table IV shows the diagnostic values of the O_1 and R phage tests on the basis of numerical and percentual data for the 3234 phage-sensitive strains. Data for phages are shown separately and together.

Among the 3234 S. strains reacting with phage, 99.1% were O_1-positive, 0.5% O_1 and R, and 0.4% were lysed by the R phage only. Percentage is so low that additional R-phage tests do not seem necessary for routine purposes.

Discussion

Sure diagnosis of S. is hampered by antigenic relationship with other genera of the family Enterobacteriaceae, especially *Arizona* and *Citrobacter*. For this reason, completion of the usual serological and biochemical tests by typing with O_1 and R phages has been proposed since 1964. The information emerging from related experiments conducted in the meantime seems to be worth of publication, because the effects and diagnostic value of O_1 and R

phages were since tested on as many as 4618 S. strains over a five-year period (1965—1970). It was found that phage typing is of a great diagnostic value in the case of S. strains belonging to the O-serogroups B, C_1, C_2 and D_1, while of little or no value in the case of O-group E_1, E_2 and E_4 strains, most of which are phage-resistant, and of doubtful value in the groups G_2 and L, in which the sensitive and resistant strains are.

S. kingston var. *kopenhagen* and *S. schleissheim* in group B, are phage-resistant, but the overwhelming majority are sensitive. Among the C_1 *S. cholerae-suis* var. *Kunzendorf*, the most frequent salmonella in Hungary, is almost always lysed. The phage sensitivity and resistance of *S. typhi suis* var. *Voldagsen* strains was 50%. Two strains of antigenic structure 6.7 fg 1.5 were phage-resistant. Many C_1 strains always lysed, as can be seen from Fig. 2b. In group C_2, 45.2% of *S. muenchen* strains and 33.3% of *S. kottbus* strains were phage-resistant, but the rest were all sensitive. In group E_1, the percentages of phage resistant strains were 89.9% for *S. anatum*, 91.5% for *S. meleagridis*, 57.1% for *S. london*, 100% each for *S. muenster* and *S. westhampton*, and 80% for *S. give*. In group E_4, 37.5% of *S. taksony* strains, but *S. senftenberg* var. *newcastle* and *S. senftenberg* were phage-resistant. Resistance percentages in the O-serogroup D_1 were 31.1% for *S. gallinarum*, 61% for *S. enteritidis*, 51.3% for *S. dublin*, 25% for *S. moscow* and 7.4% for *S. panama*. Among the group G_2 strains, 54.2 and 20% of *S. worthington* and *S. cubana*, respectively, were resistant, and in the O-serogroup L, 57.9% of the *S. minnesota* strains were not lysed by the phage.

Altogether 30% (1384 strains) of the 4618 S. isolates were phage-resistant.

99.6% of all sensitive strains were lysed by the O_1 phage and 0.4% by the R phage, thus the latter should not be obligatorily used for diagnostic phage tests.

Phage typing is a valuable complementary test in meat examinations for S. Its simplicity and economicalness are great advantages. It cannot, of course, substitute the biochemical and serological (agglutination) tests, but greatly facilitates typing wherever serological reactions with polyvalent sera are doubtful.

The use of phages in S. diagnostics resolves the problem of the differentiation of *Bethesda-Ballerup* species from S., because the former are never lysed by the O_1 phage. As the diphasic strains of the genus *Arizona* are sensitive to O_1 they cannot be differentiated from S. by the phage test alone, only serologically (agglutination reaction) because antigenically they do not fit in with the Kauffman-White scheme. Also, Arizona strains decompose lactose with some delay, do not ferment dulcitol and liquefy gelatine.

Phage typing is especially advantageous if the S. grow in R-type colonies, which cannot be used for agglutination test. Complementary phage tests

along with polyvalent sera greatly facilitate serial diagnosis in the case of outbreaks caused by S.

Typing wiht O_1 phage is useful for the diagnosis of S. strains which have lost the flagellar structure, if the strain under examination is not agglutinated by A—E+L—O serum, but behaves biochemically like a S.

Citrobacter can be differentiated from S. on the basis of growth in KCN medium, and behaviour in the lysin decarboxylase and O_1-phage tests. Unlike S. strains, *Citrobacter* do not grow in KCN medium, but their lysine decarboxylase test is positive, and most of them are lysed by the phage O_1. The biochemical subgroup I differ from the subgroup III *Arizona* strains by lack of lactose decomposition, liquefaction of gelatine, behaviour in the malonate test and decomposition of dulcitol and d-tartarate. It should be, of course, always taken into consideration that like S. strains, also *Arizona* strains are lysed by the phage O_1.

SUMMARY

The value of phages O_1 and R in salmonella diagnosis was examined on 4618 salmonella strains, out of which 70% were sensitive. Most strains of the serological groups B, C_1, C_2 and D_1 were phage-sensitive, but the great majority of E_1, E_2 and E_4 strains were wholly or partially resistant. In groups G_2 and L, the proportions of phage-sensitive and resistant strains were roughly half by half. Data of the behaviour of the individual salmonella species towards phages O_1 and R are shown in Tables and Figures. As among the large number of sensitive strains examined, 99,9% were lysed by phage O_1 and only 0,4% by phage R, the latter should not be obligatorily used for routine diagnostic examinations for salmonella. Typing by phage O_1 is, however, recommended as a reliable complementary test of routine salmonella diagnosis, especially for differentiation from the *Bethesda-Ballerup* strains of the genus *Citrobacter*, which are never lysed by O_1. The O_1 phage test is also useful for the differentiation of diphasic *Arizona* species which, although they are sensitive to O_1 as well, however, they differ from salmonellae in respect of behaviour in other tests.

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INCIDENCE OF SALMONELLAE IN FOOD PRODUCTS OF ANIMAL ORIGIN IN 1969—1970

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Data on the incidence of salmonellae in food products originating from animals in 1969/70 are reported along with related statistics from veterinary institutes for the information of workers engaged on the salmonella problem.

A total of 1 778 salmonella strains were isolated and typed over the two-year period. The incidence of salmonellae in the various sampled materials can be seen from Table I.

The distribution of species in the various samples is shown in Table II.

Salmonella cholerae-suis var. *Kunzendorf* was isolated, with the highest frequency, followed in order by *S. anatum*, *S. derby* and *S. typhi-murium*. It should be noted that in several other countries *S. typhi-murium* heads the list. Each of the above species was isolated from more than 100 samples and altogether 43 species were isolated from the different test material.

The distribution of salmonella species and serotypes found in the course of bacteriological examinations of slaughter pigs, cattle and poultry is shown in Table 3.

Altogether 19 species were isolated from pigs 14 from beef cattle, three from calves, four from horses, seven from chickens, one each from turkeys and geese, and two each from ducks and pigeons. It follows that swine are still the main reservoir of salmonellae in Hungary, though cattle also seem to be important in this respect. Aquatic fowl carry chiefly *S. typhi-murium*. From hogs *S. cholerae-suis* var. *Kunzendorf* was the commonest isolate (646 cases).

A break down of the salmonella species and serotypes cultured from various meat products and miscellaneous materials of animal or other origin is presented in Table IV.

The greatest number of salmonella species were isolated from raw meat. Other raw materials of animal or other origin contained 17 species, salted, cold-smoked and dried meat products 16, samples examined in the course of (routine) meat inspection 12, while 9 species each were isolated from semicooked and cooked meat products, 6 each from liver and meatmeal, and one from bacon.

Species originating from animal protein feeds belonged exclusively to

Table I

Distribution or salmonella isolated in various test materials in 1969—1970

Class	Origin Material	No. of contaminated samples	Percentage of all isolations (1 778)
Complementary bacteriological meat examination	hogs	820	46.11
	cattle	34	1.91
	calf	7	0.39
	horse	6	0.33
	hen, chicken	29	1.63
	turkey	4	0.22
	goose	15	0.84
	duck	6	0.33
	pigeon	6	0.33
Meat products and samples from meat processing plants	salted, smoked and dried meat products	278	15.65
	semi-finished meat products	86	4.83
	meat products ready for sale	13	0.73
	samples for meat inspection	105	5.92
Raw materials for meat industry	liver	32	1.79
	meat	254	14.28
	lard	1	0.05
Proteineous animal feed	meat meal	17	0.95
Other materials of animal or other origin		65	3.65

O-antigen groups B, C_1, E_1, E_4 and G_2, but this relatively favourable result may have been due to the lack of regular salmonella examinations in the period in question.

A break down of serotypes by test material is shown in Table V.

The framed figures in the table indicate the serotype occurring most frequently in the given host species. It should be noted that salmonella of group E_1 predominated in both meat products and in the hygienic control examinations of meat processing plants.

Discussion

The commonest isolate from the examined range of test materials in the period 1969—1970 was *S. cholerae-suis* var. *kunzendorf* (38.8%) followed in frequency by *S. anatum* (15.1%) *S. derby* (11.9%), *S. typhi-murium* (6.9%), *S. meleagridis* (5.6%) and *S. panama* (2.7%).

The isolated salmonella strains all belonged to one or other of the O-antigen groups B, C_1, C_2, D_1, E_1, E_4, G_2 and L.

Table II

Distribution of salmonella species in samples examined in 1969—1970

	Salmonella species	No of isolations	Percentage of all isolations (1 778)
More than 100 cases	*S. cholerae-suis var. Kunzendorf*	689	38.75
	S. anatum	269	15.12
	S. derby	211	11.87
	S. typhi-murium	123	6.91
10—100 cases	*S. melagridis*	100	5.62
	S. panama	51	2.86
	S. gallinarum	32	1.79
	S. cholerae-suis	30	1.68
	S. heidelberg	28	1.57
	S. minnesota	19	1.06
	S. infantis	19	1.06
	S. typhi-suis	19	1.06
	S. muenchen	18	1.01
	S. enteritidis	16	0.89
	S. abony	14	0.78
	S. bovis-morbificans	14	0.78
	S. newport	13	0.73
	S. typhi-suis var. Voldagsen	11	0.61
	S. westhampton	10	0.56
3—10 cases	*S. abortus-bovis*	9	0.50
	S. manhattan	9	0.50
	S. typhi-murium var koppenhagen	8	0.44
	O-antigen serotype B 4,12 d-	8	0.44
	S. dublin	7	0.39
	S. worthington	7	0.39
	S. isangi	6	0.33
	S. bareilly	5	0.28
	S. muenster	4	0.22
	S. stanley	4	0.22
	S. senftenberg var. newcastle	4	0.22
	S. thompson	4	0.22
2 cases	*S. brandenburg*	2	0.11
	S. cholerae-suis var. Amerika	2	0.11
	S. cubana	2	0.11
	S. paratyphi-B var. java	2	0.11
	S. reading	2	0.11
1 case	*S. blockley*	1	0.05
	S. chester	1	0.05
	S. gallinarum-pullorum	1	0.05
	S. kapemba	1	0.05
	S. kingston var. koppenhagen	1	0.05
	S. saint-paul	1	0.05
	O-antigen serotype C_1	1	0.05

Table III

Salmonella strains isolated at bacteriological meat inspection of slaughter animals

	Salmonella species and serotype O-antigen	pig				cattle		
		>10	3–10	2	1	3–10	2	1
		cases						
B	*S. abony*	—	—	—	—	—	—	1
	S. abortus-bovis		5	—	—	—	—	1
	S. brandenburg	—	—	—	1	—	—	—
	S. derby	37	—	—	—	—	—	1
	S. heidelberg	—	4	—	—	—	—	—
	S. paratyphi-B var. java	—	—	—	—	—	—	1
	S. reading	—	—	—	1	—	—	—
	S. stanley	—	—	2	—	—	—	—
	S. typhi-murium	—	8	—	—	9	—	—
	S. typhi-murium var. koppenhagen	—	—	—	1	—	—	—
C_1	*S. bareilly*	—	—	—	1	—	—	—
	S. cholerae-suis	25	—	—	—	—	2	—
	S. cholerae-suis var. America	—	—	2	—	—	—	—
	S. cholerae-suis var. Kunzendorf	646	—	—	—	6	—	—
	S. infantis	—	4	—	—	—	—	—
	S. isangi	—	—	—	—	—	—	1
	S. thompson	—	—	—	—	—	2	—
	S. typhi-suis	17	—	—	—	—	—	—
	S. typhi-suis var. Voldagsen	—	10	—	—	—	—	—
C_2	*S. bovis-morbificans*	—	—	—	—	—	—	—
D_1	*S. dublin*	—	—	—	—	4	—	—
	S. enteritidis	—	—	—	—	—	—	1
	S. gallinarum	—	—	—	—	—	—	—
	S. gallinarum-pullorum	—	—	—	—	—	—	—
	S. panama	—	4	—	—	—	2	—
E_1	*S. anatum*	44	—	—	—	—	2	—
	S. meleagridis	—	7	—	—	—	—	—
	S. muenster	—	—	—	—	—	—	1
L	*S. minnesota*	—	—	—	1	—	—	—
	Number of species isolated	5	7	2	5	3	4	7
	Total	19 species				14 species		

calf		horse		chicken				turkey	goose	duck		pigeon	
3–10	2	2	1	>10	3–10	2	1	3–10	>10	3–10	1	3–10	1
cases													
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	2	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	2	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	2	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	2	—	—	9	—	—	—	15	5	—	5	
—	—	—	—	—	—	—	1	—	—	—	—	—	1
—	—	—	—	—	—	—	1	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	2	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	4	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	2	—	—	—	—	—	—	—
—	—	—	—	13	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	1	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
1	2	2	2	1	1	2	3	1	1	1	1	1	1
3 species		4 species		7 species				1 species	1 species	2 species		2 species	

Table IV

Salmonella strains isolated from meat products and miscellaneous materials of animal or other origin in 1969—1970

	Salmonella species and O-antigen serotype	Salted, cold-smoked and dried meat products				Semi-finished meat products				Ready made meat products		
		>10	3—10	2	1	>10	3—10	2	1	3—10	2	1
		cases										
B	*S. abony*	—	3	—	—	—	—	—	—	—	—	—
	S. abortus-bovis	—	—	—	—	—	—	—	—	—	—	—
	S. brandenburg	—	—	—	—	—	—	—	—	—	—	—
	S. chester	—	—	—	—	—	—	—	—	—	—	—
	S. derby	83	—	—	—	17	—	—	—	—	—	1
	S. heidelberg	—	9	—	—	—	5	—	—	—	—	1
	S. kingston var. koppenhagen	—	—	—	—	—	—	—	—	—	—	—
	S. paratyphi-B. var. java	—	—	—	—	—	—	—	—	—	—	—
	S. reading	—	—	—	—	—	—	—	—	—	—	1
	S. saint-paul	—	—	—	—	—	—	—	—	—	—	—
	S. stanley	—	—	—	—	—	—	—	—	—	—	—
	S. typhi-murium	14	—	—	—	—	3	—	—	—	—	1
	S. typhi-murium var. koppenhagen	—	—	—	—	—	—	—	—	—	—	—
	S. 4,12 d-	—	—	—	—	—	—	—	—	—	—	—
C_1	*S. bareilly*	—	—	—	1	—	—	—	—	—	—	—
	S. cholerae-suis	—	—	—	1	—	—	—	—	—	—	—
	S. cholerae-suis var. Kunzendorf	—	3	—	—	—	—	—	—	—	—	1
	S. infantis	—	5	—	—	—	—	—	1	—	—	—
	S. isangi	—	—	—	—	—	—	2	—	—	—	—
	S. typhi-suis	—	—	—	—	—	—	—	—	—	—	—
	S. typhi-suis var. Voldagsen	—	—	—	—	—	—	—	—	—	—	—
	S. thompson	—	—	—	—	—	—	—	—	—	—	—
	S. C_1 O-group unflagellated	—	—	—	—	—	—	—	—	—	—	1
C_2	*S. blockley*	—	—	—	—	—	—	—	—	—	—	—
	S. bovis-morbificans	—	—	—	1	—	—	—	—	—	—	—
	S. manhattan	—	4	—	—	—	—	—	—	—	—	—
	S. muenchen	—	—	—	—	—	—	—	—	—	—	—
	S. newport	—	9	—	—	—	—	—	—	—	—	—
D_1	*S. enteritidis*	—	—	2	—	—	—	—	—	—	2	—
	S. gallinarum	—	—	—	—	—	—	—	—	—	—	—
	S. kapemba	—	—	—	—	—	—	—	—	—	—	—
	S. panama	13	—	—	—	—	—	—	1	—	—	—
E_1	*S. anatum*	82	—	—	—	29	—	—	—	4	—	—
	S. meleagridis	45	—	—	—	17	—	—	—	—	—	1
	S. muenster	—	—	—	—	—	—	—	—	—	—	—
	S. westhampton	—	—	—	—	—	—	—	—	—	—	—
E_4	*S. senftenberg var. newcastle*	—	—	—	—	—	—	—	—	—	—	—
G_2	*S. cubana*	—	—	—	—	—	—	—	—	—	—	—
	S. worthington	—	—	—	—	—	—	—	—	—	—	—
L	*S. minnesota*	—	3	—	—	11	—	—	—	—	—	—
	Number of species isolated	5	7	1	3	4	2	1	2	1	1	7
	Total for class of sample	16 species				9 species				9 species		

Samples for routine meat inspection				Raw materials for meat industry									Proteinaceous animal feed			Miscellaneous material			
				meat				liver				lard	meatmeal						
>10	3–10	2	1	>10	3–10	2	1	>10	3–10	2	1	1	3–10	2	1	>10	3–10	2	1
cases																			
—	8	—	—	—	—	—	1	—	—	—	—		—	—	1	—	—	—	—
—	—	—	—	—	—	—	1	—	—	—	—		—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
16	—	—	—	31	—	—	—	21	—	—	—	—	—	—	—	—	—	2	—
—	—	—	—	—	—	2	—	—	—	2	—	—	—	—	—	—	3	—	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	30	—	—	—	—	—	—	—	—	—	—	—	11	—	—	—
—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	7	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
—	3	—	—	27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	3	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	7	—	—
—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	—	—	13	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—
—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
—	—	2	—	—	—	2	—	—	—	—	—	—	—	—	—	—	5	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	17	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
36	—	—	—	63	—	—	—	—	4	—	—	1	—	2	—	—	—	—	—
—	4	—	—	23	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	2	—	—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—	—	—
—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	6	1	1	8	5	5	7	1	2	1	2	1	2	2	2	2	4	4	7
12 species				25 species				6 species				1 species	6 species			17 species			

Table V

Distribution by serotype O-antigen of salmonella strains isolated in 1969—1970

Serotype O-antigen	Samples for complementary bacteriological meat examination of slaughter animals and poultry									Samples from meat processing plants					Protein-aceous animal protein feed	Miscella-neous
	hog	cattle	calf	horse	chicken	turkey	goose	duck	pigeon	Meat prod-ucts salted, coldsmoked and dried	Semi finish-ed meat products	Ready made meat products	Samples for routine meat inspektion	Raw materials for meat industry		
B	59	13	2	4	12	—	15	5	6	109	25	4	40	99	1	20
C_1	705	11	2	1	1	—	—	—	—	10	3	2	6	37	1	7
C_2	—	—	—	—	—	4	—	—	—	14	—	—	3	20	—	14
D_1	4	7	3	—	16	—	—	—	—	15	1	2	16	20	—	24
E_1	51	3	—	1	—	—	—	1	—	127	46	5	40	107	2	—
E»	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—
G_2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	—
L	1	—	—	—	—	—	—	—	—	3	11	—	—	4	—	—

According to the findings of bacteriological meat inspections at the abattoir the predominant serotypes were C_1 in hogs, B in cattle, D in calves, B in horses, D_1 chickens, C_2 in turkeys, and B in geese, ducks and pigeons. In the meat industry in contrast group E_1 strains predominated among isolates from salted, cold-smoked and dried products semi-cooked and cooked products as well as samples sent in for routine meat inspection and raw materials, through salmonellas of group B occurred in equal numbers in samples for meat inspection. Most salmonella isolated from animal protein containing feeds belonged to group G_2 and most of those from miscellaneous samples of animal or other origin to group D_1.

During the two years *S. paratyphi*-B var. *java* was isolated for the first time in Hungary, in one case from beef cattle and in the other case from a semi-finished pharmaceutical product. *S. blockley* was first isolated in 1970, from the surface of meat imported from Romania. *S. minnesota* had likewise not been isolated before 1970, but then turned up in 19 cases: three times from smoked pork sausage, 11 times from a semi-cooked meat product, four times from the surface of raw meat (two samples of home origin and two samples of meat imported from the German Federal Republic), and once from the complementary bacteriological meat examination of a hog. Eight isolations were made in 1970 of a salmonella strain having antigenic pattern 4.12 d that is a classified in the Kauffmann-White scheme; some of the isolates derived from the surface of meat imported from the U.K., and one from a sample of unknown origin. *S. kapemba* was cultured an a single occasion in 1970, in the course of meat hygienic inspection.

The experimental data clearly indicate that efforts for the prevention and control of salmonella infections can not be slackened for the time being. The preparation of animals and poultry for slaughter could be greatly improved if the veterinarian in charge of the herd or flock were to keep the meat inspector on the abattoir informed of the salmonella status of the livestock. Lack of such information hampers the detection of symptomless carriers and excretors and may even mean that essential hygienic measures are omitted at the abattoir. Preventation of the spread of salmonella infections at the slaughterhouse requires very rigorous disinfection of hands and instruments, and a series of other measures not to be described here. It should nevertheless be pointed out as a general principle that transfer of infections by contact with abattoir personnel or instrument should be reduced to the minimum. The methods applied should always be scrutinized from this angle and all potential sources of contamination should be eliminated. If the data presented in this paper can contribute to the fulfilment of this important hygienic task, we shall be content to have achieved our object of disclosing hidden sources of salmonellosis.

SUMMARY

Forty-three salmonella species were isolated from various test materials of animal origin in 1969—70. Most of the samples were submitted from abattoir laboratories, the rest from various veterinary institutions. The isolates belonged to the O-antigen groups B, C_1, C_2, D_1, E_1, E_4, G_2 and L. Of the host species investigated hogs proved to be the main reservoir of salmonellas: 820 (46.1%) of the examined slaughter hogs were found to be carriers of 19 species of salmonella. As to other slaughter animals and poultry, 14 species were isolated from 34 (1.9%) beef cattle, three species from 7 (0.4%) calves, four species from 6 (0.3%) horses, seven species from 29 (1.6%) chickens, one species each was isolated from 29 (1,6%) chickens, 15 (0.8%) geese and four (0.2%) turkeys, and two species each were isolated from six (0.3%) ducks and six (0.3%) pigeons. The fact that symptomless carriers and excretors of salmonellae cannot be detected by traditional macroscopic methods at meat inspection seems to account for the frequent occurrence of these bacteria in meat products and meat-processing plant.

The prevention of human salmonella infections by meat or meat products requires strict hygienic measures along the possible chain of infection, with special regard to eliminating the pathogens from animals feeds. The latter have in all probability been the source of the newer salmonella serotypes not previously observed in Hungary. Five such species were encountered in the course of the present study: *S. paratyphi-B* var. *java*, *S. blockley*, *S. minnesota*, *S. kapemba*, and a fifth strain, having the antigenic pattern 4.12 d, which would not be fitted in the Kauffmann-White scheme.

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INFLUENCE OF PACKING TECHNOLOGY AND STORAGE TIME ON THE MICROBIOLOGICAL STATUS OF QUICK-FROZEN AND COOKED READY = TO-EAT FOOD

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Microbiological assays carried out during hygienic control of the production of ready-cooked-meals have often implicated bacteriological contamination following heat treatment (cooking). Careful investigations of hygiene of the manufacturing process make it quite clear that a major share of the blame for such post-contamination lies in the use of out-of-date packaging materials.

In the traditional process, where waxed cartons are used, the cooked food has to be cooled down (from 80° C to 18° C) before it can be packed into the carton, so as to avoid melting of the wax and the damage of the package. This cooling phase, however, is not only time-consuming, but it also introduces ample opportunity for the cooked food to come into contact with utensils contaminated with microbes and thereby itself becomes contaminated. As again automatic dispensing is impossible with waxed cartons, filling has to be manual, which opens yet further routes for microbial recontamination.

A drastic reduction in the possibilities of recontamination can be achieved by "hot-filling" of the cooked food — a technique already adopted successfully in several countries, including the German Democratic Republic and Sweden (Almási, 1964; Bodrossy and Benedek, 1969). By using modern dispensing equipment and packaging materials, the food can be packed at 70—80° C without pre-cooling and dispatched to the quick-freezing tunnel within one hour. The most suitable packaging material in these circumstances is the heat-resistant, boilable plastic bag. The best and most economic of such plastics, according to tests by the Central Food Research Institute, Budapest, is high-specific-weight polyethylene foil; 0.04 mm gauge foil may be used for net weights up to 350 g, and above this 0.08 mm foil (Varsányi, 1969).

In view of the evident advantages of this latter technique, we have undertaken studies to compare the microbiological properties of different meals when packed cold into cartons and hot into polyethylene bags. In addition, we have investigated the influence of storage time of the frozen product on the bacterial content of food packed by the two methods.

Material and Methods

Three meals were selected for the investigations: lights, stewed chitterling with paprika, and goulash with paprika gravy.

All three meals are cooked in open, steam-heated kettles of 300 litre capacity. The usual braising time for the chitterlings and goulash is 30 minutes,

the cooking time 2—2.5 hours; the pre-cooked material used in lights is braised for 5—10 minutes and the stewing time is 1.5—2 hours.

By the traditional method, the meals are ladled after cooking into so-called "meat-trucks" of 400-litre capacity and from there are transferred either by ladle or pump, into food coolers working with saline. Though these coolers have a capacity of 900 litres, they are filled only to 500 litres in order to achieve optimum cooling efficiency. The cooling from 80° C to 18° C takes about 30 minutes. The food is then drained of into meat trays of 20-litre capacity. Packaging of the meals proceeds from the trays either into 500-gram cartons or, for public catering purposes, into bags made from polyethilene foil of 1, 1.5 or 2 kg. Dispensing is either by ladle or hand (rubber gloves). The cooling process for goulash with paprika differs from the above in that, because of weight regulations, the gravy must be filtered off the chopped meat for separate cooling (saline cooler), while the chopped meat is transferred on uncovered aluminium trays to a cold room at 0—5° C.

After cooling, the chopped meat is dispensed into the package in the prescribed portions and then the gravy is poured back on. Obviously, this process enormously increases the chances of re-contamination.

Usually it takes the food no more than 2 hours to pass from the kettles to the freezers. The separate phases are thus:

kettle to cooler	20 minutes
cooling period	30 minutes
cooler to package	30 minutes
packaging to beginning of freezing	40 minutes.

When the hot-filling method was applied in these experiments, 500-gram portions of the meals were dispensed into preboiled plastic bags. After sampling, the bags were sealed and then transferred to the freezing-tunnel within one hour.

Samples of the three meals packed by the two methods always came from the same batch. Altogether 64 samples were taken, and in all cases bacteriological investigations were carried out immediately after freezing and following storage at —20° C for 2, 4 and 6 months. All samples reached the laboratory in the frozen state and were then allowed to thaw at +4° C for 16—24 hours before being tested. After thawing, several 5-gram portions were taken from different parts of the sample, homogenized, and made up in serial dilutions to 10^6 in physiological saline.

The culture techniques applied to determine the counts and/or titres of the various components of the microflora were as follows.

Psychrophilic and mesophilic organisms: Koch's pourplate technique

with a standard agar medium to which gelatine has been added and developed at this Institute (TAKÁCS, 1961); plate count of alive germs were determined after an incubation time of three days at 30° C.

Psychrotolerant organisms: Incubation on maltonse agar for five days at 20° C; plates were read on days 3 and 5.

Moulds and Fungi: the same method has been used as for psychrotolerant microorganisms.

E. coli I (faecal type) and coliforms: Kessler and Swenarton's gentian violet-bile-lactose-peptone broth and a modified Drigalski medium were used: titre was determined after 48-hour culture at 30° C.

Coagulase-positive staphylococci: Transfer from Bojanovszky's rhodanide enrichment medium (JANKE and DICKSCHEIT, 1969) to blood agar plate; incubation for 24 hours at 37° C.

Clostridia: Semi-solid sulfite agar, as modified by TAKÁCS and NARAYAN (1965); incubation for 48 hours at 37° C.

Salmonellae: Enrichment in Bierbauer's enrichment medium for at least 16 hours at 37° C before transfer to Drigalski and brilliant green — phenol red agar medium; incubation for 24 hours at 37° C before reading.

Proteolytic organisms: The standard agar medium was used. Colonies were poured with Clark's reagent to detect the gelatine-liquefying colonies. The count of proteolytic colonies were established by this method (TAKÁCS, 1961).

Results

The data are summarized in Table I and Figs 1—3. Cell counts and titres are given as logarithmic mean values. We have recorded the percentage reduction in the number of germs after storage at —20° C and the percentage decrease in cell count at the hot-filled products immediately after filling without storage in frozen state. The mean values of the logarithms of cell-count and titer values for cold- and hot-filled products are shown on the Figs 1—3.

If titre values were established, first of all in the case of coliform and clostridial values, mean values were calculated. The other microbiological values, which has been shown in the table. were given as the means of logarithmic values of viable cell counts. Where the cell count was less than 100, the mean logarithmic values of cell counts were calculated without using the MPN method that is why we couldn't calculate mean logarithmic cell counts less than 100 in accordance with the rules of dilution. This doesn't influence the value of the test because one must always take into consideration values higher than 100/g for the microbiological evaluation.

Table I

Technology Meal	Mean logarithmic cell count and titer values							
	Total live mesophilic and facultative psychrotolerant organisms	Coliform	Psychrotolerant organisms	Proteolytic organisms	*Staphylococcus aureus*	Moulds	Fungi	Clostridia
Cold-filled, without freeze storage								
L	3.625	10	18.200	91.2	100	100	112	31.7
SCH	6.000	192	19.600	895	100	100	100	21.5
G	7.320	21.5	8.650	252	100	100	100	50
Cold-filled, stored at — 20°C for 6 months								
L	642	3.16	1.806	6.55	100	100	100	31.7
SCH	1.162	20	5.450	20	100	100	100	21.5
G	2.140	4.44	3.340.	4.44	100	100	100	50
Percentage reduction in cell count								
L	82.3	68.4	90.1	92.8	0	0	10.8	0
SCH	80.7	97.7	72.2	97.7	0	0	0	0
G	70.8	79.4	61.4	98.2	0	0	0	0
Hot-filled, without freeze storage								
L	260	0	630	4.44	100	100	100	31.7
SCH	200	0	465	0	100	100	100	21.5
G	342	0	457	21.5	100	100	100	50
Percentage reduction in cell count								
L	92.8	100	96.5	95.1	0	0	10.8	0
SCH	96.7	100	97.6	100	0	0	0	0
G	95.3	100	94.7	91.7	0	0	0	0

Legends: L, Lights (6 samples); SCH, Stewed chitterlings (3 samples); G, Goulash (3 samples)

Conclusions

When the meals were hot-filled the cell counts of coliforms, total live mesophilic and facultative psychrophilic organism, proteolytic and psychrotolerant organisms met current hygienic requirements in (all respects) and were well below the counts established for food from the same batch packed with the traditional way. In numerical terms

(i) mean titres for coliform germs of 10—99 were diminished to 0;

(ii) total counts of live mesophilic and facultative psychrophilic germs were reduced from 10 000 and 1 000, respectively, to below 200;

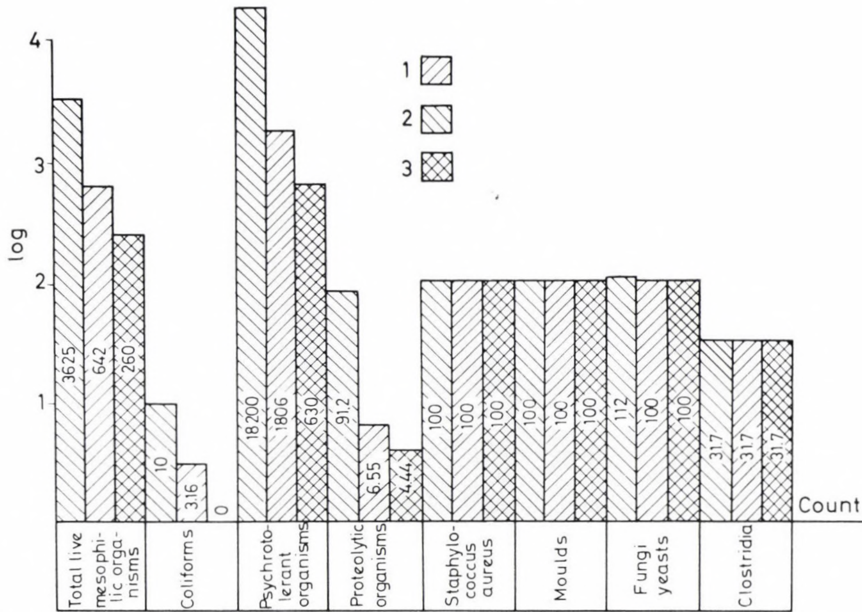

Fig. 1. Quick-frozen lights (6 samples). 1 cold-filled (+18° C), without freeze storage; 2 cold-filled (+18°C), stored at —20° C for 6 months; 3 hot-filled (+18° C) without freeze storage

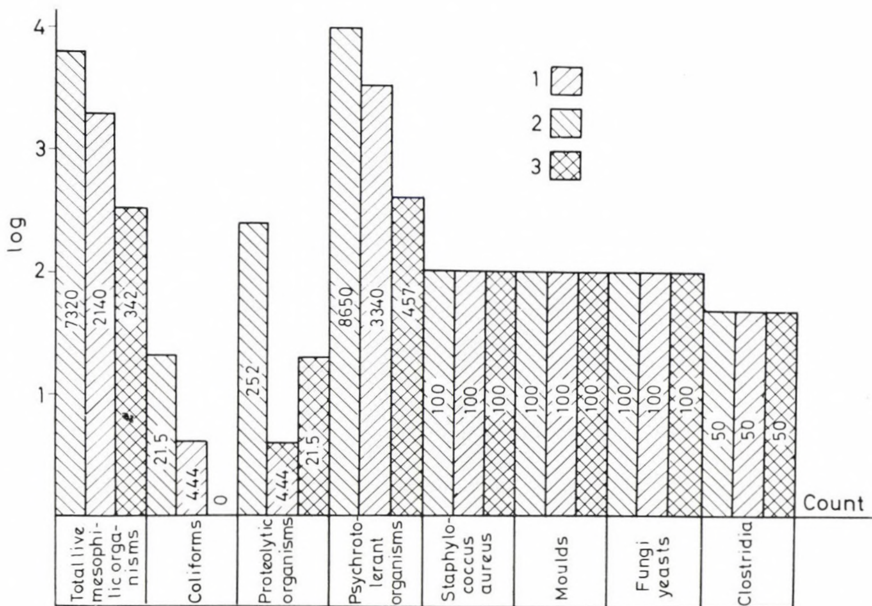

Fig. 2. Quick-frozen stewed chitterlings (3 samples). 1 cold-filled (+ 18 °C), without freeze storage; 2 cold-filled (+18° C), stored at —20° C for 6 months; 3 hot-filled (+80° C) without freeze storage

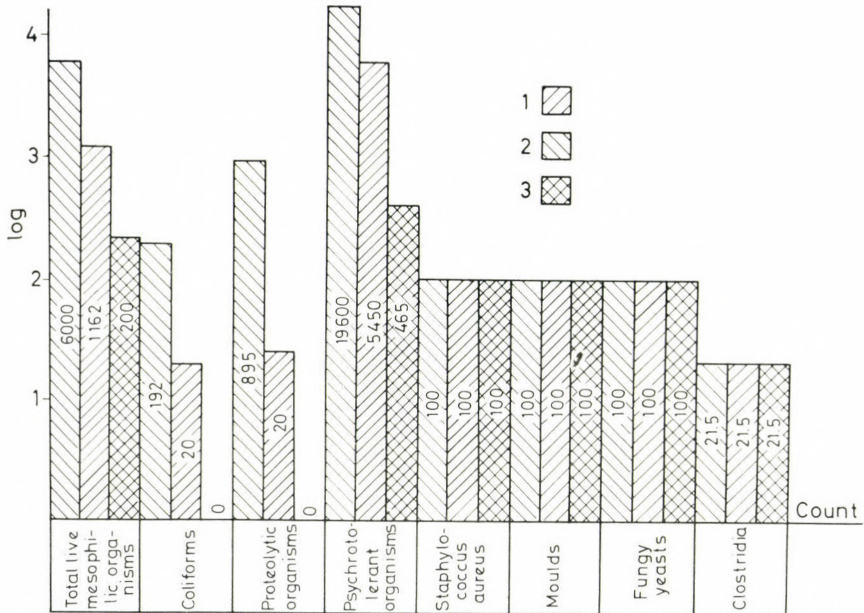

Fig. 3. Quick-frozen goulash (3 samples). 1 cold-filled (+18° C), without freeze storage; 2 cold-filled (+18° C), stored at —20° C for 6 months; 3 hot-filled (+80° C) without freeze storage

(iii) counts of proteolytic germs as high as 1 000 and 100 dropped to under 200 and zero; and

(iv) the number of psychrotolerant germs was reduced from 10 000 and 1 000 to less than 1 000.

These reductions unequivocally support the hygienic superiority of the hot-filling technology.

In cooked meals filled cold (+18° C), there was a significant decrease in the number of coliform, total live germs, proteolytic and psychrotolerant germs following six months' storage at —20° C. According to the data reported by SCHORMÜLLER (1966), storage for 115 days at —20° C resulted in a 43% drop in the counts. In our experiments the reduction in bacterial cell count were considerably higher: as much as 68.4—92.8% for lights 72.2—97.7% for stewed chitterlings, and 61.4—98.2% for goulash.

There was no measurable fall in the cell counts of hot-filled meals when stored frozen, because these were practically sterile already prior to storage. Compared with the cell counts and titres for cold-filled meals before storage, there is with all three products a 91.4—100% decrease to the advantage of the hot-filled products.

The titres of clostridia, moulds, yeasts, and *Staph. aureus* were independ-

ent of the method of packaging and duration of storage. The mean values were as follows:

clostridia less than	10 per 1 gram sample
moulds less than	100 per 1 gram sample
yeasts less than	100 per 1 gram sample
Staph. aureus less than	100 per 1 gram sample

The cell count of frozen ready-cooked food is clearly very much dependent on the technology used in its preparation. If the meal is filled into heat-resistant polyethylen foil immediately after cooking while still hot, and then quickly cooled through the zone favourable for thermophilic germs in a freezing-tunnel, the product will be practically sterile with regard to mesophilic, thermophilic, thermotolerant and psychrotolerant micro-organisms.

If on the other hand, the meal is cooled after heat-treatment and in the process comes into contact with utensils or is handled manually an appreciable contamination can be introduced into the initially sterile product. That way the heat-treated product may be recontaminated with pathogen and toxin-producing germs before freezing. The post-contamination must be anyway avoided, that's why only hot-filling has it's grounds. The significant drops in cell count following storage at —20° C, though favourable from the hygienic point of view, are very much dependent on the residual count after heat-treatment. If many germs get into the product before freezing, these germs have an opportunity to multiply and display ferment activity during thawing time and may deteriorate the product before consuming.

It must be emphasized that samples containing pathogenic and toxin-producing germs before freezing, neither freezing nor freeze storage for a long period could kill them. This was observed at a raw, frozen pork liver sample, in the case of *Salmonella*-contamination. According to our observations, there was a decrease in the count of coagulase-positive *Staph. aureus*, but not to the same extent as in the case of Gram-negative bacilli, consequently, if the product becomes contaminated with enterotoxin-producing staphylococci after heat-treatment they remain alive during freeze storage.

We have newly determined the still acceptable tolerated values in plate counts and titre for heat-treated and frozen ready products. The microbiological data obtained in this experimental series in no case exceeded the values formerly set up for these products. At our investigations the plate count and titre values were much more favourable than in our previous study (Takács, Simonffy and Imreh, 1969).

Our investigational data emphasize that the modern efficial control of frozen food, cooked food or semi-cooked frozen food cannot rest merely upon the qualification of the final product, but revealing faulty hygienic proposes

changes in processing technology as well. Modern veterinary meat control covers the prevention of mistakes as recording of mistakes.

SUMMARY

Mean logarithmic counts and titres of various microflorel components were determined in products after packaging in a cold or hot state for freeze-storage, as well as their reductions following six months' storage at —20° C. The experiments revealed that microbial contamination due to handling between cooking and freezing can be significantly diminished only by the use of hot-filling technology. The application of this method resulted in a 91.4—100% decrease in bacterial counts in comparison to those in the cold-filled, freeze-stored products. Typically the counts of non-pathogenic and non-toxogenic germs may be reduced by 51,4—98,2% during six months' storage at —20° C. These are much greater decreases than those reported in the literature, though the falls in count were much less where pathogenic and toxogenic germs are concerned. This suggests that freeze storage cannot render a product containing such microorganisms sterile but at best only diminish their number.

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MICROBIOLOGICAL STUDY OF IMPORTED FROZEN RAW FISH

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Blocks and fillets of frozen fish are subject to various kinds of natural contamination during their processing, and such contamination normally persists up to the time the fish reaches the consumer's kitchen. The actual microbiological status will depend on: 1) the extent and nature of contamination on the fishing-boat, in the filleting vat or during further processing; 2) the time elapsing before chilling or freezing and eventual consumption; and 3) the temperature effects acting on the fish during this period.

It is known that healthy muscle tissue of freshly caught sea fish contains few, if any, micro-organisms (GEE, 1930; MALTSCHEWSKY and PARTMANN, 1951). Many micro-organisms are, however, present on the skin and cutaneous mucosae (10^2—10^7/cm^2), in the gill tissue (10^3—10^6/g) and in the viscera (10^3—10^8/g) (SHEWAN, 1956). Bacteriological inspection of fish is thus essentially directed at the detection of micro-organisms that become transferred from these latter areas to muscle either as a result of faulty processing technology or through unhygienic handling by human operators (SPENCER, 1957; CASTELL, 1954; LISTON, 1955; SHEWAN et al., 1960).

Both chilling and freezing can help to reduce germ counts to some degree, for most piscine micro-organisms are killed at temperatures below zero centigrade. Gram-negative bacteria are especially sensitive, apparently pseudomonads being the first to succumb. Gram-positive organisms are less affected by cold: freezing kills the sporforming aerobes and lactobacilli but only part of the micrococci and enterococci. Fungi are even more resistant: some of the budding yeasts and molds are able to survive temperatures well below freezing.

This cold sensitivity is also of practical importance in microbiological examinations (HERSCHDORFER, 1968). Frozen fish imports to Hungary originate from the North Sea and Baltic Sea where the mean sea temperature varies between 2 and 12° C. This means that the microflora of fish from such habitats has normally to be cultured at temperatures of 0—+20° C; only about 5% of cold-adapted (psychrotolerant) organisms are able to grow at 37° C (GEORGALA, 1958; LISTON, 1955; SHEWAN and LISTON, 1956). The microflora of fish caught in warm seas (Adriatic, African and Australian coasts) is, in contrast, predominantly mesophilic.

In Hungary, the microbiological, physical and chemical properties of imported frozen fish fillets and blocks were already the subject of a recent study (BÍRÓ and KISZLER, 1971), but no independent microbiological examinations have been conducted so far, and furthermore the samples used in this earlier study were obtained from cold storehouses rather than from fish packed for retail sale in the shop.

Detailed investigations were teherefore performed in this laboratory in the frame of regular control inspections of imported foodstuffs. The aim was to qualify and quantify the microbiological status of frozen blocks and fillets of fish with a view to establishing recommended guidelines.

Materials and methods

A total of 98 samples of sea-salmon fillets, imported from Norway and Denmark, and 66 frozen gutted fish carcasses with or without the head, chiefly from the Soviet Union and Poland, were examined during the period 1969—1971. The fillet samples were delivered to the laboratory in the original 1— or 2—lb packing, the carcass (herring, stavrid, sole, mackerel) in frozen blocks.

Both fillets and blocks were transported to the country in refrigerator trucks and were stored in cold storehouses at —18—20° C and —8—12° C, respectively.

Sampling

Samples were taken in accordance with prescriptions for obtaining adequate numbers for statistical evaluation, as follows:

Sampling package unit	No. of samples:
1— 5	1
6— 10	2
11— 20	3
21— 100	5
100— 300	6
300— 700	7
700—1000	8
1000—2000	9
2000—5000	10

Method

All samples were submitted in the frozen state, and so were thawed for laboratory examination 16—24 hours at + 4° C before. Five grams of each sample were taken: in the case of whole carcases, 5 g samples were excised from several sites both the cutaneous and abdominal side. Culturing media and

dilutions were selected with regard to the expected composition and titres of the microflora.

Both fillets and blocks were examined for the following microbiological properties:

1. Psychrotolerant organisms	Koch's pour-plate technique; incubation for 48 hours at 20° C (TAKÁCS, 1961)
2. *E. coli I* and coliform organisms	McConkey's medium and modified Drigalski medium; incubation for 48 hours at 30° C
3. Coagulase-positive staphylococci	Transfer from Bojanovszky's rhodanide enrichment medium to blood agar plate: incubation for 24 hours at 37° C (JANKE and DIEKSCHEIT, 1969)
4. Enterococci	Transfer from sodium azide broth to Packer's agar; incubation for 48 hours at 37° C.
5. Clostridia	Semi-solid sulfite agar, as modified by TAKÁCS and NARAYAN (1965); incubation for 48 hours at 37° C
6. Fungi	Maltose agar; incubation for 72 hours at 37° C
7. Enterobacteriaceae	Enterobacteriaceae medium; incubation for 48 hours at 37° C (ISO, 1971)
8. Salmonellae	Enrichment in Bierbauer's enrichment medium for 24 hours at 37° C; transfer to Drigalski medium and briliant green — phenol red agar plates; incubation for 24 hours at 37° C.

Results

The results are summarized in the nine appended tables. Mean counts and titres of the different bacterial components in frozen fillets and blocks over each year are given in Tables I, II and Tables III, IV, respectively.

Counts and titres calculated for the entire three-year period of examination can be seen from Tables V and VI for fillets and from Tables VII and VIII for frozen blocks.

Table I

Mean germ counts in frozen fish fillets, calculated for one year period

Microorganism	Year	X	S
Facultative psychrophilic microorganisms	1969	18 333	12 380
	1970	19 030	32 180
	1971	93 736	195 900
Enterobacteriaceae	1969	222	234
	1970	101	33
	1971	99	1
Enterococci	1969	—	—
	1970	118	133
	1971	111	61
Molds	1969	102	5.5
	1970	266	1 281
	1971	1 455	1 633
Yeasts	1969	343	24.1
	1970	377	601
	1971	683	1 211

* X, arithmetic mean; S, standard deviation

Table II

Titres of microorganisms in frozen fish fillets, calculated for one-year period

Microorganism	Year	Titer			
		<1	1–9	10–99	100–999
E. coli I. and coliforms	1969	10 (37.03%)	9 (33.33%)	6 (22.22%)	2 (7.40%)
	1970	29 (63.04%)	13 (28.26%)	3 (6.52%)	1 (2.17%)
	1971	12 (48%)	5 (20%)	4 (16%)	4 (16%)
Saprophytic clostridia	1969	—	—	27 (100%)	—
	1970	—	—	46 (100%)	—
	1971	—	—	25 (100%)	—
Staphylococcus aureus	1969	—	—	27 (100%)	—
	1970	—	—	46 (100%)	—
	1971	—	—	25 (100%)	—

Table III

Mean germ counts in blocks of frozen fishes, calculated for one-year periods

Microorganisms	Year	X	S
Facultative psychrophilic organisms	1969	4 000	3 558
	1970	26 797	64 330
	1971	17 950	34 240
Enterobacteriaceae	1969	99	0
	1970	242	532
	1971	99	0
Enterococci	1969	99	0
	1970	136	182
	1971	99	0
Molds	1969	99	1
	1970	213	415
	1971	106	108
Yeasts	1969	174	96.2
	1970	138	189
	1971	113	53.7

Table IV

Titres of microorganisms in blocks of frozen fishes, calculated for one-year periods

Microorganisms	Year	Titer				
		<1	1–9	10–99	100–999	>1000
E. coli I and coliforms	1969	3 (75%)	1 (25%)	—	—	—
	1970	31 (64.58%)	10 (20.83%)	3 (6.25%)	4 (8.33%)	—
	1971	12 (85.71%)	2 (14.28%)	—	—	—
Saprophytic clostridia	1969	—	—	4 (100%)	—	—
	1970	—	—	40 (83.33%)	4 (8.33%)	4 (8.33%)
	1971	—	—	14 (100%)	—	—
Staphylococcus aureus	1969	—	—	4 (100%)	—	—
	1970	—	—	48 (100%)	—	—
	1971	—	—	14 (100%)	—	—

Table V

Mean germ counts in frozen fish fillets, calculated for the entire 3-year period of examination

Microorganisms	X	S
Facultative psychrophilic micro-organisms	37 895	100 400
Enterobacteriaceae	133	258
Enterococci	116	112
Molds	524	2 541
Yeasts	456	839

Table VI

Titres of microorganisms in frozen fish fillets, calculated for the entire 3-year period of examination

Microorganisms	Titer			
	<1	1–9	10–99	100–999
E. coli and coliforms	51 (52.04%)	27 (27.55%)	13 (13.26%)	7 (7.16%)
Saprophytic clostridia	—	—	98 (100%)	—
Staphylococcus aureus	—	—	98 (100%)	—

Table VII

Mean germ counts in blocks of frozen fishes, calculated for the entire 3-year period of examination

Microorganisms	X	S
Facultative psychrophilic microorganisms	23 387	32 370
Enterobacteriaceae	200	1 630
Enterococci	126	156
Moulds	184	356
Yeasts	135	165

Table VIII

Titres of microorganisms in blocks of frozen fishes, calculated for the entire 3-year period of examination

Microorganisms	Titer				
	<1	1–9	10–99	100–999	1000–9999
E. coli and coliforms	46 (69.69%)	13 (19.69%)	3 (4.54%)	4 (6.06%)	—
Saprophytic clostridia	—	—	58 (87.87%)	4 (6.06%)	4 (6.06%)
Staphylococcus aureus	—	—	66 (100%)	—	—

Table IX shows our recommendations for maximum tolerated bacterial and fungal counts in these products. The corresponding International Committee of Microbiological Specifications of Foods (ICMSF) and Portuguese standards are included for comparison.

Table IX

Proposed microbiological limits for frozen blocks and fillets of fishes, compared with the ICMSF proposals and standing Portuguese regulations

Mikroorganisms	m	c	M	ICMSF		+Portuguese standards
Facultative psychrophilic microorganisms	100.000/g	100.000—1,000.000/g	1,000.000/g	1,000.000/g		** 10^5/g 10^6/g
				x m	M	
E. coli I. and coliforms	99/g	100—999/g	1.000/g	100/ 100 g	3600/ 100 g	E. coli I. 0.1/g coliform 0.01/g
Staphylococcus aureus	99/g	100—999/g	1.000/g	100/g	1000/g	0.01/g
Enterococci	1.000/g	1.000—10.000/g	10.000/g	—	—	—
Saprophytic clostridia	99/g	100—999/g	1.000/g	—	—	—
Enterobacteriaceae	1.000/g	1.000—3.000/g	3.000/g	—	—	—
Molds	1.000/g	1.000—3.000/g	3.000/g	—	—	10^3/g
Yeasts	1.000/g	1.000—3.000/g	3.000/g	—	—	10^3/g
Vibrio parahaemolyticus For consumption in uncooked state	—	—	—	100/g	10.000/g	—

* Limits specified in the ICMSF proposal for Coliforms aply to *E. coli I.*; ** Under Portuguese regulations 10^5/g applies to all mesophilic organisms; + Portuguese regulations also resist on that the sample should not contain Salmonella or other, toxigenic pathogenic organisms; m, not hazardous to human health; c, tolerated germ content; M, hazardous, to be condemned.

Discussion

Frozen blocks and fillets of fish were examined for microbiological status to establish guidelines to hygienic qualification.

Statistical values proposed by the ICMSF, as well as m, c and M values were calculated from the arithmetic mean of counts and the standard deviations. m = a microbial count or value which, if exceeded, indicates a departure from acceptable manufacturing practice, to a degree which becomes a matter for concern with respect to health hazard and/or spoilage. YM = microbial count or value which, if exceeded indicates a condition that is cause for decisive concern with respect to health hazard and/or spoilage and thereby calls for rejection of the product. c = the maximum number of counts between m and M to be permitted for acceptance, while none may exceed M.

The maximum tolerated values proposed by us correspond with recommendations of the ICMSF, except for titres *E. coli I* and coliforms for which the ICMSF prescriptions give the "m" and "M" values for 100 g test material (Table IX). The standard prescriptions valid in Portugal set lower limit.

The same microbiological requirements can be applied for blocks and fillets of frozen fish, because the two kinds of product show no characteristic difference in a microbiological respect.

At least five random by selected samples from each lot or batch are required for the statistics of microbiological status.

Apart from the microorganisms specified in the tables, blocks or fillets of frozen fish should always be examined for pathogenic or toxigenic microorganisms which may be hazardous to human health.

The maximum tolerated plate count of alive germs of frozen fish fillet recommended have correspond with those established by KISZLER and BIRÓ (1971).

Total counts of live psychrotolerant germs in frozen blocks varied in a lesser degree between the given limits compared to similar counts in frozen fillets. The reasons are in all probability technological.

Mean titres of clostridia calculated for the entire three-year period were higher in frozen blocks than in fillets.

SUMMARY

Mean counts and titres of various microorganisms were determined in samples imported of whole and filleted frozen fish over the period 1969—71 with the aim of offer proposals for the microbiological requirements of such products. The "m", "c" and "M" values were calculated from the data and compared with similar values set forth in the International Committee of Microbiological Specifications of Foods requirements and the standard prescriptions valid in Portugal. Certain pathogenic or toxogenic organisms are hazardous to human health because their presence in frozen raw fish products automatically excludes the release for human consumption. It is proposed that microbiological qualification of frozen raw-fish products should be based on examination of at least five random samples from each lot; no more than two of the five samples could reach the "c" limit for unconditional release. The lot or batch should be condemned if a single sample reaches the value "M".

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COMPARATIVE STUDY OF FOWL PLAGUE VIRUS INFECTED AND NON-INFECTED ALLANTOIC FLUID OF CHICK EMBRYOS

By

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A comparative study of the allantoic fluid of normal chick embryos and embryos infected with fowl plague virus has been performed in order to establish the extent of involvement of the virus in cellular protein synthesis.

Materials and methods

Infected Allantoic Fluid

IAF was prepared by intra-allantoic infection of 11-to 12-day-old chick embryos with the Rostock strain of fowl plague virus (FPV).* The chick embryos were descended from strains clinically free from avian leucosis-sarcoma disease and were known to be free from CELO adenovirus, Newcastle disease virus and mycoplasmas. After harvesting the IAF was pooled, routinely clarified by low-speed centrifugation and dialysis against distilled water at 4° C for 48 hours, then divided into two parts. The first portion was lyophilized, while the second was concentrated on a DEAE-cellulose column (0.55M NaCl elution solution) and the eluted virus sedimented by high-speed centrifugation at 37,000 x g for one hour (Janetzky centrifuge). The supernatant, which was non-haemagglutinating, was concentrated by dialysis against gum arabic. In this way an about 300-fold concentration of "infective" AF-free FPV (FPV-O) was attained.

Non-infected allantoic fluid

AF was collected from uninfected 11-to 12-day-old chick embryos, clarified and lyophilized in the same manner as the first portion of IAF.

* Kindly sent to us by the Central Veterinary Laboratory, New Haw, Weybridge, Surrey.

Electrophoresis in agarose suspension

Electrophoresis largely followed the procedure of HJERTÉN (1963) but with certain modifications of our own. A 0.16% agarose suspension (Agarose, Serva, Heidelberg) was prepared in 0.05 M Na phosphate buffer (pH 7). The same buffer was used as the electrode buffer. The lyophilized AF and IAF samples submitted to electrophoresis were applied in concentrations of about 40 mg/ml. After electrophoresis, the individual fractions were removed to PVC tubes and their absorption at 280 nm measured by spectrophotometer (Model MOM 203).

Polyacrylamide gel electrophoresis

The gel utilized consist of 10% polyacrylamide gel (PCHZ, Žilina, ČSSR) with 0.2% N,N'-methylenediacrylamide (Koch-Light Lab., England), 0.04% tetramethylethyldiamine (Lachema, ČSSR) and 0.1% sodium dodecylsulphate (Sigma, USA), 0.007% ammonium persulphate (PCHZ, Žilina, ČSSR) in 0.1 M Na phosphate buffer (pH 7). As electrode buffer 0.1 M Na phosphate buffer (pH 7) containing 0.1% sodium dodecylsulphate (SDS) and 0.001 M ethylenediamine tetraacetate (EDTA) was used.

Samples in concentration of about 5 mg/ml were dissociated by solubilization with 1% SDS and 1% 2-mercaptoethanol or 1% sodium thioglycolate, either at 37° C for 3 hours or heated in a boiling water bath for 1 min. After addition of one-tenth volume of 60% sucrose solution containing 0.001 M EDTA the samples were layered onto gels in glass tubes (6×0.5 cm). Pre-electrophoresis was carried out for 1 hour at 200 V/6 tubes. Staining and destaining of the gels were performed according to GORDON (1969).

The molecular weights of the separated protein components were estimated by the method of DUNKER and RUECKERT (1965) and SHAPIRO et al. (1967). As protein markers human serum albumin (Koch-Light), bovine serum albumin (Calbiochem), cytochrome c (Calbiochem), ovoalbumin (Calbiochem) and tobacco mosaic virus were used. Quantitative determination of separated protein components was executed according to ULRYCH and NOVOTNÁ (1969) using a 1 N NaOH elution solution.

Analytical ultracentrifugation

Sedimentation analyses were made with a MOM model 3170 ultracentrifuge. The sedimentation constants were adjusted to the density and viscosity of water at 20 °C. Beforehand, the samples were dialysed against distilled water at 4° C for 48 hours.

Immunochemical analysis

The agar gel diffusion precipitation test of OUCHTERLONY (1948) was adopted, employing 1.5% Difco-Bacto agar gel in pH 8.6 veronal buffer. The centre well for antibody was 6 mm in diameter and peripheral wells for antigen 4 mm. Tests were read after several days' development at room temperature or at 37° C in a saturated atmosphere. Gels were stained with a solution of amidoblack 10 B in methanol and acetic acid (9 : 1) and differentiated by washing in the same solvent without amido black.

Preparation of antibody

Antibody was produced in adult guinea pigs. Anti-AF was prepared by intraperitoneal injection of clarified AF in five doses at three-day intervals. Serum was collected 9 days after the final injection. Anti-AF/10 was prepared in the same way except that prior to immunization AF was concentrated tenfold following the method of KOBER (cited by KEIL and GRUNBERGER, 1959). In both cases the immune serum obtained from the rabbits was concentrated fivefold by lyophilization.

Results

Electrophoresis in 0.16% agarose suspension revealed the presence of at most three protein components in proportions of AF of approximately 7, 1 and 92% (Fig. 1). The major component had an absorption curve nearly corresponding to the non-separated AF.

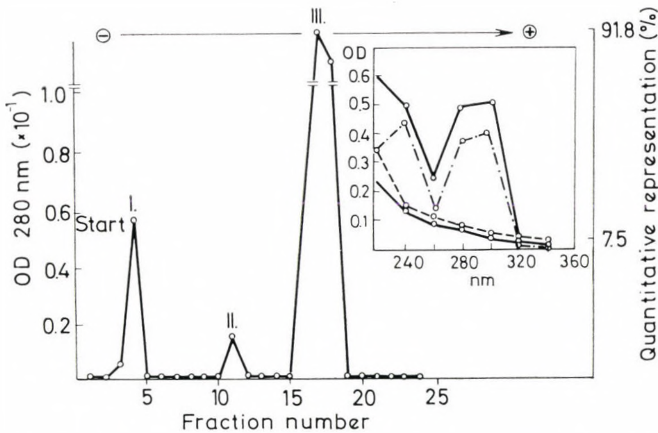

Fig. 1. Electrophoretogram of AF in 0.16% agarose suspension (duration 15 hours, current: 10 mA) the optical extinction curves of the electrophoretically separated components and their quantitative relations: ×—× AF; ○—○ I; ○– – –○ II; ○—·—○ III

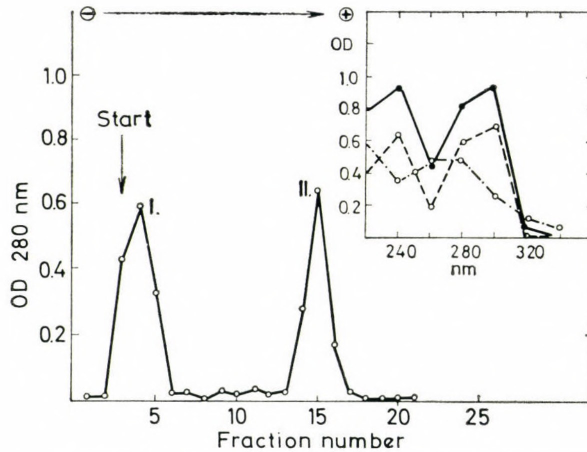

Fig. 2. Electrophoretogram of IAF in 0.16% agarose suspension (duration 15 hours, curren 10 mA) and the optical extinction curves of the electrophoretically separated components. ×—× IAF; ○—•—○ I; ○————○ II

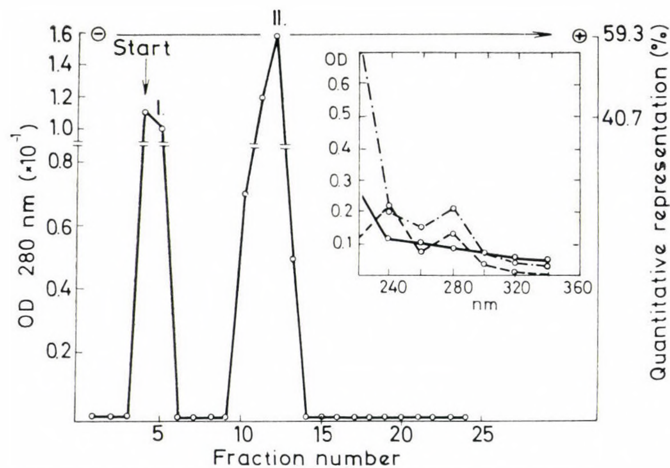

Fig. 3. Electrophoretogram of IAF—O in 0.16% agarose suspension (duration 15 hours, current: 10 mA); the optical extinction curves of electrophoretically separated components and their quantitative relations. ○—•—○ IAF; ○———○ I; ○————○ II

IAF displayed only two protein components in the electrophoresis pattern (Fig. 2), the second component having an absorption curve corresponding to the major component of AF. Study of IAF-O likewise revealed two components (Fig. 3), the first representing about 41% of the total protein and a similar absorption curve to that of the first minor component of AF, the second in an amount of about 59% but with an absorption curve differing from those of all separated components of both AF and IAF.

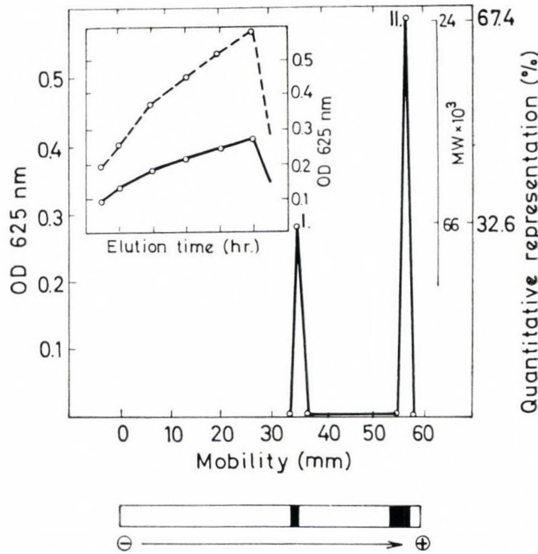

Fig. 4. Electrophoretic mobilities and quantitative relations of the electrophoretically separated components of IAF-O in 10% polyacrylamide agargel (3 hours, 2 mA/gel and estimation of MW single components and dynamics of elution of amidoblack from separated zones. ○—○ I; ○– – – –○ II

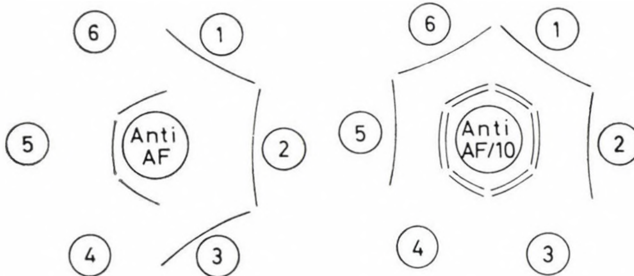

Fig. 5. Immunochemical analysis. The central well contains anti-AF, peripheral wells contain AF (1, 2, 3) and IAF (4, 5, 6)

Fig. 6. Immunochemical analysis. The central well contains anti-AF/10 serum, the peripheral wells contain AF (1, 2), IAF (3, 4) tenfold-concentrated IAF (5, 6)

AF could not be fractionated by polyacrylamide gel electrophoresis, the whole material moving as a single zone with an average electrophoretic mobility of about 1.7 cm/4 hrs. and having an estimated MW of about 90.000 daltons. IAF separated into two distinct zones: a smaller zone comprising about 33% of the material and with an estimated MW of approx. 66.000 daltons; and a major zone containing the remaining 67% of the material and having a MW of approx. 24.000 daltons (Fig. 4).

Sedimentation analysis of AF revealed two components, with sedimentation coefficients of about 25S and 4S. IAF, in contrast, banded into four com-

ponents: one with a sedimentation coefficient of around 15S; a major component with a sedimentation coefficient of about 4S; and two components with sedimentation coefficients over 15S.

Immunichemical analysis of AF with anti-AF revealed only one precipitation line, but using anti-AF/10 three lines were detected (Fig. 5 and 6). Unconcentrated IAF, too, produced only one precipitation line, against anti-AF serum and against the anti-AF/10 two lines were detected. However, when concentrated tenfold on DEAE-cellulose two distinct and one faint line precipitated with the latter serum (Figs 5 and 6).

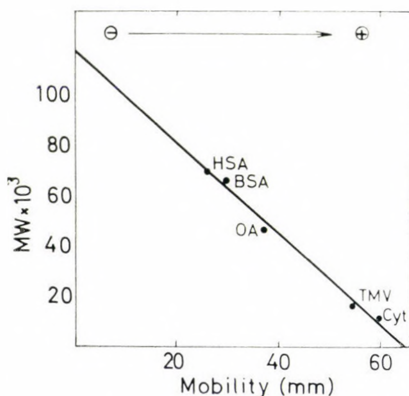

Fig. 7. The linear relationship between molecular weights (MW) of various protein fractions and their mobilities on 10% polyacrylamide gels. Electrophoresis was carried out 1% SDS, 1% 2-mercaptoethanol or sodium thioglycolate in 0.1 M Na phosphate buffer (pH 7). (HSA-human serum albumin, BSA-bovine serum albumin, OA-ovoalbumin, TMV-tobacco mosaic virus, cyt-cytochrome c)

Discussion

The finding of three protein components in AF from 11- to 12-day-old chick embryos is in agreement with the report of SMOCZKIEWICZOWA (1959) who by Tiselius electrophoresis detected components in the proportions 98.1 and 1%. According to SMOCZKIEWICZOWA this protein composition changed by the 14th day of incubation, when up to five protein components were distinguishable. IAF was found to contain at least five protein components of which only two were detectable after high-speed centrifugation. The 25S fraction of AF had an absorption curve corresponding to that of the minor protein component of IAF recovered after high speed centrifugation, which was also found to have lost its haemagglutination activity. This indicates that the alteration of the character of the absorption curve from that for the 25S component of AF was caused by the presence of virus having the same electrophoretic mobility.

Observation of the second IAF component was complicated by its low, near threshold level (about 1%); Immunochemical analysis, however, confirmed its presence. The third major component of AF had an absorption curve corresponding to that of the third component of IAF. Comparison of the two curves shows that the component present in the largest quantity influenced the absorption character of allantoic fluid irrespective of whether the chick embryos were infected with virus or not.

Table I

	Agarose electrophoresis	Polyacrylamide agar gel electrophoresis	Analytical ultracentrifugation	Immunochemical analysis	
				anti-AF	anti-AF/10
A F	3 components 7,1 and 92%	1 component MW 90,000 daltons	2 components 25S and 4 S	1 component	3 components
I A F	2 components	—	4 components	1 component	2 (or 3)* components
	—	—	15S, 4 S and two components over 15 S		
I A F—O	2 components 41 and 59%	2 components MW 66.000 (33%) and 24,000 (67%) daltons	—	—	—

* Three components detected above tenfold, only when IAF.

The collected results (Table 1) indicate that after infection with fowl plague virus the protein composition of chick embryo AF undergoes certain quantitative and qualitative changes. It is known that the protoplasmic microvilli system on the allanto-chorionic membrane can, by its secretional and resorptional activities, directly affect the elaboration and composition of allantoic fluid (Myslivečková 1958, 1963). It may be assumed therefore that the changes induced by virus infection are manifestations of the inhibition of cellular protein synthesis and a profound interference with the metabolism of the embryos.

Inhibition of protein synthesis due to viral infection has so far been described only on the level of tissue culture cells after infection with certain DNA — containing (adenovirus type 2 and 5, herpes simplex virus and vaccinia virus) and RNA — containing viruses (poliovirus 1 and 2, Mengo virus, encephalomyocarditis virus, foot-and-mouth disease virus, Sindbis virus, Venezuelan equine encephalitis virus, Newcastle disease virus, reovirus) (Martin and Kerr, 1968). Presumably the mechanism of the inhibition by fowl plague

virus will turn out to comply with prevailing ideas about how cellular protein synthesis is blocked on invasion with RNA viruses (SUMMERS et al., 1965; MARTIN and KERR, 1968; SOLOVYEV and BALANDIN, 1969). In this question, however, there is certainly difference of opinion. It demonstrates inhibition of cell proteosynthesis in chick embryo cells after infection by Newcastle disease virus (SOLOVYEV and BALANDIN, 1969).

The identity of the two components of IAF with sedimentation coefficients above 15 S remains unresolved. One of them may well represent the virus itself, while the second may be either a non-structural viral polypeptide or, perhaps, a free structural polypeptide produced in surplus in the host cell and not assembled into the virions.

Acknowledgement

The authors would like to thank Miss Maria VIRČÍKOVÁ from the Department of Epizootology, Veterinary University, Košice, for carrying out the ultracentrifugation analyses.

SUMMARY

Using electrophoretical and sedimentation analytical techniques normal allantoic fluid (AF) from 11-to 12-day-old chick embryos was shown to contain at most three protein components: (i) a component with a sedimentation coefficient of about 25S (7%); (ii) a component present in about 1% proportion; and (iii) a component with a sedimentation coefficient of about 4S (92%). Infected allantoic fluid (IAF) from chick embryos infected with fowl plague virus comprised at least five protein components: i) a component with a sedimentation coefficient of about 15S; ii) a component in just detectable amounts; iii) a component with the sedimentation coefficient of about 4S; and iv—v) two or more components with sedimentation coefficients over 15S. After high-speed centrifugation IAF only protein components with estimated molecular weights of about 66,000 and 24,000 daltons, in proportions of 33—41% and 67—59%, respectively, remained.

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INCIDENCE OF CHLORINATED HYDROCARBON PESTICIDES IN MEAT AND MEAT PRODUCTS

By

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In the past decade the scope of food testing has expanded quite considerably. Formerly hygienic control involved mostly detecting food that would be potentially detrimental to the health of the consumer owing to contamination with microorganisms or the toxins they produce. Such control depends in the first place upon organoleptic tests that are completed with laboratory investigations for revealing the microorganisms, usually on a random sampling basis. The continual efforts to increase yield in both crop cultivation and animal breeding sectors, however, have resulted in a great proliferation in the number of organoleptically undetectable, but for the consumer undesirable, chemical residues in products of animal origin. Such substances are demonstrable only by specific laboratory methods for which the hygienic control (institutions) must be specially prepared.

The World Health Organisation (WHO) and the Food and Agricultural Organisation (FAO) have repeatedly called attention to the dangers of undesirable food residues. They have warned that the quantity of herbicidal, rodenticidal, insecticidal, fungicidal and bactericidal substances in food may be so high, especially when they can accumulate in them that their incorporation is likely to be detrimental, carcinogenic or otherwise deleterious to health or working capacity.

Accordingly many countries have legislated to ensure withdrawal of all foodstuffs of animal origin that contain any of these substances if trials should suggest they may have a determinental effect on the consumer.

We have carried out investigations to determine whether and to what extent we must reckon with one class of residues — the chlorinated hydrocarbon pesticides — in the tissues of hog and cattle. In the present work the expression pesticides is used in the same sense as in the joint order No 1/1970 (251) issued by the Hungarian Ministry of Health and the Ministry of Food and Agriculture as a collective term to cover all residues herbicides, insecticides, rodenticides and other chemical substances of veterinary use or biologically active substances and their decomposition or metabolic products. In this wider sense the term includes, however, hormone preparations for promoting growth and weight gain, antibiotics, chemotherapeutics, anti-parasitic drugs, tranquillizers, or other chemical substances too that persist in animal tissues and so pass into the food of the human population.

Sampling

First of all a suitable sampling routine had to be elaborated that would ensure the collection of representative information on the pesticide level of an animal population or a batch of a product. The sampling method was devised

for the veterinary officer, who sends the samples for laboratory testing together with a report filled in according to the supplement No 7. to the Meat Hygiene Regulations of Order No. 1/1964.

The following samples must be submitted for the laboratory control: muscle and fatty tissue, liver and kidney, cocked meat products (canned and semi-preserved conserves), and otherwise preserved (salted 7 dried and ripened) products and stuffed into casings.

At abattoirs producing meat for export a representative series of homogenized muscle samples must be taken daily from the animals slaugthered throughout the day. The greatest composite sample unit is 20 000 kg, and 200 grams of muscle must be taken from every 2 000 kg meat. The samples representing each sample unit (not more than ten) are thoroughly mixed and homogenized and reduced to 100—150 g with mixing and taking the quarter of the amount and sent to the laboratory for analysis as a homogenate in a sealed container with appropriate identification kept 0—4° C by appropriate cooling.

For purposes of controlling individual hog and cattle herds a group of animals originating from the same farm forms one sample unit. In that case samples of muscle, fatty tissue and liver are taken at random. The preparation of the samples for laboratory is the same as above.

Sampling of meat products heat sterilized in containers is carried out on a random basis.

Methods

The chlorinated hydrocarbon pesticides were extracted from the homogenized samples with petroleum ether and purified on a chromatographic column. After evaporating off the solvent qualitative and quantitative determinations were carried out by thin layer- and gas chromatography (Berend et al., 1971; Csonty et al., 1969; Onley, 1964; Soós, 1969; Thompson et al., 1969; Windham, 1969).

Results

The residue concentrations are summarized in the attached five tables so as to allow ready comparison of the results obtained by the thin-layer chromatographic (TLC) and gas chromatographic (GC) methods. The figures for typical pesticide residue levels (Table V) were calculated from the mean values (X) of both TLC and GC results. Minimum and maximum values are recorded to indicate the range of contamination.

Table I

Investigations for Chlorinated Hydrocarbon Pesticides in Pig Total no. of samples: 121

Material total amount sampled	Mean values, range	Method no. of samples	DDT	DDE	DDD	Total DDT	Lindane	α-HCH	β-HCH	δ-HCH	Total HCH+ lindane
			mg/kg								
muscle 16895	$\bar{x}$	TLC 38	0.057	0.035	—	0.093	0.017	0 3	0 2	0 1	0 6
		GC 55	0.038	0.011	0.016	0.065	0.015	0.007	0 2	0	0.007
		TLC+ GC 93	0.046	0.021		0.076	0.016		0 4	0 1	0.004
	min. max.	TLC	0.02 0.16	0.00 0.10	— —	0.04 0.24	0.00 0.04	0.00 0.01	0.00 0.01	0.00 0.01	0.00 0.02
		GC	0.01 0.09	0.002 0.022	0.004 0.030	0.026 0.136	0.005 0.117	0.002 0.024	0.00 0.012	0.00 0.00	0.002 0.024
		TLC+ GC	0.01 0.16	0.00 0.10	0.004 0.03	0.03 0.24	0.00 0.12	0.00 0.024	0.00 0.012	0.00 0.01	0.00 0.024
fatty tissue 914	$\bar{x}$	TLC 4	0.34	0.14	—	0.48	0.04	0.00	0.012	0 1	0.023
		GC 10	0.507	0.195	0.057	0.759	0.087	0.038	0 2	0	0.043
		TLC+ GC	0.14	0.18		0.69	0.07	—	—	0 1	0.04
	min. max.	TLC	0.06 0.50	0.02 0.25	—	0.08 0.75	0.02 0.06	0.0 0.0	0.0 0.02	0.0 0.02	0.01 0.04
		GC	0.170 0.936	0.057 0.400	0.023 0.100	0.280 1.436	0.019 0.455	0.010 0.107	0.0 0.060	0.0 0.0	0.10 0.107
		TLC+ GC	0.06 0.936	0.02 0.40	0.023 0.100	0.080 1.436	0.019 0.455	0.010 0.107	0.0 0.060	0.0 0.02	0.010 0.107
liver 914	$\bar{x}$	TLC 4	0.023	0.01	—	0.032	0.017	0.00	0.005	0.00	0.005
		GC 10	0.025	0.015	0.024	0.060	0.012	0.006	0.005	0.00	0.012
		TLC+ GC 14	0.024	0.013		0.053	0.013	—	0.005	0.00	0.010
	min. max.	TLC	0.02 0.03	0.00 0.02	—	0.03 0.04	0.01 0.02	0.0 0.0	0.0 0.01	0.0 0.0	0.0 0,01
		GC	0.00 0.091	0.00 0.021	0.00 0.046	0.00 0.154	0.00 0.038	0.00 0.012	0.00 0.020	0.00 0.00	0.002 0 031
		TLC+ GC	0.00 0.091	0.00 0.021	0.00 0.046	0.00 0.154	0.00 0.038	0.00 0.012	0.00 0.020	0.00 0.00	0.00 0.031

Table II

Investigations for Chlorinated Hydrocarbon Pesticides from Cattle Total no. of samples: 95

Material total amount sampled	Mean values and range	Method no. of samples	DDT	DDE	DDD	DDT Total	Lindane	α-HCH	β-HCH	δ-HCH	Total HCH lindane
			mg/kg								
muscle 11451	$\bar{x}$	TLC 43	0.039	0.022	—	0.061	0.014	0 9	0 9	0	0 12
		GC 16	0.025	0.01	0.013	0.049	0.012	0.007	0 1	0	0.008
		TLC+GC 59	0.035	0.019	—	0.058	0.013	0.005	0 10	0	0 27
	min. max.	TLC	0.0 0.1	0.0 0.5	—	0.0 0.5	0.0 0.05	0.0 0.04	0.0 0.04	0.0 0.0	0.0 0.08
		GC	0.013 0.04	0.005 0.019	0.006 0.038	0.025 0.088	0.007 0.018	0.004 0.015	—	0.0 0.0	0.004 0.028
		TLC+GC	0.0 0.1	0.0 0.5	—	0.0 0.5	0.0 0.05	0.0 0.04	0.0 0.04	0.0 0.0	0.0 0.08
fatty tissue 5040	$\bar{x}$	TLC 11	0.138	0.25	—	0.39	0.118	0.078	0.092	0 1	0.172
		GC 6	0.154	0.326	0.058	0.52	0.097	0.084	0.01	0	0.092
		TLC+GC 17	0.144	0.28	—	0.44	0.11	0.08	0.06	0 1	0.014
	min. max.	TLC	0.01 0.40	0.04 0.50	—	0.10 0.90	0.02 0.50	0.0 0.5	0.0 0.30	0.0 0.02	0.0 0.062
		GC	0.08 0.315	0.138 0.750	0.017 0.158	0.256 0.890	0.037 0.138	0.020 0.158	0.0 0.02	0.0 0.0	0.020 0.158
		TLC+GC	0.01 0.4	0.04 0.75	—	0.1 0.9	0.02 0.5	0.0 0.5	0.0 0.3	0.0 0.02	0.0 0.62
liver 6240	$\bar{x}$	TLC 13	0.024	0.01	—	0.035	0.014	0.005	0.021	0 1	0.027
		GC 6	0.011	0.012	0.012	0.036	0.008	0.007	0.011	0 1	0.022
		TLC+GC 19	0.02	0.011	—	0.035	0.012	0.006	0.018	0 2	0.026
	min. max.	TLC	0.0 0.05	0.0 0.04	—	0.0 0.09	0.0 0.04	0.0 0.02	0.0 0.1	0.0 0.01	0.0 0.12
		GC	0.0 0.021	0.0 0.020	0.0 0.020	0.0 0.058	0.0 0.017	0.0 0.016	0.0 0.03	0.0 0.018	0.008 0.039
		TLC+GC	0.0 0.05	0.0 0.04	—	0.0 0.09	0.0 0.04	0.0 0.02	0.0 0.1	0.0 0.018	0.0 0.12

Table III

Investigations for Chlorinated Hydrocarbon Pesticides imported Beef and Lard.
Total no. of samples: 11

Material total amount sampled	Mean values of samples	DDT	DDE	DDD	Total DDT	Lindane	α-HCH	β-HCH	δ-HCH	Total HCH lindane
		mg/kg								
Beef (DBR) 50 000 kg	X 3	0.04	0.02	—	0.06	0.013	0.005	Ø	Ø	0.005
Beef (France) 60 000 kg	X 4	0.037	0.012	—	0.05	0.02	0.007	Ø	Ø	0.007
Beef (Holland) 10 000 kg	X 1	0.05	0.02	—	0.07	0.03	0.01	Ø	Ø	0.01
Lard (USSR) 40 000 kg	X 3	0.053	0.02	—	0.073	0.01	0.013	0.033	Ø	0.047

Aldrin + dieldrin were detected in the samples in 8 cases by TLC method:

in 2 samples of hog fatty tissue (0.02—0.04 mg/kg)
in 3 samples of bovine fatty tissue (0.02—0.04 mg/kg)
in 1 sample of bovine muscular tissue (0.01 mg/kg)
in 1 sample of canned ox tongue/totally sterilized (0.03 mg/kg)
in 1 sample of semi-preserved canned ham (0.01 mg/kg)
These quantities were within permissible levels.

Discussion

The detection of chlorinated hydrocarbon pesticid residues a 20 g sample of meat (or meat product), and a 10 g sample of fatty tissue when thin-layer chromatography is applied, while with gas chromatographic method the quantities are respectively 5 and 1 gram only. Moreover the gas chromatographic method is quicker, cheaper, and its results are more accurate. In our estimation the costs are about half these of the thin-layer chromatography. Nevertheless the gas chromatographic method has its own problems and limits. For example on a column packed with 3% SE-30, any β-, γ- and δ-HCH from only a single peak while aldrin forms a common peak with any sulphur occurring in the sample.

This difficulty can be avoided and the HCH isomers separated completely packing the column with 5% OV-17 + 5% SF-96 but such a column is not suitable for routine assays because of the long running time.

Table IV

Investigations for Chlorinated Hydrocarbon Pesticides in Canned Food. Total no. of samples: 43

Material total amount sampled	Mean values and range	Method No. of samples	DDT	DDE	DDD	Total DDT	Lindane	α-HCH	β-HCH	δ-HCH	Total HCH lindane
			mg/kg								
Semi-preserv- ed canned ham 9659	$\bar{x}$	TLC 12	0.026	0 3	—	0.033	0.009	0 1	0 1	0 1	0 2
		GC 9	0.045	0.015	0.014	0.075	0.011	0.009	0 1	0	0.01
		TLC+ GC 21	0.034	0.011		0.051	0.011	0 10	0 2	0	0 11
	min. max	TLC	0.0 0.05	0.0 0.03	—	0.0 0.08	0.0 0.04	0.0 0.01	0.0 0.0	0.0 0.0	0.0 0.01
		GC	0.023 0.086	0.008 0.019	0.006 0.024	0.039 0.128	0.007 0.016	0.005 0.016	0.0 0.013	0.0 0.0	0.005 0.021
		TLC+ GC	0.0 0.086	0.0 0.03	0.006 0.024	0.0 0.128	0.0 0.04	0.0 0.016	0.0 0.04	0.0 0.0	0.0 0.040
Semi-preserv- ed canned shoulder 3519	$\bar{x}$	TLC 6	0.204	0.04	—	0.242	0.01	0	0	0	0
		GC 3	0.046	0.016	0.019	0.074	0.008	0.006	0	0	0.006
		TLC+ GC 9	0,151	0.031	—	0.186	0.011	—	0	0	—
	min. max	TLC	0.0 1.0	0.0 0.2	—	0.0 1.2	0.0 0.025	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
		GC	0.027 0.065	0.010 0.025	0.010 0.017	0.05 0.101	0.005 0.010	0.002 0.012	0.0 0.0	0.0 0.0	0.002 0.012
		TLC+ GC	0.0 1.0	0.0 0.2	0.010 0.017	0.0 1.2	0.0 0.025	0.002 0.012	0.0 0.0	0.0 0.0	0.002 0.012
Miscellaneous totally steri- lised conserv- ed: 4 lb pork loin 4 lb chopped pork picnic ham ox tongue luncheon meat 4 lb pork shashlik pork tongue mortadella beef stew 5901	$\bar{x}$	TLC 6	0.09	0.045	—	0.135	0.023	0 1	0 1	0	0 1
		GC 7	0.062	0.044	0.026	0.133	0.016	0.009	0	0	0.01
		TLC+ GC 13	0.077	0.044	—	0.134	0.02	—	0.0	0.0	—
	min. max.	TLC	0.04 0.15	0.045 0.01	—	0.06 0.25	0.01 0.05	0.0 0.01	0.0 0.02	0.0 0.0	0.0 0.03
		GC	0.015 0.150	0.010 0.084	0.008 0.050	0.033 0.245	0.011 0.028	0.004 0.020	0.0 0.0	0.0 0.0	0.004 0.020
		TLC+ GC	0.04 0.15	0.01 0.1	— —	0.033 0.25	0.01 0.05	— —	0.0 0.0	0.0 0.0	— —

Table V

Mean concentrations of Chlorinated Hydrocarbon Pesticide (in milligram/kg body weight)

Amount sampled	Material no. samples	DDT	DDE	DDD	Total DDT	Lindane	α-HCH	β-HCH	γ-HCH	Total HCH lindane	No. of tests
16 895	pig muscle 93	0.046	0.021	0.016	0.076	0.016	0.007	0	0	0.007	
914	pig fatty tissue 14	0.46	0.18	0.057	0.69	0.07	0.038	0.	0	0.04	121
914	pig liver 14	0.024	0.013	0.024	0.053	0.013	0.006	0.005	0	0.010	
11 451	bovine muscle 59	0.035	0.019	0.013	0.058	0.013	0.005	0	0	0	
5 040	bovine fatty tissue 17	0.144	0.28	0.058	0.44	0.11	0.08	0.06	0	0.014	95
6 240	bovine liver 19	0.02	0.011	0.012	0.035	0.012	0.006	0.018	0	0.026	
50 000 kg	beef (DBR) 3	0.04	0.02	—	0.06	0.013	0.005	0	0	0.005	
60 000 kg	beef (France) 4	0.037	0.012	—	0.05	0.02	0.007	0	0	0.007	11
10 000 kg	beef (Holland) 1	0.05	0.02	—	0.07	0.03	0.01	0	0	0.01	
4 wagon	lard (USSR) 3	0.053	0.02	—	0.075	0.01	0.013	0.033	0	0.047	
9 659 db	semi-preserved canned ham 21	0.034	0.011	0.014	0.051	0.011	0	0	0	0	
3 519 db	semi-preserved canned shoulder 9	0.151	0.031	0.019	0.186	0.011	0.006	0	0	0.006	43
5 901 db	other totally sterilized conserves 13	0.077	0.044	0.026	0.134	0.02	0.009	0	0	0.01	
										Total:	270

Using this latter column a peak was obtained at the place of the standard δ-HCH. This suggests contrary to reports in the literature, that the proportion of δ-isomer among the isomers is not negligible. Further investigations must be carried out to clear up this question.

From a comparison of the recoveries obtained by thinlayer chromatographic and gas chromatographic methods it can be established that the solvent extraction step results in a 10—20% loss of active substances.

During the 8-month investigational period only one sample of semi-preserved canned shoulder contained residues of DDT over the permitted quantity. The quantity of HCH isomers was permissible.

In the future the routine assay for chlorinated hydrocarbon pesticides residues in liver samples can normally be dispensed with, because according to our investigations these residues do not accumulate in this organ. It is still important, however, to test the liver in cases of acute intoxication.

Generally speaking muscle, liver and fatty tissue contained more DDT and HCH than samples from cattle.

Furthermore, fatty tissues contained about 10 times more DDT and 5 times more lindane than muscle tissues.

The figures deriving from the investigations of large animal groups indicate a significant variation in the quantity of pesticide residues between individual farms.

Concentrations of DDT, DDE, DDD, α-, β-, γ-, δ-HCH, aldrin and dieldrin residues were determined parallelly by thin-layer chromatographic and gas chromatographic methods in altogether 93 samples of pig muscle, 14 of pig fatty tissue, 14 of pig liver; 59 samples of bovine muscle, 17 of bovine fatty tissue, 19 of bovine liver; 3 samples of beef from the GRF, 4 from France and one from Holland; 3 samples of lard from the USSR; 21 samples of canned ham, 9 of canned semi-preserved shoulders and 13 of other totally sterilised canned meat products. Sampling was carried out by collecting of composite samples and taken them at random.

A single sample of semi-preserved canned shoulder had a DDT content above the level permitted by current Hungarian regulations.

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ATTEMPTS TO IMMUNIZE GEESE WITH IRRADIATED AND NORMAL LARVAE OF *AMIDOSTOMUM ANSERIS*

By

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Considering the high incidence of amidostomosis in geese in many countries, unduly little information is available on the immunological aspects of this infection. Georgiev (1963) reported first on immunization experiments in which goslings at the age of 15 days were infected each with 25 larvae of *Amidostomum anseris* and challenged with 250 or 1000 larvae either 15, 30 or 60 days later. On the ground of worm burdens, no immunity was observed in any group of animals, neither in those which were rid of the parasites of primary infection by administration of carbon tetrachloride before the secondary infection.

Antigenic properties of adult worms of *A. anseris* were demonstrated by Bausov (1969) in double gel diffusion test in which sera of both rabbits sensitized by worm extracts and those of geese with a naturally acquired infection were employed. Although only low level of precipitating antibodies were detected in the sera of geese, it was concluded that antibodies together with other defensive mechanisms of the host might play certain role in the resistance to the establishment of larger worm populations.

On the supposition that the failure to get an immune response in Georgiev's experiments was perhaps due to the extremely small number of larvae used for primary infection, some tentative experiments have been designed to immunize geese at different age with larger numbers of radiation attenuated and normal larvae of *A. anseris*.

Material and methods

The preparation of infective stage larvae of *A. anseris*, irradiation and infection procedures were the same as described in a previous paper (Phuc and Varga, 1972). Goslings of Rajna breed were obtained from a local hatchery and were housed in a wire floor battery from the first post-hatching day to prevent spontaneous infestation with *Amidostomum*. The animals were allotted into several groups, wingbanded and fed commercial goose mash. On the first post-hatching day, two groups of goslings were dosed with larvae irradiated at 40 kilorad, and this was repeated in one of the two groups one week later. The challenge (and infection of controls) with normal larvae took place two weeks after the second inoculation (Table I). From day 14 of the challenge onwards, droppings of individual birds were daily examined for the presence of *Amidostomum* eggs by the common concentration method, and the number of eggs per gram (EPG) of faeces in samples taken from daily pooled output in each group was determined by the standard McMaster egg counting tech-

Table I

Immunization of goslings with irradiated larvae of *Amidostomum anseris*. First immunization was carried out on the first post-hatching day, and the second one week later. Challenge wit normal larvae took place at the age of three weeks, and the necropsies were performed at the age of seven weeks

No. of goslings	No. of irradiated larvae		No. of normal larvae	Necropsy worm burdens (mean ± standard deviations)	Mean worm lengths (mm ± S. D.)	
	1st dose	2nd dose			♀♀	♂♂
10	1 000	—	500	242.7±121.9[a]	19.1±1.6[b]	13.5±0.8[b]
3	1 000	—	—	32.0± 19.0	15.9±1.9	No males
11	1 000	1 000	500	336.2±192.0[a]	19.0±1.7[b]	13.6±1.0[b]
3	1 000	1 000	—	55.3± 15.0	16.6±2.1	9.3±0.5 (3 males)
11	—	—	500	193.1± 87.0	19.7±1.5	13.9±0.9

Level of significance: a, $p > 0.1$; b, $p < 0.001$

nique. Four weeks after the challenge, all goslings in these groups were killed and the gross patho-morphological changes in the gizzard examined. The worms recovered from the gizzard mucosa were sexed and their length determined by measuring 30 males and 30 females (if possible) selected at random from each animal. However, sterile females apparently arising from the irradiated larvae in the immunized groups were not measured.

At the age of 3 months, a remaining group of goslings were infected with normal larvae of *A. anseris*. After reaching maturity and discharging eggs for 4 weeks, the worms were expelled by treating the geese with 0.01% Ditriphon (trichlorphon) in the drinking water over a week as recommended by Kobulej (1970). Similar treatment was simultaneously carried out also on the uninfected controls. Following a pause of 4 days, the animals were reinfected to be cured in a similar way once more. Subsequently, both the previously infected and control geese were challenged (infected) with normal larvae, egg counts made from the 14th day, killed, etc. as afore-mentioned.

The procedure outlined for 3-month-old geese was carried through also on a group of 14-month-old geese. The latter animals were, however, not reared in the laboratory, but were as adults purchased in a farm with a history of only sporadic incidence of amidostomosis. On arrival to the laboratory, three of the twelve geese passed, however, a few eggs of *Amidostomum* in the faeces. Thus, all the twelve animals were treated with Ditriphon prior to the first experimental infection. The three geese with natural infection and three randomly selected ones constituted the group to be twice infected and treated prior to the challenge, and were later referred to as "immunized" (Table II). The remaining six geese served as control, although their being free of the infection was merely assumed.

Table II

Immunization experiments in 3-month-old and 14-month-old geese with normal larvae of *Amidostomum anseris*. Mature worms developing from the first inoculation were removed by anthelmintic medication prior to the second inoculation, and so were the latters before the third (challenge) infection took place approx. 3 months after the first inoculation. Necropsies were performed four weeks after challenge

No. of geese	Age (months)	Number of larvae per inoculum			Necropsy worm burdens (mean ± S.D.)	Mean worm lengths (mm ± S. D.)	
		1st	2nd	3rd		♀♀	♂♂
5	3	1 200	2 500	1 200	385.2±67.4[c]	11.2±3.5[c]	9.1±2.3[c]
5	3	—	—	1 200	202.4±77.0	14.4±6.8	11.6±2.7
6	14	2 000	2 000	2 000	38.8±31.3[a]	12.6±3.2[c]	9.7±2.8[b]
6	14	—	—	2 000	19.0±24.6	14.6±3.5	10.8±2.6

Level of significance: a, $p > 0.1$; b, $p < 0.05$; c, $p < 0.01$

Table III

Per cent take and length of *Amidostomum anseris* recovered from the gizzard of geese infected at different age and killed four weeks after infection

No. of animals	Age	No. of larvae	Take (mean ± S. D.)	Length of worms (mean in mm ± S. D .	
				♂♂	♀♀
4	2 days	500	77.7± 5.9	13.5±0.8	18.9±1.7
11	3 weeks	500	38.1±17.6	13.9±0.9	19.7±1.5
5	6 months	1200	16.8± 2.1	11.6±2.7	14.4±6.8
6	17 months	2000	0.9± 0.7	10.8±2.6	14.6±3.5

Groups of randomly selected geese at the age of 3 months, and 14 months, respectively, included in Table II, and in addition a group of one-day-old goslings, not included in Table I, were injected intravenously with bovine serum albumin, and bled seven days later. The presence of serum precipitins was scanned by the standard interfacial ring test.

The immunity was assessed by comparing the number and size of worms recovered after challenge in the immunized animals with those obtained from the corresponding controls.

For statistical analysis Student's t-test was applied and the correlation coefficient (r) was calculated.

Results

Each gosling immunized with single or double doses of irradiated larvae as well as the control animals commenced passing eggs of *Amidostomum anseris* in the faeces on the 14th day after challenge (infection) with normal

larvae. Although the EPG varied from 50 to 825 over the two weeks' examination period, the output of eggs in both immunized groups was comparable to that of controls. At post-mortem, severe gross patho-morphological changes characteristic of amidostomosis were found in the control and particularly in the immunized animals. Less severe lesions were also observed in goslings having been inoculated only with irradiated larvae. The latter changes were, however, more extensive than noticed in goslings inoculated with such larvae in a previous experiment (Phuc and Varga, 1972). This may be due to the larger amount of larvae used in the present experiment, and, perhaps, to some under-irradiation as some male worms were traced among the sterile females.

Data presented in Table I attest to a complete lack of immunity in terms of worm burdens. There were more parasites recovered from the immunized goslings than from controls even if the mean number of "vaccine worms" harvested from goslings having received irradiated larvae only was subtracted. The difference between the immunized and control groups was consistent although not significant as judged by the t-test ($p > 0.1$). The difference in size of worms was not conspicuous, yet, the length of both males and females was significantly smaller in the immunized groups than in the controls. Although the mean length of worms was greater in birds with least parasites than in those with heavy worm burdens, the correlation between the length and number of worms in individual hosts was not significant ($r > 1$).

Virtually the same results were obtained with older geese being two times rid of experimental infection with normal larvae by medication prior to the challenge (Table II). Eggs of *Amidostomum* in the faeces of 3-month-old geese (this denotes the age at initial inoculation only; the actual age being approx. six months) first appeared on day 15 after the challenge, whilst this took place on day 23 and 25 in the 14-month-old (in fact: 17) immunized and control geese, respectively. In the faeces of 3-month-old geese, a maximum of 175 EPG were recorded in both immunized and control animals. In view of the very small number of eggs in the faeces of 14-month-old geese, it was impossible to make reliable egg counts. Thus, the presence of eggs was demonstrated only by the concentration method yielding no obvious difference between the immunized and control animals.

At post-mortem, more or less severe lesions were observed in the immunized and control animals alike. A group of randomly selected gizzards of 3-month-old geese are shown in Fig. 1. Various burdens of worms could be harvested from the gizzard of all animals except two controls in the 14-month-old group. Despite the lack of worms in the latter two animals, lesions characteristic of amidostomosis were found in their gizzard. Among the females recovered from geese first inoculated at the age of 3 months, a 10.1% of the worms were thin, containing no eggs in the uterus and measuring 1 to 7 mm less than the rest of normal mature parasites. The sterile worms were mainly

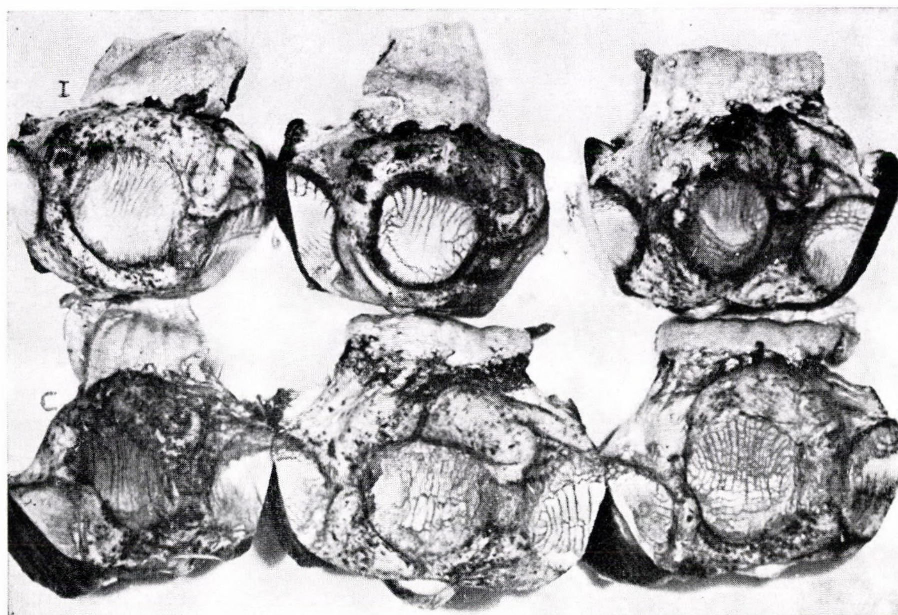

Fig. 1. Lesions in the gizzard of immunized (I) and control (C) geese four weeks after challenge at age of approx. six months. Note the uniformity of lesions in the two groups

located along the round, most dense substance of the horny lining. Similar females amounted to 10.8% in the corresponding control animals. In the 14-month-old groups, there were 58.8% and 40.9% of the females in the immunized and control geese, respectively, showing such an appearance of retarded development. Sterile females could not be encountered in the control goslings included in Table I.

According to the result of the interfacial ring test, none of the 7 goslings injected with bovine serum albumin on the first post-hatching day contained precipitins in the serum, whereas positive reactions with up to 10^{-5} dilutions of 5% bovine serum albumin were recorded for 3 of 8, and 8 of 10 geese which were injected at the age of 3 months or 14 months, respectively.

Discussion

The result of the present experiments is in full agreement with the findings of GEORGIEV (1963) who has shown that the infection of geese with *A. anseris* fails to produce protection against a subsequent re- or superinvasion. NIKOLSKIJ (1966) demonstrated that the goslings were not capable of producing humoral antibodies until an age of 20 to 30 days was reached. The lack of protective effect in the present experiments can, however, hardly be related

to the immunological unresponsiveness in the early post-hatching period of goslings, since no immunity was acquired by the older geese possessing the capacity for production of antibodies not only to so simple antigens as we used in this study, but also to antigenic substances of mature *A. anseris* (BAUSOV, 1969).

The egg counts in the faeces, lesions and the worm burdens are indicative of a complete failure to resist subsequent worm establishment. It is, however, not clear why consistently, though not significantly, more worms could be encountered in the animals with a previous experience of the infection than in those of corresponding controls. It can be left out of consideration that "vaccine worms" not affected by therapy in the 3- and 14-month-old geese might have been inclosed in the worm burdens. The efficacy of the anthelmintic treatment reported as complete by KOBULEJ (1970) was, unfortunately, not checked up by autopsy, nevertheless, disappearance of the *Amidostomum* eggs from the faeces after treatment, and their failure to reappear until the worms developing from the subsequent challenge became mature, attest to high efficacy, indeed. Moreover, the uniformity of lesions in the gizzards of immunized and control geese, in contrast with those of goslings, also suggest that the lesions were produced by, and the worms arouse only from, the challenge infection. The absence of more extensive damage to the mucosa in geese having been inoculated with normal larvae three times altogether can be regarded as circumstantial evidence in support of CVETAYEVA's (1971) observations who showed that the gross patho-morphological lesions in the lining of the gizzard could be repaired in seven days after an anthelmintic medication. Thus, it can also be inferred that the lesions in the gizzards of the two control geese containing no worms at necropsy in the 14-month-old group were liable to have been produced by the experimental infection. Eventually, there remains a guess, namely, the damaged or recently repaired mucosa may rather be favouring, instead of inhibiting, the establishment of new worm population.

The lack of previous experience with this helminth during the earlier life of 14-month-old geese was not guaranteed; in fact, there were three animals passing *Amidostomum* eggs in the faeces at the arrival into the laboratory. It is difficult to tell whether or not the rest of geese had ever been infected; anyway, the two experimental infections failed to reduce the worm burdens arising from the challenge as compared to the corresponding controls, although small per cent take of worms was exhibited in both immunized and control animals. Similar decrease in take of worms was observed also by GEORGIEV (1963) even at younger age of geese proof against spontaneous infection, and it was claimed that the take of worms and the age of host were in reciprocal proportion.

The length of worms in the immunized animals showed a negligible, though statistically significant, reduction.

In comparison with the immunization procedures, the age of the host plays apparently more important role in the establishment of *A. anseris* in geese. This is demonstrated in Table 3 in which figures representing the mean take and length of worms recovered from geese four weeks after a single infection in the course of present and an earlier experiment (PHUC and VARGA, 1972) are collated.

The remarkable decrease in length of worms recovered from the older hosts is obviously due to the increased rate of worms with arrested development in populations where slowly maturing parasites also occur.

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SUMMARY

Goslings at the age of 2 days, and older geese at the age of 3 or 14 months, were administered single or double doses of irradiated, or normal larvae of *Amidostomum anseris.* The geese were rid of the mature parasites by treatment with Ditriphon prior to each infection. On the ground of egg output, worm counts and the gross patho-morphological lesions present in the gizzards four weeks after challenge, no protective immunity could be observed in any group including a total of 54 animals. The length of worms recovered from the immunized animals was slightly reduced as compared to that of controls. A remarkable age resistance operates in elderly animals resulting in a lower take and arrested development of the parasite.

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THE ANTHELMINTIC EFFICACY OF MEBENDAZOLE AND TETRAMISOLE AGAINST *ASCARIDIA GALLI* IN CHICKENS

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The mebendazole — methyl-5(6)-benzoyl-2-benzimidazolyl carbamate — is a comparatively new anthelmintic originally synthesised by the Janssen Pharmaceutica (Beerse) and recently by the Chem. Works of G. Richter Ltd. (Budapest). It is a drab, stable substance, practically insoluble in water and having a molecular weight of 295.29. Mebendazole affects the worms by selectively inhibiting the uptake of glucose (van den BOSSCHE, 1970). According to the manufacturers' informations, an 80 to 90% of the oral dose is excreted mainly as unchanged drug with the faeces from man, dog and rat during the first three days after the administration, whereas a 30 to 50% of the dose is excreted after decarboxylation with the urine of pigs indicating a moderate absorption in this species.

The drug has been shown effective against *Enterobius vermicularis* in man (BRUGMANS et al., 1971), *Strongyloides westeri* (CALLEAR and NEAVE, 1971), *Parascaris equorum* (SAUPE and NITZ, 1972; NEAVE and CALLEAR, 1973), *Strongylus vulgaris, S. equinus, S. edentatus* and small strongyles in horses (SAUPE and NITZ, 1972; WALKER and KNIGHT, 1972; NEAVE and CALLEAR, 1973). No information has yet been available on its activity against *Ascaridia galli* in the chickens, although some action has been anticipated on the ground that, according to the manufacturers' preliminary trials, administration of mebendazole in 0.2% of the food for three days stopped the egg output of *Ascaridia* in the pheasants.

It has been satisfactorily proved by many workers (THIENPONT et al., 1966; BRUYNOOGHE et al., 1968; KLEINSCHMIDT, 1969; ENIGK, 1969; GRABER, 1969; ALERGANT, 1969a; CLARKSON and BEG, 1970; BENNEJEAN et al., 1970; BIROVÁ and BIRÓ, 1970; OVIES et al. 1971; SHOR, 1971; VELICHKHIN et al., 1972) that tetramisole at a dose level of approx. 40 mg. per kg. bodyweight administered individually, or mixed either in the food or dissolved in the drinking water, was highly effective against both mature and immature population of *A. galli.*

In the present paper, laboratory trials are described in which the anthelmintic efficacy of single doses of mebendazole and that of a 40 mg. per kg. dose of tetramisole were compared by treating chickens at different times after experimental infection with larvae of *A. galli.*

Material and methods

Both the mebendazole and tetramisole were prepared and supplied in form of powder by the Chem. Works of G. Richter Ltd. The tetramisole was

dissolved, and drench of mebendazole was prepared by agitation, in tap water before application.

PILCH-DEKALB hybrid chickens were obtained from a local hatchery, housed in a brooder, and fed half-and-half commercial chicken mash and cob-meal. At age between first post-hatching day and six weeks, groups of chickens were inoculated with larvae of *A. galli* (see Table I, II and III). The presence of mature worms in specific groups of chickens was based on detection of worm eggs in the faeces using the flotation technique. Prior to the treatment, the chickens were randomized into small groups, wing-banded and weighed. The

Table I

Anthelmintic effect of mebendazole and tetramisole against four-week-old populations of *Ascaridia galli* in chickens

Group	Dosage mg /kg	Age of chickens (days)	No. of larvae	No. of chickens	No. of worms		Per cent. reduction compared to controls
		At infection		Infected/Total	Mean	Range	
Mebendazole	2000	7	750	0/5	0	0	100
Mebendazole	1000	7	750	0/5	0	0	100
Mebendazole	0.1% of food fed for 3 days	7	750	0/5	0	0	100
Tetramisole	40	7	750	1/5	7.2	0— 36	90.7
Controls	—	7	750	5/5	77.2	19—110	—
Mebendazole	500	21	750	0/5	0	0	100
Mebendazole	250	21	750	0/5	0	0	100
Mebendazole	100	21	750	0/5	0	0	100
Controls	—	21	750	5/5	15.4	1—27	—

appropriate dosage per kg. body-weight was individually administered to the chickens by intubation of the crop on one occasion at one, four, seven or eight weeks, respectively, after the infection (Table I, II and III). A single group of animals were treated with 0.1% of mebendazole in the ratio fed for three days.

On day 6 after the medication, the chickens were killed following a starvation period of 12 hours'. The worms and larvae were removed from the lumen of the intestine by flushing with tap water, were harvested and counted. Larvae in the gut contents were traced under a dissecting microscope. After flushing, the intestine was cut into 5 cm. pieces, pressed in compressorium and larvae remaining in the mucosa were also counted under dissecting microscope. The efficacy of the anthelmintic treatment was evaluated by comparing the mean worm burdens, including the "mucosa larvae", recovered from the treated and untreated control birds.

Table II

Efficacy of mebendazole and tetramisole against mature *Ascaridia galli*

Group	Dosage mg./kg.	Age of chickens (days) At infection	No. of larvae At infection	No. of chickens Infected/Total	No. of worms Mean	No. of worms Range	Per cent. reduction compared to controls
Mebendazole	500	7	750	0/6	0	0	100
Mebendazole	250	7	750	0/6	0	0	100
Mebendazole	100	7	750	0/6	0	0	100
Tetramisole	40	7	750	0/5	0	0	100
Controls	—	7	750	4/6	5.8	0— 13	—
Mebendazole	200*	1	1000	0/10	0	0	100
Mebendazole	100*	1	1000	0/10	0	0	100
Mebendazole	50*	1	1000	0/10	0	0	100
Tetramisole	40	1	1000	4/10	2.4	0— 17	91.8
Controls	—	1	1000	8/10	29.4	0—159	—
Mebendazole	50	1	1000	1/10	0.1	0—1	99.4
Mebendazole	25	1	1000	3/10	0.6	0—3	96.4
Tetramisole	40	1	1000	2/10	0.3	0— 2	98.4
Controls	—	1	1000	8/10	16.6	0—55	—

* Groups treated 7 weeks after infection; other groups were treated 8 weeks after infection

Results

The arrangement and the result of the anthelmintic tests are summarized in the Table I, II and III.

In the first, tentative experiment mebendazole was given at a rather high dosage rate (Table I) to explore how such doses are tolerated and whether a simple treatment proves effective against the immature parasites at all. No toxicity was noticed following the treatment; at the same time, a 100% efficacy was observed in all groups except for those treated with tetramisole. A complete anthelmintic effect was achieved in other groups even if the dosage rate was reduced to 1/20, i.e. 100 mg. per kg. bodyweight.

Both mebendazole and tetramisole proved highly effective against the mature worms (Table II). Worms passing in the faeces of the chickens were observed over 2 and 4 days after treatment with tetramisole and mebendazole, respectively.

A remarkable action against the one-week-old (2nd stage) larvae was shown in groups treated with either drug (Table III).

Table III

Efficacy of mebendazole and tetramisole against one-week-old larvae of *Ascaridia galli*

Group	Dosage mg./kg.	Age of chickens (days)	No. of larvae	No. of chickens Infected/Total	No. of worms		Per cent. reduction compared to controls
		At infection			Mean	Range	
Mebendazole	250	42	900	0/5	0	0	100
Mebendazole	100	42	900	1/5	0.2	0—1	99.5
Tetramisole	40	42	900	0/5	0	0	100
Controls	—	42	900	6/6	45.3	6—214	—
Mebendazole	200	1	500	0/5	0	0	100
Mebendazole	100	1	500	0/5	0	0	100
Mebendazole	50	1	500	0/5	0	0	100
Tetramisole	40	1	500	1/5	0.4	0—2	99.8
Controls	—	1	500	6/6	283.5	113—423	—
Mebendazole	40	1	500	6/6	167.7	38—427	17.7
Mebendazole	20	1	500	7/7	196.4	42—301	5.5
Mebendazole	10	1	500	7/7	278.6	76—430	0
Controls	—	1	500	7/7	203.7	129—345	—

Discussion

The result of the present study is based on experiments including limited numbers of animals in a group, yet, it seems to be beyond doubt that the mebendazole is a highly effective drug against *A. galli* in the chickens, and the efficiency obtained with tetramisole corresponds to that reported earlier by other investigators.

The mebendazole at doses of 50 mg. per kg. bodyweight and upwards attests to an excellent anthelmintic activity against both mature and immature parasites. The efficacy of mebendazole surpasses markedly that of thiabendazole, another benzimidazole derivate, reported to exhibit only a 57% efficiency against one-week-old larvae at dosage rate of 1000 mg. per kg. bodyweight (Long and Wakelin, 1964). Mebendazole seems to meet many demands put on up-to-date anthelmintics; the most noteworthy being that a single treatment is satisfactory to remove virtually all worms of *Ascaridia* irrespective of their stage of development. Thus, its application makes both the control and treatment a distinct possibility. Mebendazole has a wide therapeutic index; in the present study a single dose of 2000 mg. per kg. bodyweight caused no untoward effect. Attention should, however, be paid to an observa-

tion, namely, that administration of the drug in the food at 500 ppm for 4 days, or at 125 ppm for 1 month may diminish the egg production in laying hens (Temmerman, 1971).

In the present trials relatively large doses of mebendazole were applied to procure excellent and consistent results in the chickens as compared with the standard 100 mg. dose reported as effective against *Enterobius vermicularis* in humans (Brugmans et al., 1971) and either standard 4000 mg. (Saupe and Nitz, 1972) or 5—10 mg. per kg. bodyweight dose levels used with success against strongyle and parascarid nematodes in the horses (Callear and Neave, 1971; Walker and Knight, 1972; Neave and Callear, 1973). Although the enormous gap between the result of treatment by 40 and 50 mg. per kg. (Table III) certainly arises from some technical error, a dose of not less than 50 mg. per kg. is recommended for obtaining good results with single mebendazole treatment in the chickens.

In agreement with others' findings, cited earlier, the tetramisole proved very potent against *A. galli* also in the present study. However, a 100% activity was not consistently attained with a 40 mg. per kg. dose, and the therapeutic index is less wide than that of mebendazole. Thus, it has been suggested that tetramisole should be administered at 50 mg. per kg. dosage rate in ascaridiosis (Graber, 1969). A further increase of the dose level cannot be advised. Although a single dose of 550 mg. per kg. (Graber, 1969), and 2750 mg. per kg. (Alergant, 1969b) was only reported as toxic, a dosage of 80 mg. per kg. bodyweight applied in the food may decrease the food consumption of the chickens (Velichkhin et al., 1972). A transient anorexia and decrease of egg production in hens was observed even after a treatment with 60 mg. per kg. dose of tetramisole (Dovadola and Carlotto, 1970). The egg production, weight gain and food conversion rate in the broiler chickens was not influenced by a single or repeated treatment with tetramisole at dosage rate of 40 mg. per kg. (Marsboom and Thienpont, 1967). Bruynooghe et al. (1968) claimed an increased hatching rate from eggs laid after chickens being treated with tetramisole at dosage rate of 40 mg. per kg. but this was not confirmed by other studies; in fact, Bennejean et al. (1970) reported on a slight decrease instead.

It is a notable advantage of the application of tetramisole over that of mebendazole that the former drug can easily be administered in the drinking water. Although the time needed for the intake of drug containing water is extended by 8 hours, the delayed water consumption causes no interference with the efficiency of the treatment (Bruynooghe et al., 1968; Bennejean et al., 1970).

Bearing in mind that a single dosage of tetramisole at 25 mg. per kg. yields a 91—98% anthelmintic efficacy against *A. galli* (Clarkson and Beg, 1970), it seems worthy to be examined whether 25 mg. per kg. dosages given

on two consecutive days either in the food or in drinking water would prove a more advisable alternative of the therapy. It is expected that such regime might entail less untoward consequencies and a more perfect cure.

SUMMARY

Between the age of day 1 and 42, a total of 211 chickens were artificially infected with larvae of *Ascaridia galli* on one occasion, and groups consisting of 5 to 10 animals were individually administered single doses of mebendazole, or tetramisole at dosage rate of 40 mg. per kg. bodyweight 8, 7, 4 or 1 week after infection. The anthelmintic efficacy of the drugs was assessed by comparing the worm burdens in the treated and control animals at post-mortem carried out 6 days following the treatment,

The per cent. reduction of worms was as follows:

a) *mature worms*

tetramisole: 100; 91.8; 98.4,
mebendazole at 100 mg. per kg.: 100; 100,
mebendazole at 50 mg. per kg.: 100; 99.4,
mebendazole at 25 mg. per kg.: 96.4,

b) *4 weeks after infection*

tetramisole: 90.7,
mebendazole at 100 mg. per kg.: 100,

c) *1 week after infection*

tetramisole: 100; 99.8,
mebendazole at 100 mg. per kg.: 99.5; 100,
mebendazole at 50 mg. per kg.: 100.
mebendazole at 40 mg. per kg.: 17.7,
mebendazole at 20 mg. per kg.: 5.5,
mebendazole at 10 mg. per kg.: 0

No side-effects could be noted in any of the chickens given a single dose of mebendazole as high as 2000 mg. per kg.

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PATHOLOGICAL CHANGES CAUSED BY TREMATODES IN THE URINARY SYSTEM OF A *CONSTRICTOR CONSTRICTOR*

By

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(Received February 25, 1973)

Kidneys of giant snakes (*Boidae* and *Pithonidae*) are frequently invaded by flukes of the genus of *Styphlodora* Looss, 1899. Nearly 20 species of *Styphlodora* have been described from various reptile hosts so far. Despite high incidence of these trematodes, little is known about the pathology of the infection. The reason for this may well be that gross pathological changes are not invariably caused by this parasite. There has been only a single work published (Grünberg and Kutzer, 1964) dealing with pathological alterations produced by *Styphlodora renalis*, *S. elegans* and *S. horridum* in total of 8 giant snakes (4 *Python sebae*, 1 *P. molurus*, 1 *P. reticulatus*, 1 *P. regius* and 1 *Constrictor constrictor*, respectively). According to this report, the invasion brings about urinogenic nephritis, formation of urate deposits, and always results in uricaemia.

Our case provides a further information on the pathological significance of the species of genus *Styphlodora*.

Material and methods

Carcase of an approximately 5-year-old *Constrictor constrictor* has been delivered to the of Department Pathology for routine diagnostic procedure. Parts of kidney showing pathological changes were fixed in 10% formaldehyde and then embedded in paraffin. The histological cuts were stained with haematoxiline-eosine, according to van Gieson and Giemsa, and, in addition, the PAS, Schultz-Schmidt, Perls and Kossa tests were performed with them.

Results

Pathological changes were detected only in the urinary system of the snake. The left kidney was normal, while the size of the right one markedly diminished and showed greyish-white, congested, cigar-shaped appearance (Fig. 1). The capsule of the latter kidney was thickened and the contracted lobes united so that the normal lobed structure was difficult to recognize. On cutting, small cracking was noticed, and on the cut surface numerous sub-

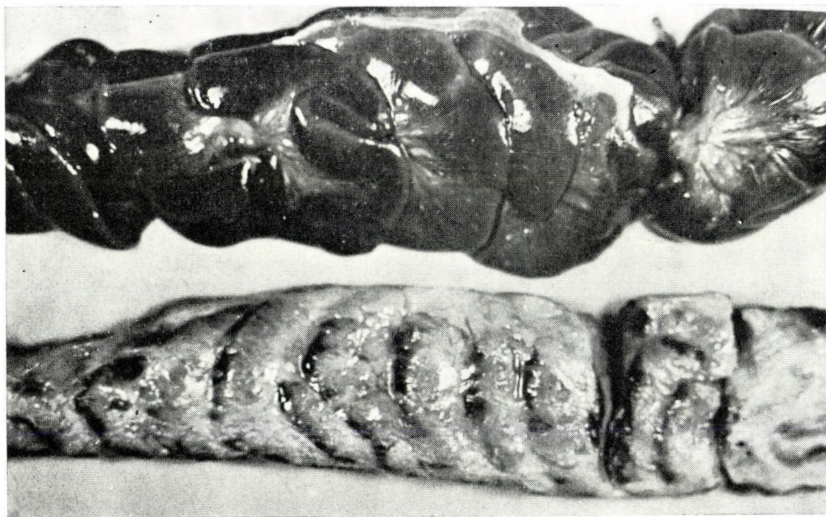

Fig. 1. Above the normal left, below the markedly deminished right kidney

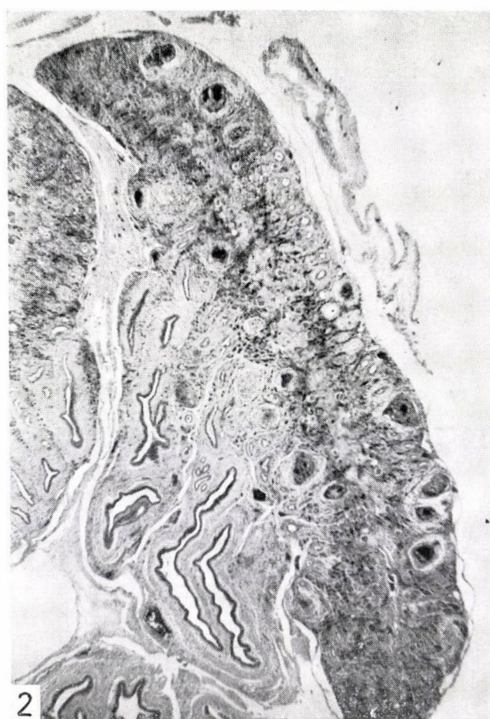

Fig. 2. In a lobe in large number of collecting tubes pathological changes can be seen. Haem.-eos

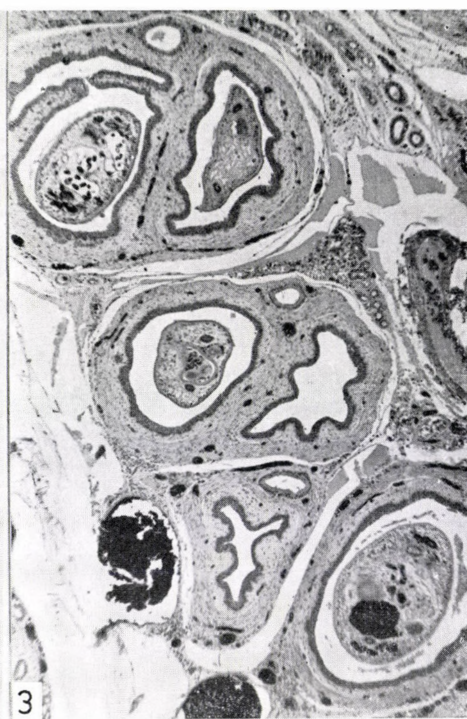

Fig. 3. Live parasites in the main collecting tubes. Haem.-eos. ×50

tle stripes and foci were shown being surrounded by fibrous tissue and containing greyish-white debris.

The proximal portion of the right ureter was largely dilated. Its wall was about 2 to 3 mm thick and the lumen filled with pastery mass and sticky mucous matter containing also many trematodes. The wall of the left ureter was but slightly thickened, and a few trematodes were covered by some mucus in the lumen.

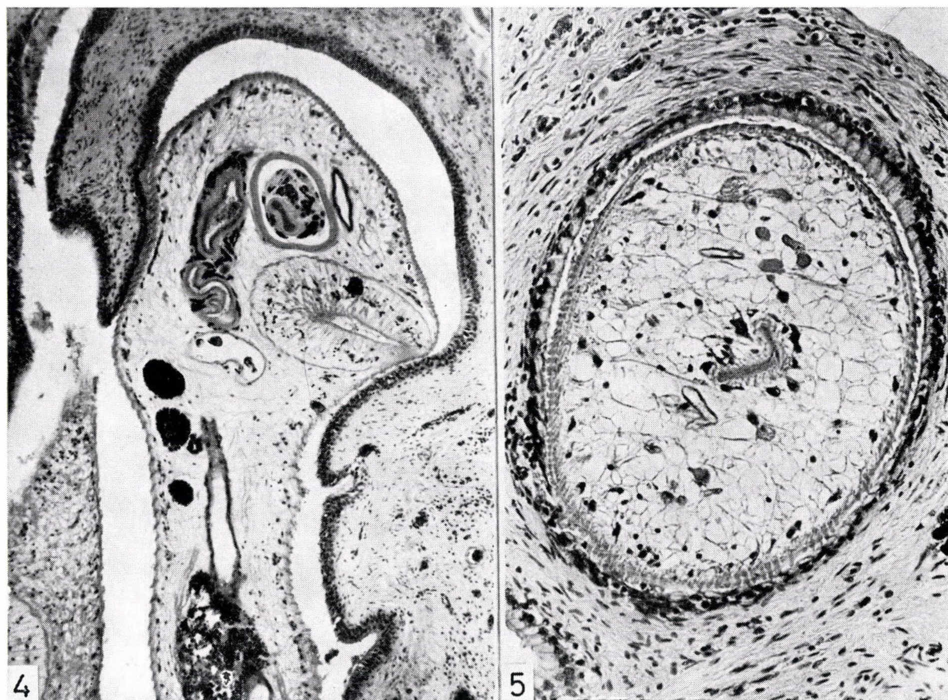

Fig. 4. Living fluke cut longitudinally in the main collecting tube. Haem.-eos. ×110

Fig. 5. Transversal cut of a fluke entirely filling up the lumen of a urinary tube with undamaged epithelial lining. Haem.-eos. ×200

Characteristic histopathological lesions were produced by the species of *Styphlodora condita* De Faria, 1911 in the urinary tube system (chiefly in the main collecting tubes and collecting tubules (Fig. 2) and in the ureter of right kidney. A number of intact parasites were found in the larger tubes (ureter and main collecting tubes), although they failed to block completely the lumen (Fig. 3). The epithelial lining was undamaged in some of these areas (Figs 4, 5). In sites where the ventral suckers of the flukes attached to the wall of the tubes mushroom-shaped protrusions of the wall could be observed (Fig. 6). Epithelial proliferation or regressive alterations extending to larger

or smaller portions of some tubes were also seen (Fig. 7). The latter were represented by formation of vacuoles and other necrobiotic changes within the epithelial cells, and desquamation, respectively (Fig. 8). More or less extensive acute cellular infiltration (Fig. 7) or proliferation of budding connective and young fibrous tissue was shown in the interstitial tissue around the larger tubes with more severe regressive changes within the epithelial cells. Among the inflammatory cells there were many of them containing eosinophilic granules

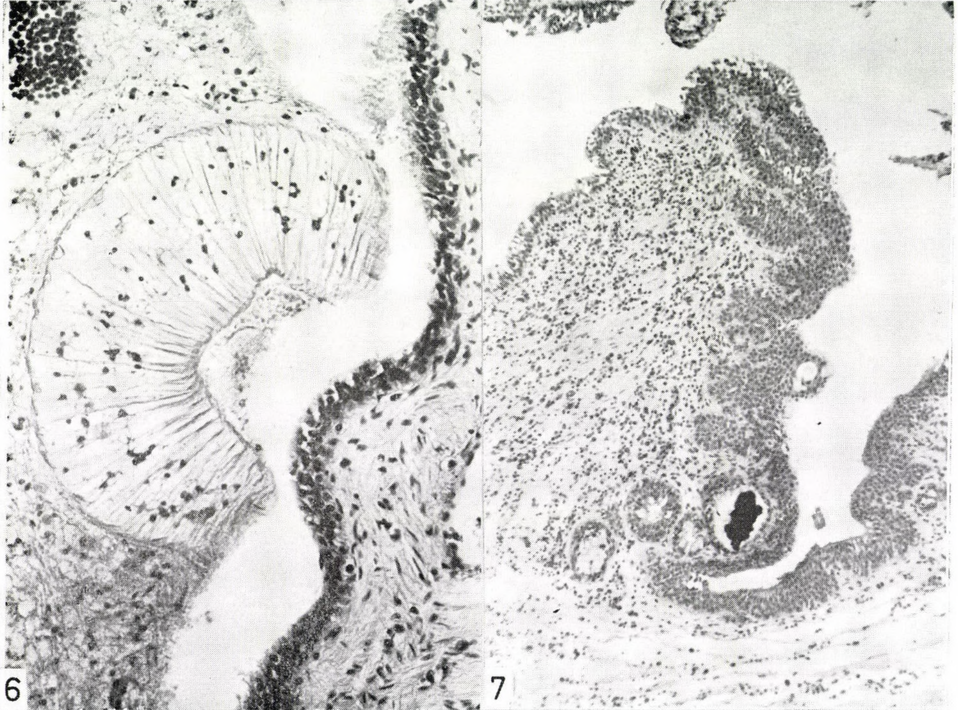

Fig. 6. Mushroom like protrusion of the wall of a urinary tube on the level of ventral succer. Haem.-eos. $\times 200$

Fig. 7. Intensive epithelial proliferation in a larger urinary tube. Inflammatory infiltration in the surrounding tissue. Haem.-eos. $\times 75$

(Fig. 9). Proliferation of the connective tissue was most conspicuous in the ureter resulting in the thickening of its wall.

More severe lesions were found in the narrower tubes (mainly in the collecting tubules). The lumen was generally much dilated and filled with amorphous lumpy material (Fig. 10), staining purplish red with haematoxylin and eosin, in which eggs and debris of parasite origin were occasionally embedded (Fig. 11). On the ground of histochemical examination, the bulk of the amorphous material consisted of urates (bright green colour in Schultz-

Schmidt test; Fig. 12), and contained minor amounts of calcium salts (positive Kossa test; Fig. 13) and mucopolysacharids (positive PAS test; Fig. 14) the latter deriving probably from disintegrated parasites. The narrower collecting tubules were in places derived of epithelial lining being replaced by giant cells in some areas (Fig. 15). The wall of the tubules consisted of fibrous tissue stretching also into the interstitial tissue. Among other inflammatory cells in the budding tissue, there was an accumulation of cells with eosino-

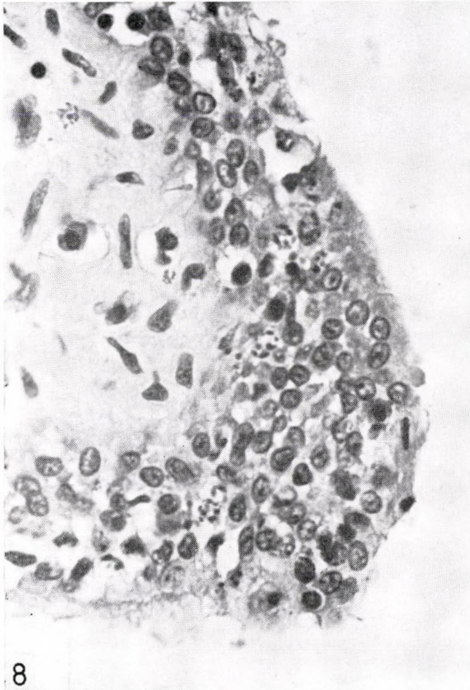

Fig. 8. Regressive changes in the proliferating epithel. Haem.-eos. ×275

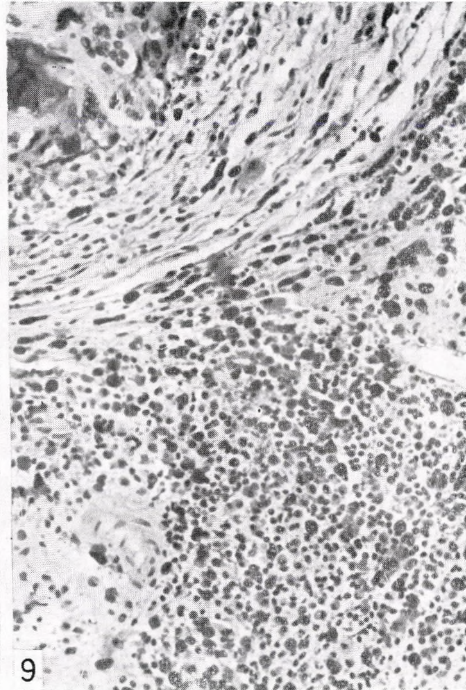

Fig. 9. Great number of big cells with eosinophile granulation are seen among the inflammatory cells surrounding the damaged urinary tube. Haem.-eos. ×200

philic granules. Extensive proliferation of the interstitial connective tissue accounts for the atrophy of many of the urinary tubules adjacent to the glomeruli while large amounts of urate granules were deposited both within the cytoplasm of the epithelial cells and in the lumen of intact tubules (Fig. 16). Atrophy was observed in some of the glomeruli whereas thickening of the Bowman's capsules due to the proliferation of the connective tissue was seen in others (Fig. 17).

Discussion

The microscopical lesions indicate that the trematodes inhabited mainly the lower portions (ureter, main collecting tubes and distal part of collecting tubules) of the urinary tubes of *Constrictor constrictor*. More or less intact parasites were recovered from larger urinary tubes (ureter and main collecting tubes) where generally minor regressive and inflammatory changes occurred

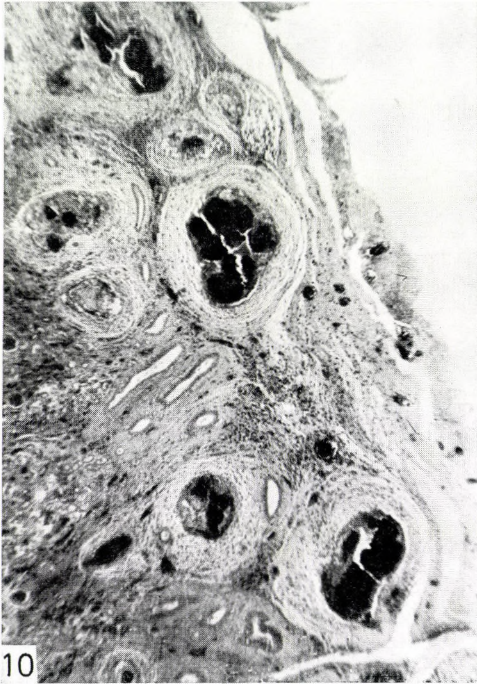

Fig. 10. Concrements in the lumen of main collecting tubes. Haem.-eos. ×50

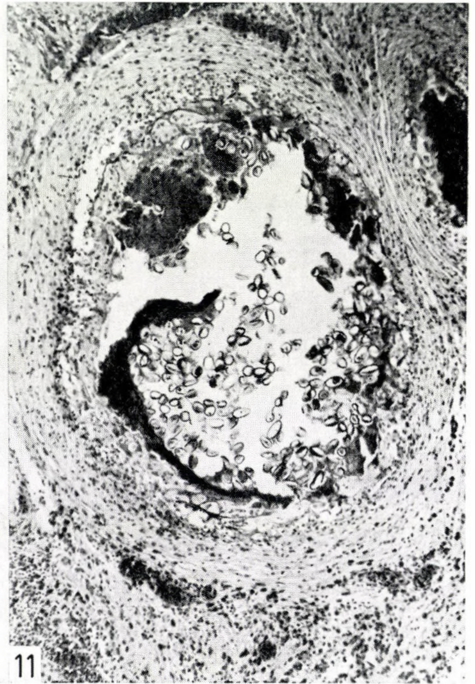

Fig. 11. The remainders of a fully desintegrated parasite in a urinary tube with heavy regressive alterations. Haem.-eos. ×110

only. Large amounts of deposits enough to block the lumen here were not produced either. However, as a result of mechanical and toxic effect of the flukes severe regressive and inflammatory changes were brought about in the proximal narrow portions of the urinary tubes (in the collecting tubules). The acute interstitial inflammation was followed by chronic changes including proliferation of the connective tissue. Accumulation of cells with eosinophilic granules was a characteristic feature of the infiltrated area. The chronic alterations in the interstitial tissue created nephrosclerosis, and the pressure of the budding tissue caused atrophy of the tubules. Moreover, large amounts of

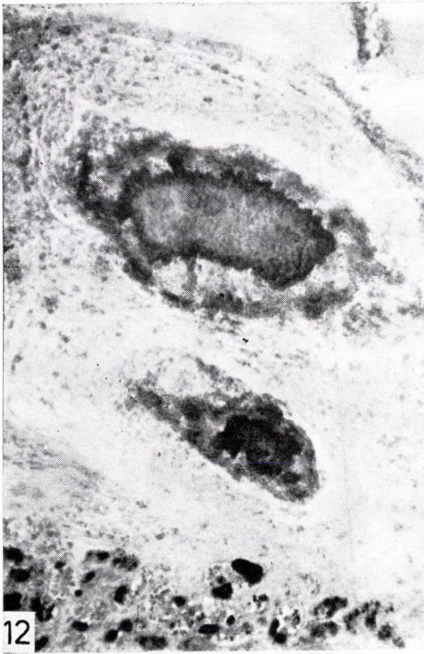

Fig. 12. The concrements consist mainly of urate salts stained darker. Schultz-Schmidt's test. ×110

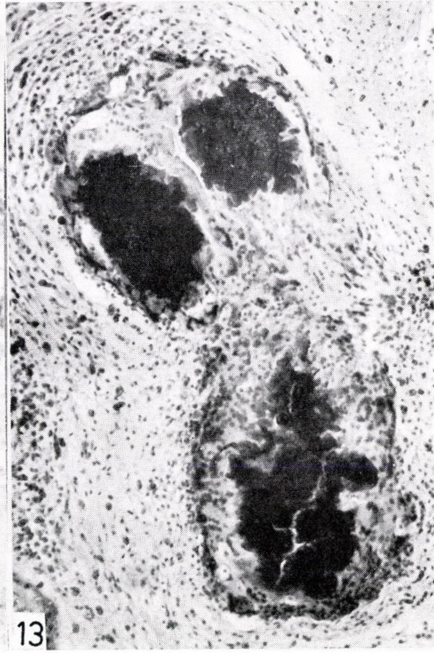

Fig. 13. Calcic salts in the concrements. Kossa's test. ×110

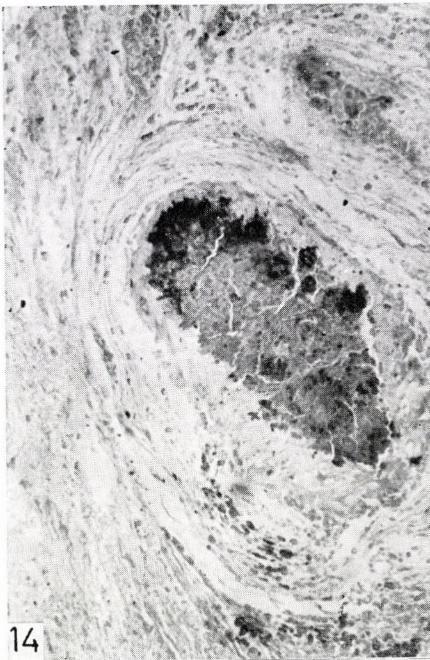

Fig. 14. PAS positive material in the concrements. ×110

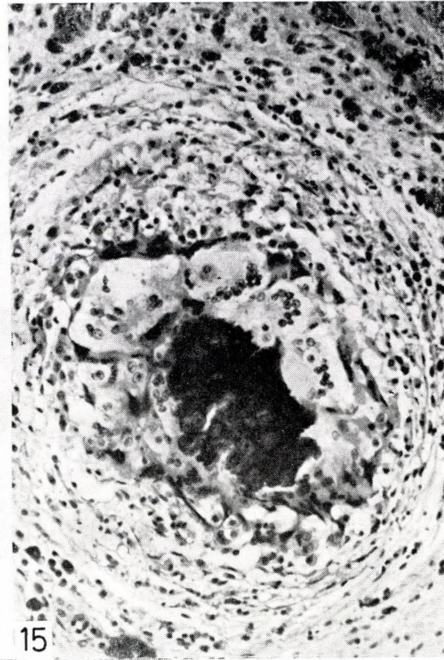

Fig. 15. Giant cells around the concrements on place of the damaged epithelial lining. Haem.-eos. ×200

deposit were produced here which entailed obstruction of the narrow tubules. Disintegrated parasites, cells and cellular detritus may have acted as a nucleus for the formation of deposits consisting mainly of urates and partly of calcium salts. It seems characteristic of this infection that the narrower the urinary tubes are the more severe lesions occur.

In accordance with the studies of GRÜNBERG and KUTZER (1964), our case suggests that flukes belonging to the genus of *Styphlodora* should not be considered as harmless parasites, since heavy infection of the proximal narrow portion of the urinary tubes may cause severe pathological condition and even death of the snakes.

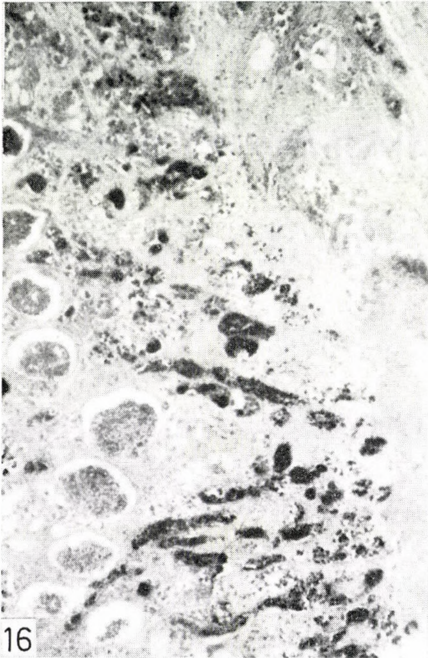

Fig. 16. Granules consisting of urate salts in the connecting part. Schultz-Schmidt's test. ×110

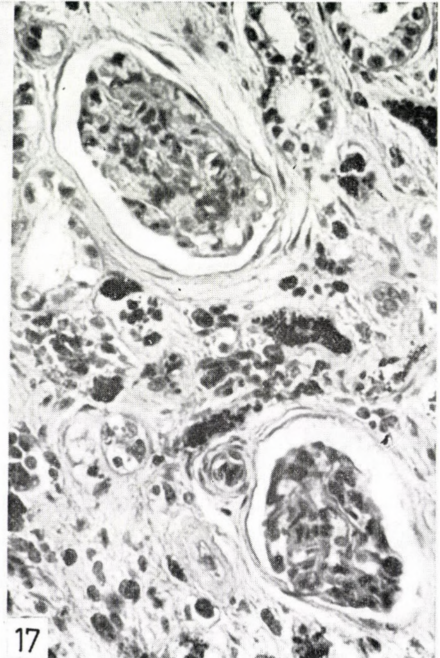

Fig. 17. Bowman's capsule has thickened and starting sclerotic alterations can be seen in the glomeruli. Haem.-eos. ×200

It remains, however, to be answered whether the death of our *Constrictor constrictor* was due to the lesions afore-mentioned. It has been assumed that alterations in the right kidney were, indeed, severe hardly enabling the kidney to perform even a decreased function. This suggestion can be explained by both the marked sclerosis in certain areas of the kidney and the complete stop of the function of glomeruli and that of tubules, respectively, on account of the mechanical obstruction of the collecting tubules. If this sort of changes

had occurred also in the left kidney it would certainly have given rise to uraemia. The normal function of the left kidney was not necessarily impaired as several flukes were only found in the ureter, and the kidney looked otherwise normal. Anyway, pathological changes beside those of the kidneys, which might account for the death of the snake, were not encountered in other organs of the carcase.

SUMMARY

Lesions produced by flukes of the species of *Styphlodora condita* De Faria, 1911 in the urinary tubes (ureter and collecting tubes) of an approximately 5-year-old *Constrictor constrictor* have been described. Pathological changes associated with the presence of the parasites were as follows: regressive changes and deposits in the urinary tube system, interstitial nephritis and nephrosclerosis. The severe alterations observed were liable to result in hypofunction of the kidney.

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ACTA VETERINARIA

ТОМ. 23 — ВЫП. 2

РЕЗЮМЕ

ДЕЙСТВИЕ ОДНОЙ БЕЗАНТИБИОТИЧЕСКОЙ КОРМОДОБАВКИ НА КОЛИБАЦИЛЛЕЗ И РАЗВИТИЕ ТЕЛЯТ И ПОРОСЯТ

Б. ЮХАС, Л. БОНЭ и З. КУНФИ

Тестмикс 232 (Стимулекс фабрики БИОФАК, Копенгаген) является препаратом, который не содержит антибиотика. Авторами доказано, что заблаговременным применением и соответствующей дозировкой препарата можно лечить колибациллезные гастроэнтериты новорожденных и отечную болезнь телят и поросят. Препарат с успехом можно применить и для профилактических целей. Привесы и поедаемость корма у животных, обработанных препаратом Тестмикс 232 или получавших этот препарат с кормом-стартером, формировались лучше, чем у контрольных таковых.

ЗНАЧЕНИЕ ФАГОВОЙ САЛМОНЕЛЛЕЗНОЙ O_1 И R ПРОБЫ ДЛЯ ЛАБОРАТОРНОЙ ПРАКТИКИ

Я. ТАКАЧ и ДЬ. НАДЬ

Применением сальмонеллезного штамма 4618 изучена эффективность фаговой салмонеллезной O_1 и R пробы в улучшении диагноза на салмонеллез. 29,96% изученных штаммов были резистентными к фагу и 70,03% дали положительную реакцию с фагом. Лучшие результаты получены с салмонеллезными видами D, C_1, C_2, D серологической группы O. Обнаружено, что салмонеллезные виды E_1, E_2 и E_4 серологической группы O только на 50% резистентны к фагу. Таблицы и рисунки информируют и о салмонеллезном фаге O_1 и R. На основании анализа богатых данных авторы считают лишним пользоваться в практической диагностической работе кроме салмонеллезного фага O_1 и салмонеллезным фагом R. Фаг R — в случаях, когда не получено эффекта от фага O_1 — вызывал бактериолиз только в 0,37%-ах случаев. Авторы рекомендуют включить салмонеллезный фаг O_1 в диагностическую практику, ибо он может быть очень полезным в случаях дифференциации фагустойчивых видов Bethesda Ballerup из рода Citrobacter от салмонелл. При помощи фаговой пробы O_1 двухфазовые виды из группы Arizona тоже диагносцируемы, ибо у них фаг O_1 тоже вызывает бактериолиз.

ОБНАРУЖИВАЕМОСТЬ САЛМОНЕЛЛ В ПРОДУКТАХ ЖИВОТНОГО ПРОИСХОЖДЕНИЯ В ГГ 1969—1970

ТАКАЧ Я. и Б-НЭ НАДЬ ДЬ.

В годах 1969—1970 в продуктах животного происхождения авторами типизировано 43 вида салмонелл. Большинство материала для исследования поступало из лабораторий при бойнях, его меньшая часть из других ветеринарных учреждений.

Изолированные виды принадлежали к O-антигенным группам B, C_1, C_2, D_1, E_1, E_4, $Б_2$ и L. Среди убойных животных свинья является наибольшим резервуаром салмонелл; из этого животного в 820 случаях (46,11%) изолировано всего 19 видов рода Salmonella. Из крупного рогатого скота в 34 случаях (1,91%) изолировано 14 видов, из телят в 7 случаях (0,39%) — 3, из лошадей в 6 случаях (0,33%) —4, из куриных в 29 случаях (1,63%) —7, из гусей в 15 случаях (0,83%) —1, из индеек в 4 случаях (0,22%) —1, из уток в 6 слу-

чаях (0,33%) —2, из голубей в 6 случаях (0,33%) тоже 2 вида. Макроскопическими методами классической ветсанэкспертизы нельзя распознать бессимптомных носителей и выделителей салмонелл. Этим объясняется тот факт, что при регулярном контроле мясных продуктов и рабочих мест большинство этих видов можно обнаружить. В борьбе с инфекциями людей при каждом звене инфекционной цепи необходимо строго соблюдать требования гигиены, но особенно важно подчеркнуть значение обезвреження от салмонелл кормов животного происхождения. Вероятно они являются источником появления с года в год новых типов, раньше не обнаруженных в нашей стране. В указанный выше период обнаружены 5 новых вида, а именно, S. paratyphi — B. var. java, S. blockley, S. minnesota, S. kapemba и новый салмонеллезный вид с антигенной структурой 4,12d пока не вкладывающийся в схему Кауфман—Хвайт.

ФОРМИРОВАНИЕ МИКРОБИОЛОГИЧЕСКИХ СВОЙСТВ ЗАМОРОЖЕННЫХ БЫСТРЫМ СПОСОБОМ ГОТОВЫХ ПИЩЕВЫХ ПРОДУКТОВ В ЗАВИСИМОСТИ ОТ СПОСОБА УПАКОВКИ И СРОКА ХРАНЕНИЯ

Я. ТАКАЧ, Л. БЕНЕДЕК и Э. ИМРЕ

Изучено формирование микробиологических свойств быстрым способом замороженных готовых пищевых продуктов в зависимости от способа упаковки и срока хранения. При этом сравнивались средние логарифмические показатели титров и количества микробов в продуктах, наполненных холодным фаршем и храненных в замороженном виде с таковыми продуктов, наполненных горячим фаршем и храненных в замороженном виде. Определили, насколько снизились средние логарифмические показатели засоренности микробами (в %-ах) продуктов, наполненных холодным фаршем после 6-месячного хранения при температуре —20 °C в сравнении с таковыми в продуктах, наполнявшихся холодным фаршем и не хранившихся в замороженном виде. Кроме этого определили, в сколько %-ов ниже средние логарифмические показатели засоренности микробами продуктов, наполнявшихся горячим фаршем и нехранившихся в замороженном виде в сравнении с таковыми продуктов, наполнявшихся холодным фаршем и хранившихся в замоиоженном виде. Этими исследованиями доказано, что загрязнение микробами от орудий т человека после обработки температурой и до замороження можно снизить до минимума dолько применением технологии горячего наполнения. Обнаружено, что эта технология снижает количество микробов на 94,1—100% по сравнению с продуктами, наполнявшимися холодным фаршем и хранившимися в замороженном виде. Обнаружено дальше и то, что количество непатогенных, токсин непродуцирующих микробов, характеризующих гигиеническое состояние продуктов, за время 6-месячного хранения при температуре —20 °C снизилось на 61,4—98,2%. Это намного выше процентного значения, фигурирующего среди литературных данных. Необходимо подчеркнуть однако и то, что из процентного снижения показателей количества непатогенных и токсин непродуцирующих микробов не явствует, что такое же положение будет и в случаях, если в продуктах имеются патогенные или токсин продуцирующие микробы.

Таким образом при наличии патогенных и токсин продуцирующих микробов заморože ние и хранение при температуре —20 °C не может быть условием декларации годным для потребления в пищу данного продукта. Из этого явствует, что снижение количества микробов за время хранения в замороженном виде еще не является доказательством того, что засоренный патогенными микробами продукт полностью освобождается от них; в лучшем случае снизится их количество.

МИКРОБИОЛОГИЧЕСКОЕ ИЗУЧЕНИЕ ИМПОРТНОЙ ЗАМОРОЖЕННОЙ РЫБЫ И РЫБНЫХ ПРОДУКТОВ

Я. ТАКАЧ и Э. ИМРЕ

В материале трех лет (1969—71) определялись средние показатели количества микробов и их титра, их распределение и процентное соотношение в замороженной рыбе и рыбном филэ. На основании полученных данных высчитаны значения m c, и M, чтобы дать рекомендации к разработке микробиологических требований к замороженной рыбе и рыбному филэ. Рекомендуемые нами значения m, c, и M мы сравнили с соответствующими значениями ICMSF (International Committee of Microbiological Specifications of Foods) и таковыми португальского стандарта. Ради лучшей наглядности крайние значе-

ния микробиологической оценки собраны в таблице 9. В этой таблице нефигурирующие патогенные, токсин продуцирующие микробы, вредные для здоровья человека, само собой разумеется, не должны быть ни в замороженной рыбе, ни в рыбном филэ. Рекомендуется, чтобы при оценке замороженного филэ и рыбных продуктов изучались минимально по 5 образца, среди которых выраженные в с терпимые значения могут быть максимально в двух образцах и в трех они должны быть ниже терпимых значений, отраженных в с. Если среди 5 образцов даже одно характеризуется значением, отраженным в **М**, то от пользования партией, из которой брали образцы, нужно отказаться.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ АЛЛАНТОИДНОЙ ЖИДКОСТИ ВИРУСОМ ЧУМЫ ПТИЦ ЗАРАЖЕННЫХ И НЕЗАРАЖЕННЫХ ЭМБРИОНОВ ЦЫПЛЯТ

Ф. ЛЕШНИК и О. Й. ВРТЯК

Применяя технику электрофореза и седиментационной аналитики выявлено, что нормальная аллантоидная жидкость 11—12-дневных эмбрионов цыплят содержит три протеиновых фракции: 1. компонент с коэфициентом седиментации 25S (5%); 2. компонент налицевствующий в соотношении 1%; 3. компонент с коэфициентом седиментации 4S (92%). Аллантоидная жидкость зараженных вирусом чумы птиц эмбрионов цыплят содержит не меньше пяти протеиновых компонентов: 1. компонент с коэфициентом седиментации около 15S (7%); 2. компонент, обнаруживаемый в следах; 3. компонент с коэфициентом седиментации около 4S; 4—5. два или больше компонентов с коэфициентом седиментации выше 15S. После ультрацентрифугирования в зараженной аллантоидной жидкости остались только два протеиновых компонента, молекулярного веса 66000 и 24 000 далтонов, при соотношении 33—41 и 67—59%, соответственно.

ИЗУЧЕНИЕ СОДЕРЖАНИЯ ХЛОРИРОВАННЫХ УГЛЕВОДОРОДОВ В МЯСЕ И МЯСНЫХ ПРОДУКТАХ

З. ШИМОНФИ, Э. ЯНЧО и Я. ТАКАЧ

В согласии с совместным распоряжением министерств здравоохранения и сельского хозяйства и пищевой промышленности авторами изучалось содержание ДДТ, ДДЭ, ДДД α, β, γ и δ изомеров гексахлорана, алдрина и диелдрина в мясе и мясных продуктах. Исследования проводились методами тонкослойной и газовой хроматографий. В изученном материале было 93 образца свинины, 14 образцов свиного жира, 14 свиных печеней, 59 образцов говядины, 17 образцов говядиного сала, 19 печеней крупного рогатого скота, 3 образца импортного мяса (ФРГ), 4 образца импортного мяса (Голландия), 3 образца импортного свиного жира (СССР), 21 коробка консервной ветчины, 9 коробок консервной лопаточной части, 13 коробок других консервов. Образцы брались единым способом.

Авторами обнаружено, что, согласно дейсттвующему распоряжению, только в одной коробочной ветчинной консерве было содержание ДДТ выше уровня, которое является терпимым.

ОПЫТ ИММУНИЗАЦИИ ГУСЯТ ПРИ ПОМОЩИ ОБЛУЧЕННЫХ И НОРМАЛЬНЫХ ИНВАЗИОННЫХ ЛИЧИНОК

Amidostomum anseris

Ц. В. ФУК и И. ВАРГА

Гусята возраста 2 дней и 3—14-месячный гусиный молодняк инвазировались разовыми дозами облученных и нормальных личинок Amidostomum anseris. До этого животных освободили от половозрелых амидостом дегельминтизацией Дитрифоном (хлорофос). На основании выделения яиц, количества нематод и патологоанатомических изменений в мышечном желудке при вскрытии 4 недели после реинвазии не удалось обнаружить наличия иммунитета нив одной среди групп, насчитывающих всего 54 животных. Размеры нематод, обнаруженных в желудках иммунизированных птиц были немножко меньше таковых нематод из неиммунизированных животных. Имеется заметная возрастная резистентность, обусловливающая поселение меньшего количества и замедление развития нематод.

АНТГЕЛЬМИНТНАЯ ЭФФЕКТИВНОСТЬ МЕБЕНДАЗОЛА И ТЕТРАМИЗОЛА ПРИ АСКАРИДИОЗЕ ЦЫПЛЯТ

И. Варга

Всего 211 цыплят возраста от 1 по 42 дня искусственно инвазировано инвазионными личинками Ascaridia galli и группы животных по 5 и 10 цыплят дегельминтизированы индивидуально разными разовыми дозами мебендазола и тетрамизола в дозе 40 мг/кг 8, 7(а), 4(б) и 1(в) недель спустя от инвазии. Эффективность дегельминтизации оценена на основании вскрытия после 6 дней от дегельминтизации животных и сравнения количества аскаридий, обнаруженных в дегельминтизированных и контрольных животных. Обнаружена следующая И. Э. (в %-ах) от вмешательства:

а) половозрелые нематоды
тетрамизол: 100; 91,8; 98,4
мебендазол при дозе 100 мг/кг: 100; 100
мебендазол при дозе 50 мг/кг: 94,4; 100
мебендазол при дозе 25 мг/кг: 96,4

б) 4 недели после инвазии
тетрамизол: 90,7
мебендазол при дозе 100 мг/кг: 100

в) 1 неделю после инвазии
тетрамизол: 99,8; 100
мебендазол при дозе 100 мг/кг: 100; 99,5
мебендазол при дозе 50 мг/кг: 100

У цыплят не обнаружено побочных явлений даже от разовых доз мебендазола порядка 2000 мг/кг.

ПАТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В ПРОТОКАХ ВЫДЕЛИТЕЛЬНОЙ СИСТЕМЫ CONSTRICTOR CONSTRICTOR, ВЫЗВАННЫЕ ТРЕМАТОДАМИ

Э. ТУРИ и Т. КОБУЛЕЙ

Описываются патологические изменения в протоках выделительной системы (мочеводы, собирательные каналы) приблизительно 5-летнего удава Constrictor constrictor, вызванные трематодами рода Styphlodora condita. Трематоды вызывают следующие изменения: регрессивные изменения, отложения в протоках выделительной системы, интерстициальный нефрит и нефросклероз. Причиной тяжелых изменений явилась гипофункция пораженной почки.

D, C_1, C_2, D E_1, E_2 E_4 Bethesda Ballerup Citrobacter Arizona S. paratyphi — B. var. java, S. blockley, S. minnesota, S. kapemba 4.12d
ICMSF (International Committee of Microbiological Specifications of Foods)
Amidostomum anseris.
Ascaridia galli Constrictor constrictor, Styphlodora condita.

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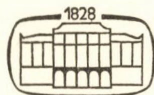

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ДИНАМИКА ИЗМЕНЕНИЙ СЫВОРОТОЧНЫХ БЕЛКОВ (PHASIANUS COLCHICUS) В ПЕРИОД ПОСЛЕИНКУБАЦИОННОГО ОНТОГЕНЕЗА

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(Поступило 22 марта 1971 г.)

Вв едение

В последние годы в сельском хозяйстве применяется неисчислимое количество химических веществ, направленных на защиту урожая от разнообразных вредителей. Наряду со своим бесспорно защитным и полезным действием, химическое вмешательство может неблагоприятно воздействовать на биологию охотничьей дичи, живущей в химически обработанном биотопе. Для определения степени отрицательного влияния химизации на хозяйственные потери у дичи надо знать основные физиологические и биохимические показатели дичи, применимые для изучения также ее субклинического повреждения под влиянием химизации. Большинство биохимических показателей даже у здоровых животных меняется под влиянием роста, созревания, питания, больше того, они могут зависеть от сезона отлова, пола и др. факторов.

В нашей работе мы устремились на изучение тех показателей, надежность которых в указанном аспекте нами уже ранее проверена как на домашней птице, так и на фазанах (Сова и Коудела, 1969; Сова с сотр., 1971; Коудела и Сова, 1970) в послеинкубационный период.

Материал и методика

Из фазанов (Phasianus colchicus) Научно-исследовательского института лесного и охотничьего хозяйства в Збраславе в период от июня до августа 1970 г. в возрасте 0, 3, 8, 14, 21, 29, 45, 65 и 96 дней убивали всегда по 10 цыплят разного пола сначала декапитацией, а позже уколом в сердце. Для исследований использовали сыворотку крови и печень, которые сразу же препарировали и до начала исследований хранили на сухом льду.

Фазанята вылуплялись в инкубаторе и на первой неделе жизни содержались в брудере при 30 °C в течение дальнейших 3 недель — в выгуле на деревянном полу под брудергаусом, а начиная с месячного возраста они свободно выходили в выгулы.

Таблица I

Количество всего сывороточного белка (г%) и белковых фракций (%, г%) в крови

Возраст фазанов (в днях)	n % г%	К-во всего белка			Альбумин		
		x̄	s	v	x̄	s	v
0	10 %				42,6	1,22	2,9
	г%	2,62	0,18	6,3	1,11	0,05	4,7
3	10 %				41,0	0,28	0,7
	г%	2,35	0,07	3,0	0,97	0,04	3,2
8	10 %				40,0	0,29	0,7
	г%	2,65	0,10	3,8	1,06	0,05	3,4
14	10 %				39,7	0,30	0,8
	г%	2,60	0,23	8,9	1,03	0,10	9,2
21	10 %				40,1	0,81	2,0
	г%	2,92	0,10	3,3	1,17	0,03	2,9
29	10 %				39,9	0,99	2,5
	г%	3,14	0,09	2,8	1,25	0,02	1,7
45	10 %				39,7	0,41	1,0
	г%	3,88	0,19	4,9	1,54	0,08	5,0
65	10 %				39,2	0,71	1,8
	г%	3,80	0,19	4,9	1,49	0,08	5,2
96	10 %				41,6	2,38	5,0
	г%	4,11	0,20	4,9	1,71	0,14	8,0

С первого дня жизни цыплят кормили гранулированной смесью БЖ-1 (гранулят размера 2×5 мм) в течение 7 недель, а затем БЖ-2 (гранулят размера 3×6 мм). До 6-недельного возраста им добавляли тысячелистник (**Achilles millefolium**) и крапиву (**Urtica dioica**), а спустя 6 недель в выгул им давали и салат (**Lactuca sativa**), где они могли дополнить свой рацион ограниченным количеством травы и насекомыми.

В период онтогенеза у фазанов определяли количество всего сывороточного белка по биуретовому методу (Горжейши, 1964), белковые фракции при помощи бумажного электрофореза (при 300 V по 6,5 часа), оксалат-цитрат-веронал буфер при pH 8,6 и мощности ионов 0,06. Для окраски применяли бромфенолевую синь; фракции оценивались при помощи экстинционного регистратора ERI-65 и выражались как в относительных %, так и в г%.

Статистическая обработка результатов осуществлялась в Вычислительном институте Сельскохозяйственного института в Праге на Минске 22, программа стандартная, S-метод (Шеффес, 1963). Результаты сведены в таблице I и рисунках 1 и 2.

Статистическая оценка дана в таблицах II—IV.

Дискуссия

В доступной литературе мы не нашли данных о показателях всего белка (ВБ) и белковых фракций у молодых фазанов в период онтогенеза. Имеются, напр., данные Бекер и Манвел (1966), изучавших, помимо

фазанов в период онтогенеза

α-глобулин			β-глобулин			γ-глобулин		
$\bar{x}$	s	v	—	s	v	$\bar{x}$	s	v
18,9	1,09	5,8	20,6	0,66	3,2	17,9	0,43	2,4
0,5	0,07	13,2	0,54	0,05	8,4	0,49	0,02	4,9
18,6	0,85	4,6	19,8	0,85	4,3	20,6	0,28	1,4
0,44	0,04	8,1	0,47	0,01	1,5	0,49	0,01	1,4
19,0	0,51	2,6	19,6	0,59	3,0	21,6	0,95	4,4
0,50	0,03	6,6	0,52	0,03	6,5	0,57	—	—
19,1	0,74	3,9	20,1	0,44	2,2	21,0	1,37	6,5
0,49	0,06	11,5	0,52	0,05	9,6	0,55	0,04	7,6
18,1	0,81	4,5	19,5	1,39	7,1	22,3	2,03	9,1
0,53	0,03	5,9	0,57	0,05	8,9	0,63	0,06	9,6
18,2	1,12	6,2	19,7	0,67	3,4	22,2	0,96	4,3
0,57	0,05	8,2	0,61	0,00	4,4	0,70	0,04	5,2
17,8	0,55	3,1	19,7	0,30	1,5	22,7	0,73	3,2
0,69	0,02	2,9	0,76	0,05	6,2	0,88	0,06	6,7
19,1	0,36	1,9	20,4	0,47	2,3	21,2	0,99	4,6
0,73	0,05	6,7	0,78	0,05	6,5	0,80	0,04	5,0
18,2	0,91	5,0	18,3	1,43	7,8	21,8	2,2	0,1
0,77	0,05	6,7	0,75	0,05	6,9	0,89	0,07	7,4

прочего, у 12—14-недельных фазанов некоторые сывороточные фракции (α-глобулин, γ-глобулин, трансферин, гаптоглобин, церулоплазмин), но не с точки зрения онтогенеза и другой методикой. Результаты приводятся в табл. I и снимках 1 и 2, где показано, что у молодых фазанов, как и у других цыплят (Коудела с сотр., 1971) после вылупления можно наблюдать слабое понижение ВБ, что с возрастом постепенно растет. На 5-й день жизни у куриных цыплят ВБ равняется $2{,}46 \pm 0{,}22$ г%-ам, а у 3-дневных фазанят — $2{,}35 \pm 0{,}07$ г%-ам, у 2-месячных цыплят он же равняется $4{,}85 \pm 0{,}51$ г%-ам, а у 2-месячных фазанят — $4{,}11 \pm 0{,}2$ г%-ам. Если учесть некоторую разницу в диете одних и других, то между уровнем ВБ у фазанят и цыплят имеется совсем незначительная разница.

Анализ дисперсии при двойной сортировке показал значительные различия ($p < 0{,}005$) в г% общей протеинемии между последующими днями послеинкубационного онтогенетического ряда (см. табл. II).

С белковыми фракциями получается иная картина. Относительные %-ы альбумина у фазанят слабо понизились с $42{,}6 \pm 1{,}22$ в день вылупления до $39{,}2 \pm 0{,}7$ на 65-ый день жизни. Наоборот получаются абсолютные показатели уровня альбумина (благодаря постепенному росту ВБ): в первые 14 дней он был почти одинаковым, а при дальнейших измерениях его абсолютное количество возросло до 1,71 г% у 96-дневных фазанов, значит по сравнению с показателем при вылуплении 1,11 г% оно увеличилось более, чем на 60%. Эти данные не аналогичны тем, которые мы наблюдали у цыплят, где отмечено

Таблица II

Статистически достоверная разница в количестве всего белка в крови между фазанятами разного возраста ($p < 0,05$)

Возраст в днях	0	3	8	14	21	29	45	65	96
0	×	—	—	—	—	+	+	+	+
3	—	×	—	—	—	+	+	+	+
8	—	—	×	—	—	+	+	+	+
14	—	—	—	×	—	+	+	+	+
21	—	—	—	—	×	+	+	+	+
29	+	+	+	+	+	×	+	+	+
45	+	+	+	+	+	+	×	—	—
65	+	+	+	+	+	+	—	×	—
96	+	+	+	+	+	+	—	—	×

неравномерное колебание альбумина в период послеинкубационного онтогенеза в масштабе от 39,5% в 0-ой день по 50,5% на 17-ый день.

При помощи анализа дисперсии двойной классификацией были установлены статистически достоверные различия ($p < 0,05$) в г% альбумина между последующими днями послеинкубационного ряда (см. табл. III).

Если показатели протеинемии выражались в относительных %-ах, значительные различия наблюдались между 0-ым и 65-ым днями жизни.

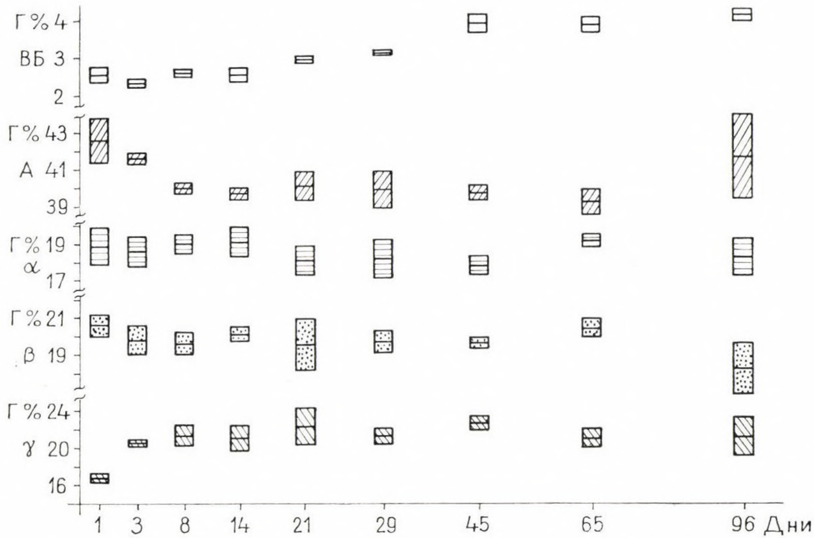
α-глобулин в относительных %-ах колебался в незначительном масштабе ($18,9 \pm 1,1\%$ в 0-ой день до $18,2 \pm 0,9\%$ на 96-ой день с минимумом $17,8 \pm 0,6\%$ на 45-ый день и максимумом $19 \pm 0,4\%$ на 65-ый день).

При выражении в г% в ходе развития α-глобулины постепенно возросли, что соответствует постепенному повышению ВБ. При вылуплении уровень

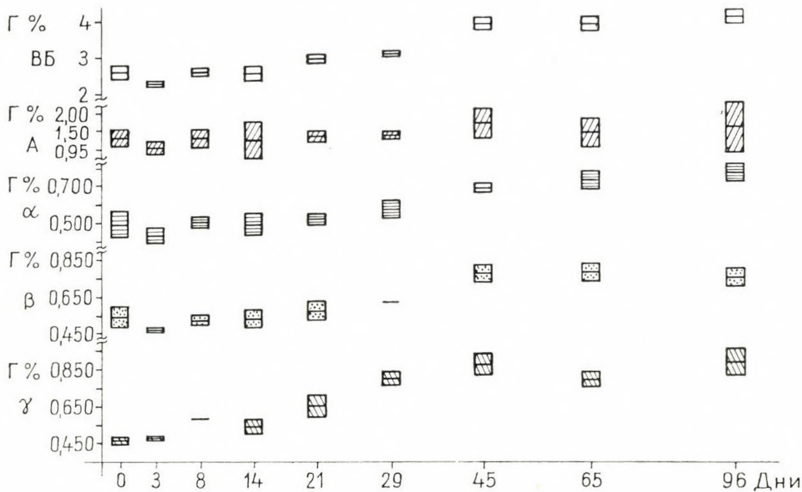
Таблица III

Статистически достоверная разница в количестве сывороточного альбумина (+) (в 2 %) между разными возрастными группами фазанят ($p < 0,05$)

Возраст в днях	0	3	8	14	21	29	45	65	96
0	×	—	—	—	—	—	+	+	+
3	—	×	—	—	—	+	+	+	+
8	—	—	×	—	—	—	+	+	+
14	—	—	—	×	—	—	+	+	+
21	—	—	—	—	×	—	+	+	+
29	—	+	—	—	—	×	+	+	+
45	+	+	+	+	+	+	×	—	—
65	+	+	+	+	+	+	—	×	—
96	+	+	+	+	+	+	—	—	×



Сн. 1. Весь белок и белковые фракции в крови фазанят в период раннего онтогенеза в течение послеинкубационного развития
Белковые фракции выражены в относительных %-ах



Сн. 2. Весь белок и белковые фракции у фазанят в период раннего онтогенеза в течение послеинкубационного развития
Белковые фракции выражены в г%-ах

α-глобулина равняется 0,5 $\pm$ 0,07 г%-ам; в возрасте 96 дней — 0,77 $\pm$ 0,05 г%-ам (следовательно примерно на 50% выше).

Анализ дисперсии показал при двойной классификации статистически достоверные различия ($p < 0{,}05$) в г% α-глобулина между последующими днями послеинкубационного онтогенетического ряда (см. табл. IV).

Таблица IV

Статистически достоверная разница в количестве сывороточного α-глобулина (+) (в г%) между разными возрастными группами фазанят ($p < 0{,}05$)

Возраст в днях	0	3	8	14	21	29	45	65	96
0	×	—	—	—	—	—	+	+	+
3	—	×	—	—	—	—	+	+	+
8	—	—	×	—	—	—	+	+	+
14	—	—	—	×	—	—	+	+	+
21	—	—	—	—	×	—	+	+	+
29	—	—	—	—	—	×	+	+	+
45	+	+	+	+	+	+	×	—	—
65	+	+	+	+	+	+	—	×	—
96	+	+	+	+	+	+	—	—	×

Если показатели β-фракции выражались в относительных %-ах, не было никаких значительных различий между отдельными возрастными группами.

Если сравнить диагнозы фазанят и цыплят, то видно, что у цыплят значения β-глобулина в среднем на 6% ниже (при вылуплении 12,9 $\pm$ 3,2%, а в возрасте 60 дней 12,3 $\pm$ 3,5%; Коудела и сотр., 1971).

α-фракция колебалась в относительных %-ах примерно в масштабе 2% с максимумом при вылуплении в 20,6 $\pm$ 0,7% и минимумом 18,3 $\pm$ 0,7% в возрасте 96 дней. В абсолютных значениях наблюдается такое же повышение, как и у α-глобулина: с 0,54 $\pm$ 0,05 г% при вылуплении до 0,75 $\pm$ 0,05 г% спустя 3 месяца (следовательно, почти 50%).

Анализ дисперсии при двойной классификации показал, что существуют статистически достоверные различия ($p < 0{,}05$) в г% β-глобулина между последующими днями послеинкубационного онтогенетического ряда (см. табл. V).

При выражении β-фракции в относительных %-ах значимые различия доказаны лишь между 0-ым и 96-ым днями жизни.

У петушков легхорн белый в этом возрасте значения γ-глобулина на 3—5% ниже, причем у цыплят наблюдаются отклонения порядка 12,8 $\pm$ 96% (на 1-ый день жизни) до 18 $\pm$ 1% (на 8-ой день).

У фазанят γ-глобулин в период онтогенеза незначительно повысился с 17,9 $\pm$ 0,4% в 0-ой день до прибл. 22% между 21-м и 96-ым днями. В абсолют-

Таблица V

Статистически достоверная разница в количестве сывороточного β-глобулина (+) (в г%) между разными возрастными группами фазанят ($p < 0,05$)

Возраст в днях	0	3	8	14	21	29	45	65	96
0	×	—	—	—	—	—	+	+	+
3	—	×	—	—	—	—	+	+	+
8	—	—	×	—	—	—	+	+	+
14	—	—	—	×	—	—	+	+	+
21	—	—	—	—	×	—	+	+	+
29	—	—	—	—	—	×	+	+	+
45	+	+	+	+	+	+	×	—	—
65	+	+	+	+	+	+	—	×	—
96	+	+	+	+	+	+	—	—	×

ных значениях подобно другим глобулиновым фракциям наблюдается повышение: с $0,5 \pm 0,02$ г% до $0,89 \pm 0,07$ на 96-ый день жизни (значит, почти на 80%).

Анализ дисперсии при двойной классификации показал статистически значимые различия ($p < 0,05$) в г% γ-глобулина между последующими днями послеинкубационного ряда (см. табл. VI).

Таблица VI

Статистически достоверная разница в количестве сывороточного γ-глобулина (+) (в г%) между разными возрастными группами фазанят ($p < 0,05$)

Возраст в днях	0	3	8	14	21	29	45	65	96
0	×	—	—	—	+	+	+	+	+
3	—	×	—	—	—	+	+	+	+
8	—	—	×	—	—	—	+	+	+
14	—	—	—	×	—	+	+	+	+
21	+	—	—	—	×	—	+	+	+
29	+	+	—	+	—	×	+	—	+
45	+	+	+	+	+	+	×	—	—
65	+	+	+	+	+	+	—	×	—
96	+	+	+	+	+	+	—	—	×

Если значения γ-глобулина выражались в относительных %-ах, значимые различия устанавливались лишь между 0-ым днем и 21, 29, 45, 96 днями жизни. В отличие от наших данных, полученных у цыплят (Коудела с сотр., 1971), у фазанят мы отметили более низкий % γ-глобулина (у цыплят 26—35%, а у фазанят 17—21%).

В нашей предыдущей работе (Сова и сотр., 1971), посвященной изучению действия ДДТ на фазанов, мы определяли у здоровых взрослых фазаньих самок и ВБ и электрофореограммы. Данные не отличаются ВБ 3,43 ± 0,21 г%, альбумины 1,36 ± 0,1 г% α-глобулины 0,74 ± 0,08 г%) от данных 2-месячных цыплят этой системы.

Данные, полученные у фазанов, можно конечно сопоставить с данными почти что необозримого уже ряда результатов, полученных у птицы. Но это не является целью обзора, мы стремились дать лишь ориентировочное обозрение сывороточных белков двух близкородственных групп. Параллельная статистическая оценка ясно показала, но надо учитывать не только выражение белковых фракций в относительных %-ах, а исходить из значений всего белка, которые в ранний послеинкубационный период онтогенеза имеют сильно вохсодящую тенденцию.

РЕЗЮМЕ

У 90 фазанов, разделенных на 9 равных групп, в возрасте 0, 3, 8, 14, 21, 29, 45, 65 и 96 дней при помощи бумажного электрофореза определяли количество всего сывороточного белка и белковые фракции.

На 3-м дне жизни ВБ понизился с 2,62 ± 0,18 г% при вылуплении до 2,35 ± 0,07 г% с последующим медленным повышением до 4,11 ± 0,2 г% на 96-й день жизни.

Значения относительного %-а альбумина медленно понизились с 42,6 ± 1,22 % в день вылупления до 39,2 ± 0,7 % к 65 дню жизни. Уровень в течение первых 14 дней был в абсолютных значениях выравненным (0,97—1,11 г%) и постепенно возрастал до 1,71 ± 0,14 г% на 96-й день.

Диапазон колебания показателей α-глобулина незначителен: 18,9 ± 1,1% в 0-й день до 18,2 ± 0,9% к 96-му дню жизни, с минимумом 17,8 ± 0,6% к 45-му дню жизни и с максимумом 19,1 ± 0,4% к 65-му дню жизни. При выражении в г% α-глобулиновая фракция возросла в период онтогенеза с 0,44 ± 0,04 г% к 3-му дню до 0,77 ± 0,05 г% к 96-му дню жизни.

β-глобулины обнаружены в пределах 20%, с максимумом при вылуплении в 20,6 ± ± 0,7% и минимумом в 18,3 ± 0,7% в возрасте 96 дней. Абсолютные показатели возросли незначительно: с 0,5 ± 0,05 г% при вылуплении до 0,75 ± 0,05 г% спустя 3 месяца.

Показатели γ-глобулина незначительно повысились с 17,9 ± 0,4% в 0-й день до приблизительно 22% между 21 и 96-м днями жизни. Абсолютные значения этой фракции возросли с 0,49 ± 0,02 г% в 0-й день до 0,89 ± 0,07 г% на 96-й день жизни.

CHANGES OF SERUM PROTEINS IN PHEASANT DURING POSTINCUBATION ONTOGENESIS

Z. SOVA, D. TREFNÝ, B. PUJMAN, J. JANDA AND Z. NĚMEC

In 9 age groups of pheasants, each having 10 birds, at the age of 0, 3, 8, 14, 21, 29, 45, 65 and 96 days, total plasma proteins and protein fractions were determined by paper electrophoresis. Plasma protein level decreased from the values obtained at hatching (2.62 ± 0.18 g%) to 2.35 ± 0.07 g% on the third day of age; this decrease was followed by a gradual increase up to the value of 4.11 ± 0.2 g% at the age of 96 days.

Albumin expressed as relative percentage showed some decreasing trend (from 42.6 ± 1.22% on the day of incubation to 39.2 ± 0.7% on the 65th day of age). In the absolute values the levels for the first 14 days were well-balanced (0.97—1.11 g%) with a gradual increase up to 1.71 ± 0.14 g% at the age of 96 days.

α-globulins fluctuated in a narrow range — from 18.9 ± 1.1% on the 0th day to 18.2 ± 0.9% on the 96th day, the minimum value occurring on the 45th day — 17.8 ± 0.6% — and the maximum on the 65th day — 19.1 ± 0.4%. The expression in g% indicates

that during ontogenesis the α-fraction increased from 0.44 ± 0.04 g% (the third day of age) to 0.77 ± 0.05 g% (96th day of age).

β-globulins fluctuated in the range of 20% with maximum at incubation ± 20.6 ± 0.7%, and minimum at the age of 96 days — 18.3 ± 0.7%. The absolute values showed some increase from 0.54 ± 0.05 g% at hatching to 0.75 ± 0.05 g% after 3 months.

γ-globulins slightly increased from 17.9 ± 0.4 (0th day) to values about 22% between the 21st and 96th days of age. In absolute values the γ-globulin fraction increased from 0.49 ± 0.02 g% (0th day) to 0.89 ± 0.07 g% (96th day of age).

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DATA ON MINERAL COMPONENTS OF THE HORNY PART OF THE FOOT OF CATTLE, SHEEP AND SWINE

By

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During the recent decades extensive studies have been conducted all over the world on the mineral contents of soil, plants as well as of the hair and other structures of animals. The vast literature of microelement research is now practically unmanageable.

Many investigators, among them Hungarians, demonstrated a close relationship between the microelement contents of soil and vegetation and pointed to the influence of the mineral content of fodder plants on the mineral supply of animals (Anke, 1965; Cunningham et al., 1958; Hennig, 1970; Marek et al., 1930; Mócsy et al., 1960; Modor et al., 1965; Szentmihályi, 1963; Tölgyesi, 1960, 1965). Little is, however, known about the mineral and microelement components of the horny structures, especially of the hoof and claws as well as about the relationship of the mineral content with the elasticity and strength of the horny matter (Kile, 1954).

We generally agree that the resistance of the hoof and claws plays a decisive role in the prevention and development of various diseases of the foot. The factors determining the resistance of the horny matter are, however, not yet known in full detail. Closer investigations into these factors are of immediate interest, with special regard to intensive farming, because the animals managed under such conditions are deprived of nearly all benefits of natural life, above all of free movement and sunshine.

In this laboratory, healthy-appearing horny parts of the feet of adult cattle, sheep and swine were analysed for mineral composition to assess the normal mineral status. As calcium, phosphorus and sodium are the main mineral components of the foot horn, and magnesium, copper, zinc and iron play an important role in keratinization, these minerals were determined quantitatively. Zinc and calcium determinations are also important with regard to the role of these minerals in swine parakeratosis.

Materials and methods

The samples were taken from the wall of the external surface of the horny part of the fore- or hind foot. Filth was removed by washing in running warm water. The washed specimens were placed in an oven of about 200°C for a few minutes, after which the horny casing could easily be detached from the bone.

The samples were then cut into 2—3 mm long pieces, shaken for 10 minutes in tap water, twice for 30 minutes in a detergent containing neither anions nor any compound of phosphorus, rinsed in distilled water and dried to constant weight at 115°C. Pieces weighing 0.5—1.0 g were digested in a 6 : 1 mixture of nitric acid and perchloric acid. Appropriate dilutions of the digestion product were used for determinations of the different elements. Sodium, magnesium, calcium, zinc and iron were determined by atomic-absorption spectrophotometry, phosphorus was determined with the method of BELL and DOISY (1920) and copper by photometry, in the form of its zinc-dibenzyl-dithiocarbamate compound (BROWN et al., 1962).

Samples from 24 Hungarian Spotted cattle, 31 Merino sheep and 30 Cornwall swine were examined in this series.

The concentrations of the elements were expressed as $\mu g/g$ dry material (ppm). Mean values (M) and standard error of the mean (±S.E.M.) were calculated for each mineral, and the corresponding figures were tabulated. Apart from the numerical values, curves were plotted for each mineral in each species, to facilitate comparison. Interspecies variations were analysed by Student's test.

All data presented in this paper relate to the external part of the wall; this is important because the mineral contents are different in the regions of the sole and frog and also differ between young and adult animals. Results of determinations in the latter regions and at different ages will be reported in another paper.

Results and conclusions

Calcium

Next to phosphorus, calcium was found to be the other main mineral component of the claw of all three species. Interspecies variations were slighter than with the other elements examined. The lowest concentration was measured in swine (1141 ± 34 ppm), the highest (1431 ± 46 ppm) in sheep and the value for cattle (1267 ± 53 ppm) was intermediate between the two.

According to WEISER et al. (1965), the calcium contents of the different parts of the horse hoof range from 294 to 665 ppm. KILE (1954), pursuing the relationship between the fragility of the human nail and calcium content, determined the latter as 680—2130 ppm and gave it for the nails of cattle and dogs as 1350—1400 and 660—10 900 ppm, respectively. ANKE (1965) determined 722—2310 ppm, POWELLEIT (1966) 1870—2020 ppm in the hair of swine from different breeds. STRAIN et al. (1966) found 180—1517 ppm, GOLDBLUM et al. (1953) 700—4900 ppm in human hair and the latter authors demonstrated 940—5900 ppm in human nails. It is remarkable that swine hair as well as human hair and nails contain more calcium than the horn of the hoof or claw.

Table I

Concentrations of Ca, P, Mg, Na, Fe, Zn and Cu in the horny part of the cattle, sheep and swine, expressed as μg/g related to dry material (M ± S.E.M.)

Species	n	Ca	P	Mg	Na	Fe	Zn	Cu
Cattle	24	1267±53	1169±84	188±10	454±19	85±5.6	142±3.9	3.8±0.5
Sheep	31	1431±46	1523±53	240±10	535±20	110±9.5	88±2.1	16.0±1.2
Swine	30	1141±34	1002±32	234±13	309±10	61±5.3	92±2.2	5.1±0.2

Phosphorus

We found, in accordance with other investigators, a great interspecies variations in the phosphorus content of the horny parts. The foot horn of sheep contained one and a half times more phosphorus (1523 ± 53 ppm) than that of the swine (1002 ± 32 ppm) and the value for cattle (1169 ± 84 ppm) was intermediate between the two, differing significantly from both.

According to the available data, the phosphorus content is lowest in human hair (25.8—140 ppm; GOLDBLUM et al., 1953), and relatively low in horse hoof (134—209 ppm; WEISER et al., 1965) and swine hair (138—516 ppm; ANKE, 1965; POWELLEIT, 1966), comparated to the maximum level (2482—2863 ppm) measured in the horny part of the goat's claw (ANKE, 1971).

Magnesium

Highly similar values were found for swine and sheep, 234 ± 13 and 240 ± 10 ppm, respectively, both differing significantly from the value for cattle (188 ± 10 ppm).

Table II

Comparison of the Ca, P, Mg, Na, Fe, Zn and Cu concentrations measured in the horny part of the foot of cattle and sheep

Ele-ments	Cattle		Sheep		t	P
	n	M ± S.E.M. ppm	n	M ± S.E.M. ppm		
Ca	21	1267 ±53	26	1431±46	2.55	<0.05
P	21	1169 ±84	31	1523±53	3.75	<0.001
Mg	24	188 ±10	31	240±10	3.68	<0.001
Na	24	454 ±19	31	535±20	2.91	<0.01
Fe	24	85 ± 5.6	31	110± 9.5	2.14	<0.05
Zn	24	142 ± 3.9	31	88± 2.1	13.09	<0.001
Cu	16	3.8± 0.5	22	16± 1.2	8.27	<0.001

A similar magnesium level was demonstrated in the hoof of the horse (WEISER et al., 1965) and a much higher concentration in swine hair, ranging from 314—786 ppm, depending on colour and body region, according to ANKE (1965) and from 510—570 ppm according to POWELLEIT (1966). GOLDBLUM et al. (1953) give the magnesium contents of human hair and nails as 10—101 and 23—110 ppm, respectively. SCHROEDER (1969) found 37.3—182.9 ppm in human hair.

Sodium

Mean sodium contents differed significantly in the examined three species. The mean values for the two ruminants, cattle (454 $\pm$ 19 ppm) and sheep (535 $\pm$ 20 ppm), were closer to each other than either was to the mean value for swine (309 $\pm$ 10 ppm).

The hoof of the horse contains much less sodium (90—263 ppm; WEISER et al., 1965), whereas the hair of swine considerably more (919—1259 ppm) according to ANKE (1965); 1300—1690 ppm according to POWELLEIT (1966).

Iron

The nails of cattle and swine contain less iron than zinc (85 $\pm$ 5.6 and 61 $\pm$ 5.3 ppm, respectively). According to the literature, the iron content varies greatly with the type of the horny structure and exceeds the zinc content only slightly, if at all. The iron concentrations determined by ANKE (1965) in the hair of white and pigmented swine of different ages ranged from 20 to 445 ppm and CREMER (1953) found 130—170 ppm in human hair.

Zinc

Zinc levels were nearly identical in the nails of swine and sheep (92 $\pm$ 2.2 and 88 $\pm$ 2.1 ppm, respectively), but much higher in those of cattle (142 $\pm$ 3.9 ppm). WEISER et al. (1965) found zinc contents to range from 41—146 ppm in different parts of the horse hoof, and a research group in Jena (GDR) determined 52—66 ppm in the nail of the goat. Similar concentrations were measured in the hair of swine and cattle (ANKE, 1965; MARTIN et al., 1969; MILLER et al., 1965) and much higher levels in human hair (SCHROEDER et al., 1969; STRAIN et al., 1966).

Copper

The copper contents of the horn of the foot also differed greatly between the species; sheep had about four times more (16.0 $\pm$ 1.2 ppm) than cattle (3.8 $\pm$ 0.5 ppm). The latter value is in good accordance with the corresponding data of CUNNINGHAM et al. (1958) and KERK (1970), (4.3—5.5 and 4.0 ppm,

Table III

Comparison of the Ca, P, Mg, Na, Fe, Zn and Cu concentrations measured in the horny part of the foot of swine and cattle

Elements	Swine		Cattle		t	P
	n	M ± S.E.M. ppm	n	M ± S.E.M. ppm		
Ca	27	1141 ±34	21	1267 ±53	2.06	<0.05
P	27	1002 ±32	21	1169 ±84	2.01	≈0.05
Mg	30	234 ±13	24	188 ±10	2.87	<0.01
Na	30	309 ±10	24	454 ±19	7.09	<0.001
Fe	29	61 ± 5.3	24	85 ± 5.6	3.06	<0.01
Zn	26	92 ± 2.2	24	142 ± 3.9	11.36	<0.001
Cu	30	5.1± 0.2	16	3.8 ± 0.5	2.62	<0.05

respectively). ANKE (1971) found 2.3—2.7 ppm copper in the nail of the goat and somewhat more in bovine hair (8.1—13.9 ppm, ANKE, 1965). Human hair and nails contain much more copper than the above values (CUNNINGHAM et al., 1958; SCHROEDER et al., 1969).

As can be seen from the above data, the concentrations of several mineral components of the horny part of the foot differ between species.

The mean values shown in the Tables are regarded as characteristic of the species or breed and will be used as references in further studies on mineral contents of the horny part of the foot of animals of different species and ages. They also will serve as a basis of comparison in investigations of the changed

Table IV

Comparison of the Ca, P, Mg, Na, Fe, Zn and Cu concentrations measured in the horny part of the foot of swine and sheep

Elements	Swine		Sheep		t	P
	n	M ± S.E.M. ppm	n	M ± S.E.M. ppm		
Ca	27	1141 ±34	26	1431±46	5.58	<0.001
P	27	1002 ±32	31	1523±53	8.11	<0.001
Mg	30	234 ±13	31	240±10	—	n. s.*
Na	30	309 ±10	31	535±20	10.06	<0.001
Fe	29	61 ± 5.3	31	110± 9.5	4.43	<0.001
Zn	26	92 ± 2.2	31	88± 2.1	—	n. s.*
Cu	30	5.1± 0.2	22	16± 1.2	10.38	<0.001

* Not significant.

nail and of the effects of mineral supplements in various diet formulas. They are taken into consideration in studies of the tensile strength, and resistance to wear and pressure of the horny part. The main aim of these studies is to find out whether the mineral components of the horn of the foot can be altered by dietary measures and whether this alteration can be utilized to prevent certain diseases of the foot through increasing the elasticity and strength of the horny part.

SUMMARY

Ca, P, Mg, Na, Fe, Zn and Cu were determined in apparently healthy horny parts of the foot of cattle, sheep and swine. The results were tabulated and compared with data from other authors. The values obtained are regarded as characteristic of the species of breed and will be used as references in determinations of mineral contents of changed nails as well as in studies on the mineral composition of the horny part in different breeds and at different ages and in relation to various mineral supplements.

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VERGLEICHSUNTERSUCHUNGEN MIT IN DIE KNOCHENHÖHLE EINGEPFLANZTEN BIOPLAST- UND METALLIMPLANTATEN

Von

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Im Laufe der Entwicklung der Traumatologie wurden zur inneren Fixierung der Knochenfrakturen verschiedene Stoffe erprobt. Außer einigen Metallen und Legierungen fanden organische tierische und Pflanzenstoffe, Kunststoffe sowie Auto- und Heterotransplantate eine Anwendung. Die abweichenden physikalischen, chemischen und biologischen Eigenschaften der erwähnten Stoffe ermöglichten, daß zu bestimmten Zwecken immer andere Stoffe angewandt werden.

Bei einigen Krankheiten ist die ständige Anwesenheit der in den Organismus eingepflanzten Fremdsubstanz erforderlich, bei anderen pathologischen Prozessen besteht dagegen die wichtige Anforderung, daß die intraoperativ eingepflanzte Substanz — Endoprothese oder Transplantat — nur eine gewisse Zeitlang im Organismus verbleibe. Die Regenerationsbereitschaft des Organismus besitzt nämlich — in Anwesenheit einiger inneren Fixatoren — die Fähigkeit den neuen Stoff der erwünschten Funktion entsprechend zu umwandeln. Sobald dies eintrifft, wird die Anwesenheit des inneren Fixators überflüssig oder manchmal sogar schädlich, so daß sich seine Entfernung empfiehlt. Laut TSCHAKLIN (1964) verursacht die Entfernung einiger implantierten Substanzen manchmal größere Schwierigkeiten, als die Osteosynthese selbst.

Seit Einführung der operativen Reposition werden zur Stabilisation der frakturierten Knochen weitläufig Metallegierungen angewandt. Nach SERSER (1966) hat sich unter den Metallegierungen zur inneren Fixation von Knochenfrakturen die Magnesiumlegierung am besten bewährt. Diese Substanz entspricht den Anforderungen ohne Schädigung des Organismus und ist auch deshalb vorteilhaft, weil sich ihre spätere Entfernung erübrigt. Eine alte Bestrebung ist weiterhin die Verwendung von Substanzen, die nach Erfüllung ihrer Funktion auf Wirkung zytologischer oder humoraler Prozesse aus dem Organismus eliminiert werden.

Jene aus natürlicher Grundsubstanz bestehenden, kunstoffartigen Produkte, welche aus Fibrin, Albumin, Myosin (GERENDÁS und Mitarb., 1955; GERENDÁS, 1959; GERENDÁS, 1961) bzw. Gelatine (HORVÁTH und PÁRKÁNY, 1971: PÁRKÁNY, 1967) menschlichen oder tierischen Ursprungs hergestellt werden, nennt man Bioplaste. Die gemeinsame Eigenschaft der Substanzen von Bioplast-Typ ist, daß sie aus dem Organismus resorbiert werden und ihre Resorptionszeit mit chemischer Vorbehandlung reguliert werden kann (GERENDÁS, 1961).

Nach Erfüllung ihrer biologischen Funktion erübrigt sich somit ihre Entfernung, wodurch der Patient von der erneuten Operation befreit wird.

Das Fibrin-Bioplast wurde auf zahlreichen Gebieten verschiedener chirurgischen Eingriffe erprobt (ÁFRA, 1960; ÁFRA und Mitarb., 1955; ÁFRA und FÉNYES, 1956; ÁFRA und Mitarb., 1957; DROBNY und Mitarb., 1962, 1964; FRANKL, 1955; KOVÁCS und GERENDÁS, 1960, 1961; B. KOVÁCS und Mitarb., 1965, 1965, 1967; PATAKY und Mitarb., 1956; REMETE und Mitarb., 1956, 1956; ZINNER und Mitarb., 1955, 1956; ZINNER und BIRÓ, 1959). Die sich auf das Gelatine-Bioplast beziehenden Prüfungen befinden sich gegenwärtig in Gang.

In vorliegender Arbeit haben wir die Knochenmarkhöhlenreaktion der sich langsamer und rascher resorbierenden Fibrin-Bioplaste mit der entsprechenden Reaktion des aus rostfreiem Stahl verfertigten KIRSCHNERschen Drahts von identischem Ausmaß verglichen. Die Beobachtungszeit erstreckte sich auf 150 (Fibrin-Bioplast) bzw. 60 (KIRSCHNERscher Draht) Tage.

Material und Methodik

In der ersten Versuchsserie wurden 35 Meerschweinchen untersucht: In die Markhöhle des rechten Femurs von 28 Tieren wurde ein Bioplast mit einem Kreisdurchmesser von 20×2 mm implantiert, während bei 7 Tieren ein KIRSCHNERscher Draht von identischem Maß zur Anwendung kam. Die Versuchstiere wurden in 7 Gruppen eingeteilt: Je zwei Tieren jeder Gruppe wurde ein auf langsamere Resorption (170—200 Tage) eingestelltes und je zwei Tieren ein auf raschere Resorption (120—150 Tage) eingestelltes Bioplast implantiert, während bei je einem Tier in die Markhöhle des Femurs ein KIRSCHNERscher Draht eingepflanzt wurde.

Der rechte Schenkelknochen der Meerschweinchen wurde mittels longitudinaler Haut- und Muskelinzision freigelegt, der Knochen unterhalb des Trochanters mit der Rippenschere quer durchgetrennt und das Bioplast bzw. der KIRSCHNERsche Draht in die Markhöhle eingeschoben. Die Tiere wurden am 3., 6., 9., 16., 30., 45. und 60. postoperativen Tag getötet und die entsprechenden Organe pathologisch-anatomisch bzw. pathohistologisch aufgearbeitet.

Zur zweiten Versuchsserie gehörten 60 Meerschweinchen: Die einzelnen Gruppen bestanden aus je zwei Tieren, bei denen ein sich langsam und ein sich rasch resorbierendes Bioplast implantiert worden war. Die Tiere wurden am 3., 6., 9., 16., 30., 45., 60., 80., 90., 100., 110., 120., 130., 140. und 150. postoperativen Tag getötet.

Im Laufe der dritten Versuchsserie wurde in die Markhöhle der Tibia von 6 Hunden ein Bioplast mit einem Kreisdurchmesser von 120×3—4 mm implantiert. Die rechte Tibia wurde nach der Freilegung im mittleren Drittel quer durchgesägt, die Enden reponiert, sodann das proximale Ende der Epiphyse angebohrt und das Bioplaststäbchen in die Markhöhle eingeführt. Am 90., 120. und 150. Tag wurden je ein Verscuchshund, bei denen ein sich rasch bzw. langsam resorbierendes Bioplast implantiert worden war, getötet.

Im Laufe der Versuche fanden bei sämtlichen Tieren mit Immundiffusions- und anaphylaktischen Verfahren immunbiologische Untersuchungen statt.

Anläßlich der Sezierung der operierten Extremitäten der Versuchstiere wurden die von den Weichteilen befreiten Knochen teils longitudinal aufgesägt, teils wurden aus der Diaphyse 1 cm größe Quersegmente ausgeschnitten. Die ausgesägten Knochensegmente wurden in 10%iger Formalinlösung fixiert, in 5%iger Salpetersäurelösung dekalziniert und schließlich in Gelatine bzw. Paraffin eingebettet, sodann die Schnitte mit Hämatoxylin-Eosin, Orcein sowie Azan-Färbung und nach VAN GIESON gefärbt.

Ergebnisse

Bei der Sektion der operierten Extremitäte ließ sich beobachten, daß die auf langsame Resorption eingestellten Bioplaststäbchen und auch der KIRSCHNERsche Draht innerhalb der Markhöhle — bereits 9—16 Tage nach der Einpflanzung — eine elastische gräulich-braune Kapsel umgibt. Die die Metallimplantate einhüllende Kapsel hat sich bis zum 45.—60. auf die Einpflanzung folgenden Tag verdickt und konnte aus der Markhöhle mit einer Pinzette herausgehoben werden; in der Umgebung der Kapsel ließ sich wenig gräulichbraunes Exsudat beobachten. Falls sich um das Bioplast überhaupt eine Kapsel bildet, ist diese nicht mit Exsudat umgeben und kann aus dem Markkanal — da sie mit dem Endosteum zusammenhängt — nicht einmal nach der longitudinalen Aufsägung hervorgehoben werden.

Histologisch konnte das Bioplaststäbchen — und zwar sowohl das auf langsame, als auch das auf rasche Resorption eingestellte — am 3.—6. postoperativen Tag in der Markhöhle des Femurs in seiner ganzen Ausbreitung vorgefunden werden; mit Eosin färbte sich das Implantat homogen rot. Die freien Räume der Markhöhle waren mit Exsudat, roten und weißen Blutkörperchen und einigen feinen Fibrinfäden ausgefüllt. In der unmittelbaren Nähe des KIRSCHNERschen Drahts entwickelte sich nebst Rundzellen, Leukozyten und Fremdkörperriesenzellen eine aus zirkulär angeordneten Fibroblasten bestehende dünne Schicht.

Am 9.—16. postoperativen Tag waren auf der glatten Oberfläche des sich langsam resorbierenden Bioplasts mit Rundzellen infiltrierte Knochenmarkreste ersichtlich; in der Umgebung setzte sich die Entwicklung einer mit dem Endosteum zusammenhängenden zellarmen, blassen Bindegewebekapsel in Gang. In der Nachbarschaft des Metalldrahts ließ sich in dieser Periode in Begleitung einer aus Granulozyten und Fremdkörperriesenzellen bestehenden Zellreaktion, die Entwicklung einer Bindegewebekapsel beobachten. Diese Kapsel hing mit dem Endosteum nicht zusammen. Von der ungleichmäßigen

Oberfläche des sich rasch resorbierenden Bioplasts haben sich mit Granulozyten umgebene kleine Partikel abgelöst. In diesen Fällen waren keine, auf die Bildung einer Bindegewebekapsel weisende Zeichen vorzufinden.

Bis zum 30.—45. Tag hat sich das sich rasch resorbierende Bioplaststäbchen verdünnt, seine Oberfläche wurde ungleichmäßig, in die Bioplastsubstanz drangen stellenweise Granulozyten ein. Augenfällig war außerdem die Änderung der Färbung der einzelnen Abschnitte des eingepflanzten Bioplaststäbchens: Das sich resorbierende Bioplast hat sich mit Hämatoxylin im Gegensatz zum ursprünglichen rosarot, blau gefärbt. Mit Azan-Färbung schlug die Farbe einzelner Bioplastgebiete aus hellrot in ein dunkles bläulich-lila um, was darauf hinweist, daß sich das intakte Bioplast mit Azokarmin hellrot und das sich resorbierende mit Anilinfarbe bläulich-lila färbt. Diese Erscheinung entsteht annehmbar auf Wirkung der fermentativen Tätigkeit der Phagozyten und auf diese Weise wird das Bioplast zur Resorption geeignet. Auf den Präparaten der sich rasch resorbierenden Bioplaste konnten nach 45 Tagen nur an den Randteilen des Implantats kleinere Ausfressungen beobachtet werden. In der Umgebung des Stäbchens vollzog sich die Bildung einer aus Angio-Fibroblasten und feinfaserigen Bindegewebe bestehenden lockeren Kapsel. Bei den Tieren, bei denen ein KIRSCHNERscher Draht implantiert wurde, bestand die den Nagel umgebende Kapsel aus zellarmen, dicht angeordneten, reifen Bindegewebeelementen und dichterer Kollagenstruktur. Die Kapsel grenzte sich vom Metall scharf ab und hing mit dem Knochengewebe ebenfalls nicht zusammen.

Bei den am 60. Tag getöteten Tieren hat sich der Umfang des sich rasch resorbierenden Bioplasts wesentlich verkleinert. Die in die Substanz des Stäbchens eingedrungenen zahlreichen neutrophilen Granulozyten haben die einheitliche Substanz des Bioplasts zerstört. Auf der Oberfläche des sich langsam resorbierenden Bioplasts ließen sich Arrosionen und die Ablösung kleinerer Partikel beobachten. In der Umgebung bildete sich eine mit dem Endosteum eng zusammenhängende Bindegewebekapsel, die sich nicht einmal in Richtung des Bioplasts scharf abgrenzte. Die Kapsel bestand in dieser Periode bereits aus zwei Schichten: Die innere Schicht war von kollagenöser Struktur, während in der äußeren Schicht osteoide Elemente und Knochenbalken erschienen. Bei den Tieren, bei denen die Implantation eines Metalldrahts stattfand, bestand die in der Markhöhle befindliche Bindegewebekapsel in dieser Zeit aus Kollagenfasern und reifen Bindegewebselementen. In der Umgebung des Metallimplantats hat sich dieser Zustand des weiteren nicht mehr geändert.

In der Markhöhle des Femurs der am 80.—110. Tag getöteten Meerschweinchen waren lediglich fragmentierte Teilchen der einheitlichen Substanz der sich rasch resorbierenden Bioplast-Nägel zu beobachten, während die Stelle der sich resorbierenden Bioplast-Abschnitte faseriges Bindegewebe einnahm. Das histologische Bild der sich langsam resorbierenden Bioplast-

stäbchen war ähnlich wie das der sich rasch resorbierenden Stäbchen am 16. postoperativen Tag, aus dem das Bioplast umgebenden Granulationsgewebe bildete sich jedoch dichtes fibrotisches Gewebe, an dessen Randteilen bereits neugebildete Knochenbälkchen erschienen.

Am 120.—130. Tag befanden sich die Auflockerung bzw. Fragmentierung der sich rasch resorbierenden Bioplastsubstanz bereits in einem bedeutend fortgeschrittenen Stadium. Im faserreichen Angio-Fibroblastgewebe ließen sich nurmehr kleinere Teilchen des Bioplaststäbchens erkennen. Zu dieser Zeit begann sich die Färbung der Randteile des sich langsam resorbierenden Bioplasts zu ändern — so wie das beim sich rasch resorbierenden Implantat am 30.—45. Tag in Erscheinung trat. Auf der inneren Fläche der sich früher entwickelten Bindegewebekapsel war dagegen granulozytäre und histiozytäre Infiltration zu beobachten.

Am 140.—150. postoperativen Tag blieben nur mehr winzige Fragmente der sich rasch resorbierenden Bioplast-Nägel zurück. Anstelle des Bioplasts erschien ein zahlreiche elastische und Kollagenfasern enthaltendes, gefäßreiches Bindegewebe. An den Randteilen des zerfaserten Bindegewebes bildeten sich kleine Knochenbalken, unter denen sich auch aktives Knochenmark befand. Die Phagozytose der Randteile des sich langsam resorbierenden Bioplasts wurde in diesem Zeitpunkt ausgeprägt, worauf die Zerbröckelung und Zackigkeit der bis dahin glatten Oberfläche hinwiesen. Die innere, zellreiche Fläche der das Bioplast umgebenden, mit dem Endosteum eng zusammenhängenden Kapsel drang unter die Bioplast-Fragmente ein, während auf den äußeren Teilen auch weiterhin reifes, faseriges Bindegewebe zu erkennen war.

Das histologische Bild der Umgebung der in die Tibia von Versuchshunden eingepflanzten Bioplast-Nägel zeigte folgendes:

90tägiger Befund; auf langsame Resorption eingestelltes Bioplast: Die Länge des Bioplaststäbchens blieb soz. unverändert, es hat sich aber auf die Hälfte bzw. das Zweidrittel seines ursprünglichen Durchmessers verdünnt. Die Nagelränder waren rundherum zackig und in der Substanz bildeten sich kleinere Spalten. Die Bioplastsubstanz war mit einer bindegewebigen Kapsel umgeben, unter den Bindegewebefasern erschienen stellenweise mit Leukozyten und Histiozyten umgebene, abgespaltete, basophil gefärbte Bioplast-Partikel. Auf rasche Resorption eingestelltes Bioplast: Unter die zerbröckelten Fragmente des Bioplast-Stäbchens sind neutrophile Granulozyten und Histiozyten eingedrungen, in ihrer Umgebung befand sich gefäßreiches Angiofibroblast-Gewebe. Im peripheren Abschnitt des Markkanals bildeten sich faseriggeflechtartige Knochenbälkchen.

120tägiger Befund; auf langsame Resorption eingestelltes Bioplast: Das Implantat war in Stücke zerfallen. Unter den Fragmenten ließ sich verhältnismäßig zellarmes, faseriges Bindegewebe beobachten, welches nebst Leukozyten vornehmlich Histiozyten und stellenweise auch Fremdkörperrieesenzllen ent-

hielt. Auf rasche Resorption eingestelltes Bioplast: Anstelle des Nagels konnten nur basophil gefärbte Bioplast-Schollen beobachtet werden. Die Markhöhle war größtenteils mit zellarmem, faserigem Bindegewebe ausgefüllt, an den Randteilen ließ sich die Entwicklung von spongiösem Knochengewebe erkennen.

150tägiger Befund: die Stelle des Bioplasts nahmen fibrinöses Bindegewebe, Fettgewebe, aktives Knochenmark und sich neugebildete kleine Knochenbalken ein. Ausschließlich in der Knochenmarkhöhle jener Tiere, bei denen ein sich langsam resorbierendes Bioplast implantiert worden war, ließen sich vereinzelt basophil gefärbte Bioplast-Partikelchen vorfinden. Die Markhöhle war auch in diesen Fällen mit faserigem Bindegewebe, Fett und aktivem Knochenmark ausgefüllt.

Besprechung

Aufgrund der Bewertung der Untersuchungsergebnisse darf festgestellt werden, daß die in die Markhöhle der Röhrenknochen implantierten Fibrin-Bioplast-Nägel, von der chemischen Vorbehandlung abhängig, nach kürzerer bzw. längerer Zeit resorbiert werden. Das erste Zeichen des Ingangsetzens der Resorption war die mit sämtlichen angewandten Färbungsverfahren beobachtete, sich aber mit der Azan-Färbung in besonders intensiver und demonstrativer Form meldende Farbenveränderung. Die Randteile des sich früher azidophil gefärbten Bioplasts zeigen auf einmal eine basophile Färbung. In die eine Farbenveränderung zeigenden Bioplastteile (die wahrscheinlich eine pH-Veränderung erlitten haben) dringen Zellelemente, in erster Linie neutrophile Granulozyten, sodann eine rege Phagozytose aufweisende Gewebemakrophagen von histiozyten Typ ein.

Der Abbau des in die Markhöhle des Femurs von Meerschweinchen eingepflanzten, auf rasche Resorption eingestellten Bioplasts setzt sich annähernd am 16., auf die Implantation folgenden Tag in Gang, während die totale Resorption zwischen dem 120. und 150. Tag eintrifft. Beim sich auf langsame Resorption eingestellten Bioplast melden sich die Initialzeichen des Abbaus am 60. postoperativen Tag, die totale Resorption ist aber nicht einmal am 150. Tag beendigt; in dieser Zeit befindet sich das Implantat in einem Zustand, welchen das sich rasch resorbierende Bioplast am 45.—60. Tag erreicht hat.

Das in die Markhöhle der Tibia von Hunden implantierte, auf rasche Resorption eingestellte Fibrin-Bioplast wird infolge der aktiven Tätigkeit der Leukozyten und der Zellen des Angiofibroblast-Gewebes im Verlauf von 90—120 Tagen und das sich langsam resorbierende im Verlauf von 120—140 Tagen fast vollkommen resorbiert.

Anstelle des sich resorbierenden Bioplast-Nagels entwickelt sich faserreiches, dagegen aber verhältnismäßig zellarmes Bindegewebe. Im selben Maße

wie sich die Bioplast-Resorption abspielt, vollzieht sich die Obliteration der Stelle des Nagels. Nach vollkommener Resorption des Bioplasts nehmen seine Stelle elastische und Kollagenfasern enthaltendes Bindegewebe, Fettgewebe und aktives Knochenmark ein. Auf den an den Knochen grenzenden Bindegewebegebieten befinden sich kleine Knochenbalken und es spielt sich eine rege Knochenbildung ab. In der Umgebung des sich rasch resorbierenden Bioplasts bildet sich keine dermaßen zellarme Bindegewebekapsel, wie um das sich langsam resorbierende Bioplast und besonders um das Metallimplantat. Beim auf lange Resorptionszeit eingestellten Bioplast läßt sich zwar eine bindegewebige Demarkation beobachten, in der späteren Periode, sobald die Resorption des Bioplasts an Intensität gewinnt, wird die sich entwickelte Kapsel mit Granulozyten und Rundzellen infiltriert und dadurch mehr oder minder aufgelockert. Von diesem Zeitpunkt an, besteht die Kapsel aus zwei Schichten, die innere verfügt über eine faserige kollagenöse Struktur, während die äußere aus spongiösem Knochengewebe aufgebaut ist.

Die bindegewebige Einkapselung des eingepflanzten KIRSCHNERschen Drahts setzt sich bereits am 6. Tag in Gang, auf dem Gebiet der Zellreaktion sind zahlreiche Fremdkörperriesenzellen ersichtlich. In der späteren Periode lindert sich die entzündliche Zellreaktion, die Kapsel wird ausgeprägter, während der mit dem Metall in Berührung stehende, innere Teil der Bindegewebekapsel eine membranartige Demarkation zeigt. In der Umgebung des Metalls bleibt die Bindegewebekapsel auch weiterhin unverändert bestehen, im Falle des sich langsam resorbierenden Bioplasts lockert sich dagegen parallel mit dem Fortschreiten der Resorption auch die Kapsel auf und wird mit den in der Resorption eine Rolle spielenden Zellelementen infiltriert. Schließlich verdünnt sich die fibröse Schicht und die Knochenmarkhöhle füllen neugebildete spongiöse Knochenfalken, faseriges Bindegewebe und aktives Knochenmark aus.

Letzten Endes verhält sich das Bioplast in der Knochenmarkhöhle nicht als ein Fremdkörper und wird von der chemischen Vorbehandlung abhängig in kürzerer oder längerer Zeit resorbiert; an seiner Stelle entwickelt sich aktives Knochenmark und es scheint, daß es auf die Knochenregeneration und die Entwicklung der Kallusbildung anregend einwirkt. Unsere Forschungen erlauben die Feststellung, daß das Fibrin-Bioplast für den Organismus von immunbiologischem Standpunkt aus ungefährlich ist (HORVÁTH und Mitarb., 1969, 1969; HORVÁTH und Mitarb., 1971). Laut der Ergebnisse von B. KOVÁCS und Mitarb. (1965, 1965) verhält sich das Fibrin-Bioplast im Organismus auf gewebefreundliche Weise.

ZUSAMMENFASSUNG

Im Schenkelknochen von Meerschweinchen und Hunden kam zur Fixierung der Bruchenden experimenteller Knochenfrakturen eine sich resorbierende Substanz, Bioplast zur Anwendung. Die mittels chemischer Vorbehandlung auf kürzere und längere Resorptionszeit eingestellten Bioplast-Nägel verhielten sich in der Knochenmarkhöhle auf gewebefreundliche Weise und wurden von der Vorbehandlung abhängig in kürzerer oder längerer Zeit resorbiert. Die die Resorption begleitenden Gewebserscheinungen waren günstiger, als im Falle der kontrollhalber angewandten Metallnägel. Die Untersuchungen sprachen dafür, daß durch Bioplast die Knochen- bzw. Markhöhlenregeneration und die Kallusbildung vorteilhaft beeinflußt werden. Die Bestrebungen der Verfasser richten sich auf die Herstellung von Bioplast mit entsprechender Festigkeit und auf die Ersetzung der Metallnägel mit Bioplast.

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A SELECTIVE MEDIUM FOR THE ISOLATION OF *CORYNEBACTERIUM PYOGENES*

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Isolation of the bacterial agent from exudates or carcases may fail. Inappropriate storage or lasting shipment of the test materials favours the growth of saprophytic organisms, especially in the summer season. The isolation of the causative agent is often greatly hampered by a high degree of saprophytic contamination.

Such difficulties were encountered in this laboratory in diagnostic isolation experiments of *Corynebacterium pyogenes* from tonsils, milk, faeces, purulent necrotic tissue or from organs of putrefied carcases. A new medium component has therefore been employed, which does not interfere with *Corynebacterium* growth, but inhibits the multiplication of a wide range of other microorganisms.

Several materials are known to inhibit the growth of Gram-positive bacteria, but there are very few chemical compounds and antibiotics whose presence in the medium inhibits Gram-negative and other saprophytic organisms without suppressing *Corynebacterium* growth as well.

SYNDER and LICHTENSTEIN (1940) added sodium azide to the medium to prevent the outgrowth of *Escherichia coli*, salmonellae and putrefying bacteria, as well as thes warming of proteuses, if these were present.

PACKER (1943) used crystal violet in various media for the suppression of Gram-negative organisms.

SZENT-IVÁNYI (1951) combined sodium azide with crystal violet in a selective medium which made possible the safe isolation of *Erysipelothrix rhusiopathiae* and pathogenic udder streptococci from test materials highly contaminated with saprophytic and putrefying organisms.

Inhibition of the growth and /or the swarming of proteuses was reported with chloral hydrate (KRÄMER and KOCH, 1931), sodium-phenyl-ethyl-barbiturate (LEISTNER, 1950) and sterogenol (cetyl-pyridine-bromide solution in ethanol) (BANDY, 1964). Gram-negative bacterial growth could be inhibited with potassium tellurite (LOVREKOVICH et al., 1935), guanofuracine (5-nitro-2-furfurylidine-aminoguanine-hydrochloride) (SEELIGER, 1961) and a combination of glycine anhydride and lithium chloride (McBRIDE and GIRARD, 1961).

The antibiotic era has markedly extended the range of selective media of which only two are mentioned here, the neomycin-containing medium of FORRAY and SZÁZADOS (1969) and the polymyxin-containing medium of SKOVGAARD (1968), for the isolation of *E. rhusiopathiae* and corynebacteria, respectively.

Experimental

No chemical compound except potassium tellurite proved to be suitable for selective *Corynebacterium* isolation in our hands, because the applied concentrations inhibited also corynebacteria along with the other organisms. On the basis of SKOVGAARD's investigations, a total of 220 *Corynebacterium* and other strains, isolated from different species, were then tested for sensitivity to polymyxin, in order to assess the value of this antibiotic in selective media.

Initially liquid media, later solid media, were used. The liquid medium was a common broth of pH 7.4, containing 1% peptone and 0.5% NaCl. The composition of the solid medium was the same, with 2% agar added for solidification. Ten per cent horse serum and 10% sheep blood were added for every 100 ml of solid and liquid medium, respectively.

Polymixin B (Pfizer GmbH, Karlsruhe) was added to the medium simultaneously with the serum or blood, in concentrations ranging from 25 to 2000 IU/ml. Five ml of liquid medium and 10 ml of solid medium was distributed per tube and Petri dish, respectively. At the beginning of the experiments, 0.1% Tween 80 was also added, but later it was omitted, because it soon caused haemolysis and failed to promote bacterial growth.

The media were inoculated with various strains from our collection and incubated at 37°C. The results were read after 24, 48 and 72 hr. Growth in liquid media containing no Polymyxin served as control. The control growth was designated with three crosses (+++), absence of growth with minus (—) and suppressed growth with one (+) or two crosses (++).

The results are summarized in Table 1.

The 25 IU/ml concentration of Polymyxin, recommended by SKOVGAARD, only reduced the counts of pasteurellae, salmonellae and bacteria of the *E. coli*-group. Growth inhibition of the other saprophytic or pathogenic organisms required a much higher level of this antibiotic, and the concentration capable of inhibiting *E. rhusiopathiae* and enterococci suppressed *C. pyogenes* as well. The broth medium containing serum and an optimal concentration of Polymyxin B, was intended to serve as an enrichment medium, promoting *Corynebacterium* growth through suppression of the accompanying flora. But neither *E. rhusiopathiae*, nor enterococci were inhibited and some cases not even certain *Proteus* strains.

The broth was therefore replaced by a 10% serum containing solid medium; in this, the antibiotic was as active as in the broth and it had the advantage over the latter that colony morphology could also be studied.

At Polymyxin concentrations of 300 IU/ml or higher, *C. pyogenes* failed to grow for the first 24 hr, but this initial inhibition disappeared by 48—72 hr. This property of the corynebacteria was utilized to differentiate them from *E. rhusiopathiae* and enterococci, which grew vigorously already at 24 hours.

Table I

Bacterial strains examined	Bacteriostatic effect of different concentrations IU/ml of polymyxin in a broth medium containing 10% serum																																
	25			50			100			200			300			500			700			1000			2000			1230			Kontroll		
Time of reading (hours)	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
1. *C. pyogenes*	+	+++	+++	+	+++	+++	+	+++	+++	+	+++	+++	—	+++	+++	—	+++	+++	—	+++	+++	—	++	+++	—	—	++	—	++	+++	+	+++	+++
2. *Erysipelothrix rhusiopathiae*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	—	+	++	+	+	+++	+++	+++	+++
3. *Enterococcus*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	—	+	+	++	+++	+++	+++	+++	+++
4. *Streptococcus pyogenes anim.*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	++	+++	+	++	+++	—	—	+	—	—	—	—	—	—	—	—	—	+++	+++	+++
5. *Staphylococcus aureus*	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+	+	++	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
6. *Staphylococcus albus*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+	++	++	—	—	++	—	—	—	—	—	—	—	—	—	+++	+++	+++
7. *L. monocytogenes*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
8. *Salmonella typhi-murium*	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
9. *E. coli*	—	—	+	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
10. *P. multiseptica*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
11. *Bacillus subtilis*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
12. *B. mesentericus*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++	+++
13. *P. vulgaris*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+	+	++	—	+	+	—	—	+	—	+	+	+++	+++	+++

The optimal concentration of polymyxin was found to be 1230 IU/ml. In the second series of the experiments, 0.01% potassium tellurite was also added to the medium, because many *Proteus* strains had been resistant to Polymyxin. The applied concentration of potassium tellurite prevented the swarming of *Proteus* organisms even if it failed to inhibit their growth; it did not affect the development of *C. pyogenes*, facilitating rather the diagnostic work by causing a brownish discoloration of the colonies of the latter.

Table I clearly indicates that Polymyxin and potassium tellurite failed to inhibit the growth of *E. rhusiopathiae* and enterococci in the selective solid medium. These organisms grew out by the 24th hr, at 37°C, their colonies grew larger and assumed a black shade from the tellurite by 48 hr, whereas *C. pyogenes* did not grow out in the first 24 hr of incubation and its young colonies were of a light greysh or brownish colour. The saprophytic, Gram-positive and Gram-negative organisms listed in Table I usually failed to grow in the Polymyxin-containing medium. Potassium tellurite prevented the swarming of the Polymyxin-resistant Proteus strains.

The Polymyxin B and potassium tellurite containing blood agar plates have been used with success for diagnostic isolations of Corynebacteria from various test materials including exudates highly contaminated with saprophytic organisms as well as putrefied organs and could also be used for the identification of carriers.

SUMMARY

A selective medium, containing 1230 IU/ml Polymyxin B and 0.01% potassium tellurite, is described for the isolation of *Corynebacterium pyogenes*. Potassium tellurite inhibits the growth of the Polymyxin-resistant proteus strains, or at least their swarming.

Of the organisms tested, the growth of *Erisypelothrix rhusiopathiae* and enterococci could not be fully suppressed, but since these bacteria form colonies already after 24 hours of incubation at 37°C and the colonies assume a black hue from the tellurite, they can easily be differentiated from *C. pyogenes*, which does not grow out on the selective medium until after 48—72 hours and its colonies are light brown.

The selective medium proved to be suitable for diagnostic isolations of *C. pyogenes* from exudates contaminated with saprophytic bacteria and from putrefied organs.

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INCIDENCE OF MAEDI (CHRONIC PROGRESSIVE INTERSTITIAL PNEUMONIA) AMONG SHEEP IN HUNGARY

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The chronic interstitial pneumonia of sheep is a slowly progressive viral disease from which the animals do not usually recover. Its main clinical signs are dyspnoea (= Maedi in Icelandic language) which becomes more difficult day by day, and a gradual emaciation. Grossly and microscopically the condition is characterized by formation of lymph follicles in the pulmonary parenchyma, proliferation and metaplasia of alveolar epithelial cells, marked hyperplasial enlargement of the peribronchial lymph nodes and encephalomyelitis accompanied by nerve fibre demyelinization and glia cell proliferation.

Literature

The disease was first observed by Marsh (1922) in North America. In the meantime it was recognized in South Africa (Shirlaw, 1959), France (Siguardardottir and Thormar, 1964), Holland (De Vriss, 1959; Grommers, 1964), Iceland (Sigurdsson, 1954), Great Britain (Stamp and Nisbet, 1963), German Federal Republic (Flir, 1970; Straub, 1970; Weiland and Behrens, 1970), German Democratic Republic (Seffner and Lippmann, 1967) and recently also in India (Tiwari and Pandit, 1967). The disease has been given different names in the different countries, such as "progressive sheep pneumonia", "Montana sheep disease" in America, "interstitial pneumonia", "Laikipia", "Graaf-Reinet Disease" in South Africa, "La Bouhite" in France, "Dampigheid", "Zwoegerziekte" in Holland, "interstitial sheep pneumonia", "Maedi" in Iceland, "atypical pneumonia" in Great Britain and "Maedi", "Visna/Maedi" or "progressive interstitial pneumonia" in the two German republics. It is, however, certain from the descriptions of the gross and microscopic lesions that the various names designate one and the same disease.

The etiology of the condition seems to be clear. Most investigators have classified its causal agent as an RNA virus, resembling the agents of avian and murine leucosis. The virus causes characteristic cytophathic changes — multinucleated giant cell (syncytium) formation — in cell cultures prepared from choroid plexus of sheep, adrenal cortex of lamb and bovine tracheal epithelium (Sigurdardottir and Thormar, 1964). The virus particles are demonstrable in the cytoplasm by immunofluorescence as soon as 24 hours after infection. The cell nuclei show no fluorescence, indicating that virus synthesis takes place in the cytoplasm. The agent is sensitive to ether, chloroform, formaldehyde, ethanol and phenol (Straub, 1970), and induces virus-neutralizing antibody response in the diseased sheep (Thormar, Gislason and Helgadottir, 1966).

Like leucosis, Maedi chiefly spreads vertically, from generation to generation. Since, however, the diseased sheep shed the virus with respiratory exudation and saliva, horizontal spread by contact can also take place, especially in crowded flocks. Certain authors (Seffner and Lippmann, 1967; Straub, 1970) have attributed a carrier role to apparently healthy rams. According to an earlier view, only ewes are susceptible to the causal agent. Indian authors (Tiwari and Pandit, 1967) reported the occurrence of Maedi among goats. Certain

breeds were found to be more susceptible than others (SEFFNER and LIPPMANN, 1967). The disease was transferable to sheep and goats by intranasal and intracerebral infection (SIGURDARDOTTIR and THORMAR, 1964). Owing to the extremely long latency period (occasionally 2—3 years), however, biological tests are of no value for routine diagnostic purposes.

Clinically the disease is characterized by an obdurate dry cough and an afebrile state, marked loss of condition and a 2—8 months long course which is usually irresponsive to all kinds of therapy and nearly always ends fatally (MARSH, 1923; SIGURDARDOTTIR and THORMAR, 1964; STRAUB, 1970). The condition becomes particularly severe with advancing pregnancy and parturition. A concurrent bacterial infection may shorten the course and cause a temperature elevation. The most typical gross and microscopic lesions develop in the lungs, peribronchial lymph nodes and central nervous system (MARCH, 1923: SIGURDARDOTTIR and THORMAR, 1964; STRAUB, 1970).

Results

In a Cooperative Farm in South Hungary, several ewes of the Merino-like breed developed in succession signs indicative of pneumonia. For the last two years, Suffolk and Hampshire rams imported from Great Britain had been used for artificial insemination of the ewes. Later the incidence of the disease was noted in the sheep flock of the neighbouring cooperative farm as well as in another farm of West Hungary, in which the sheep also were of a Merino-like breed. The symptoms, course and epizootology of the disease were studied by the authors on the spot. Carcases of 2 spontaneously died and 4 emergency-slaughtered animals were examined for gross and microscopic lesions as well as microbiological and parasitological status in the laboratories of the Central Veterinary Institute and 10 blood samples were used for serological and haematological tests. Detailed case histories were available from two farms.

Clinical observations

The first symptom was a dyspnoea, scarcely noticeable at the beginning, but becoming more severe with time. After an increased physical exertion, *e.g.* when the animals were forced to move, the respiration became still more difficult; the head was stretched forward an the nostrils widened at inhalation. An abdominal respiration set in, with a frequency up to 100—120 per minute. Dyspnoea was accompanied by a weak but obdurate, dry cough. In advanced cases a serous nasal discharge appeared. Severely ill animals lagged behind the rest of the flock, moved reluctantly and often collapsed from loss of strength. There was no temperature elevation throughout the course. Neither the appetite, nor urine and faeces discharge changed notably, yet there was a progressive loss of condition and anaemia developed. A slight leucocytosis (16 000—17 000 leucocytes per mm^3) and relative lymphocytosis were observed. Most animals died 2—6 months after the onset of the first symptoms. All diseased animals died which had not been emergency-slaughtered in the meantime. In one farm, 35 ewes from a flock of 420 became ill in the

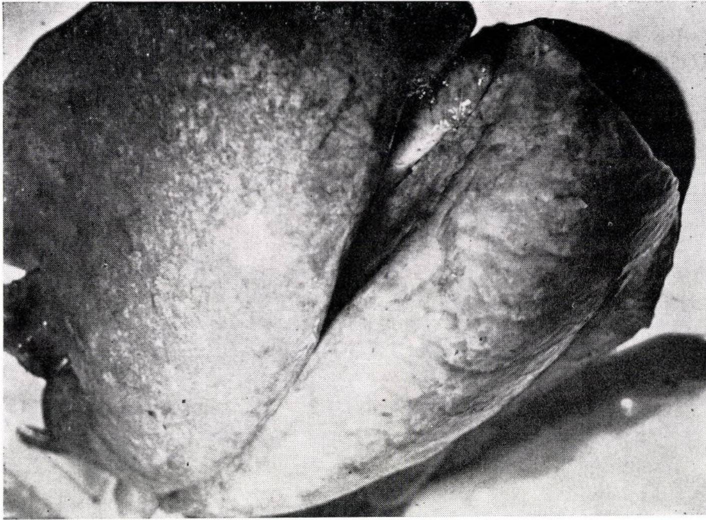

Fig. 1. Enlarged lung and mediastinal lymph node

period from September 1971 to February 1972, and by then 10 had already died and four had to be emergency-slaughtered. In the other farm, four ewes of 250 were emergency-slaughtered during the same period.

The disease did not affect the breeder rams and young lambs.

Gross lesions

Lungs removed in an earlier stage of the disease (from emergency-slaughtered animals) were only partly changed, showing grayish-red spots of atelectasia, as elastic as a rubber sponge, chiefly in the accessory lobes and in the adjacent parts of the diaphragmal lobes. Inside the changed areas there were small, scattered, light-red air spaces and light grayish-pink or grayish-red miliary foci, more compact than the surrounding tissue and in places confluent. The air spaces and airless foci rendered the cut surface of the lung characteristically variegated. In the advanced stage, the pulmonary atelectasia was almost complete, the lung enlarged, weighing 1600—2000 g instead of the normal 600—800 g (Fig. 1); the homogeneously grayish-red parenchyma became more compact, less elastic and resisted tearing. Also the miliary foci felt more compact, but not even those localizing directly under the pleura bulged above the surface. The bronchial walls thickened, the peribronchial and mediastinal lymph nodes enlarged several times, their colour changed to grayish-white and their cut surface resembled that of bacon.

Microscopic lesions

In accordance with the symptoms and gross lesions, microscopic changes were chiefly found in the lungs. An inflammatory cell infiltration of the alveolar walls (Fig. 2) was already observed in the lungs of animals emergency-slaughtered in an early stage of the disease. The infiltrating cells were lymphocytes, histiocytes, plasma cells and a few neutrophil granulocytes (Fig. 3); their proliferation and migration resulted in thickening of the wall and constriction of the lumen of the alveoli. Parallel to the establishment of interstitial pneumonia, foci consisting of lymphoid cells arose in the parenchyma, above all along the larger vessels, respiratory bronchioles and bronchi (Fig. 4). In advanced cases, these lymph follicles increased in size and number (Fig. 5), causing thickening of the alveolar wall and constriction of the lumen, until the alveolar structure was practically lost. The lining epithelial cells of the alveoli degenerated, grew larger and became desquamated from the wall into the lumen (Fig. 6). Inflammatory cells and other mononuclear elements, infiltrating the interalveolar tissue, also appeared in the alveolar lumen (Fig. 7). The interstitial pneumonia progressed to fibrosis (Fig. 8), resulting in further constriction of the alveolar lumina. In the fibrotic areas, the elastic fibres showed no change, but the argyrophilic fibres thickened and increased in number, as demonstrated by Gömöri's silver impregnation technique (Fig. 9). Proliferation of smooth muscle cells and, occasionally, of the alveolar and bronchiolar epithelium was observed (Fig. 10), as well as an adenomatous change of the minor airways (Fig. 11). In other cases, round or oval, concretion-like basophilic bodies, some of which showed concentrical layers and some contained calcium salts, were seen in the bronchial cavities among proliferating epithelial cells and inflammatory cells (Fig. 12). The alveolar wall and bronchiolar lumen contained, apart from degenerated epithelial cells and inflammatory cells, giant cells (Figs 13 and 14); these were either large, binucleated or bizarrely shaped, multinucleated forms. The walls of the minor airways appeared intact, without even an inflammatory change. In the brain, above all in the olfactory lobe and Ammon's horn, there was a circumscribed non-purulent lymphocytic encephalitis, accompanied by glia cell proliferation (Fig. 15).

Microbiological and parasitological examinations

No pathogenic bacteria could be isolated from parenchymatous organs, brains, changed lymph nodes and lungs except one lung. The latter contained pasteurellae which probably originated from a secondary infection. Microscopic examinations and infection experiments in chick embryo for the demonstration of Bedsonia also had negative results and no complement-fixing (CF) antibodies were found in the sera of the diseased animals.

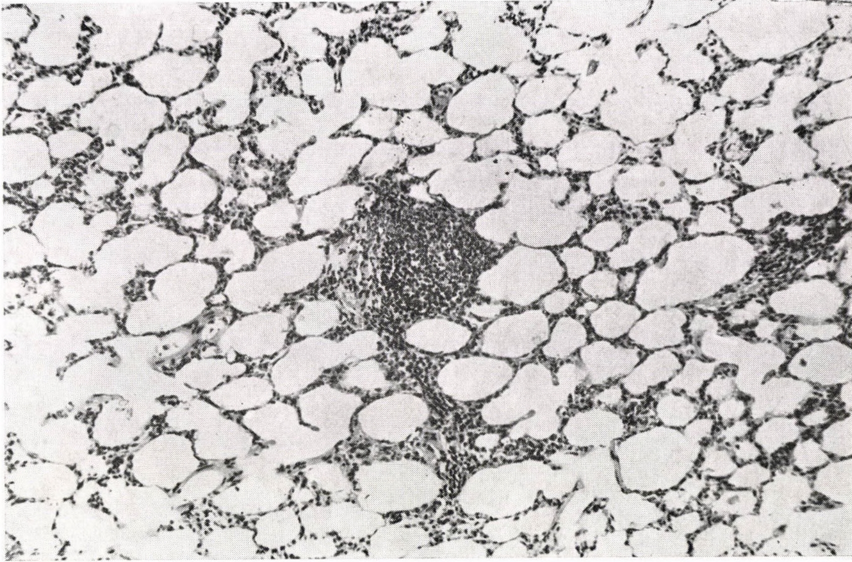

Fig. 2. Detail of lung parenchyma from a diseased sheep. Early stage of interstitial pneumonia with lymph follicle formation. Haemalaun and eosin stain, ×95

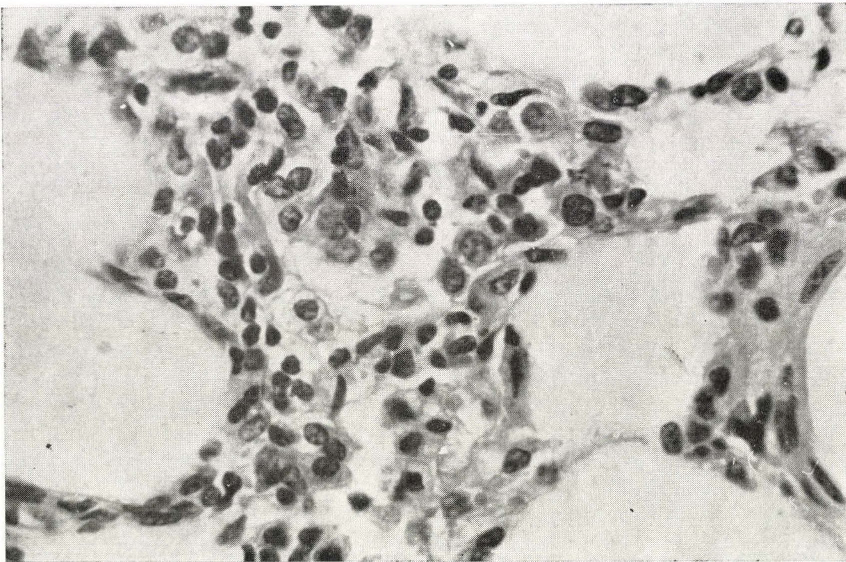

Fig. 3. Detail of Fig. 1. The alveolar walls are thickened and infiltrated by inflammatory cells, ×600

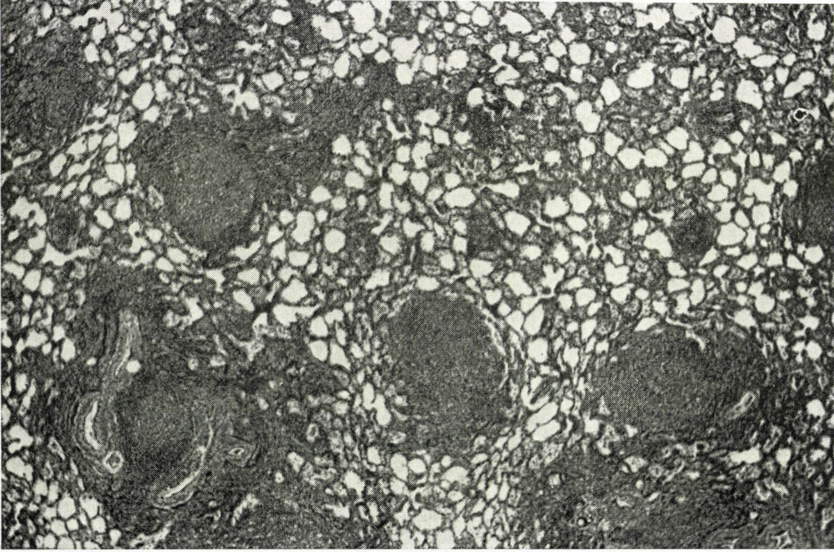

Fig. 4. Lymphoid foci along airways and blood vessels as well as in the pulmonary parenchyma. Haemalaun and eosin stain, ×45

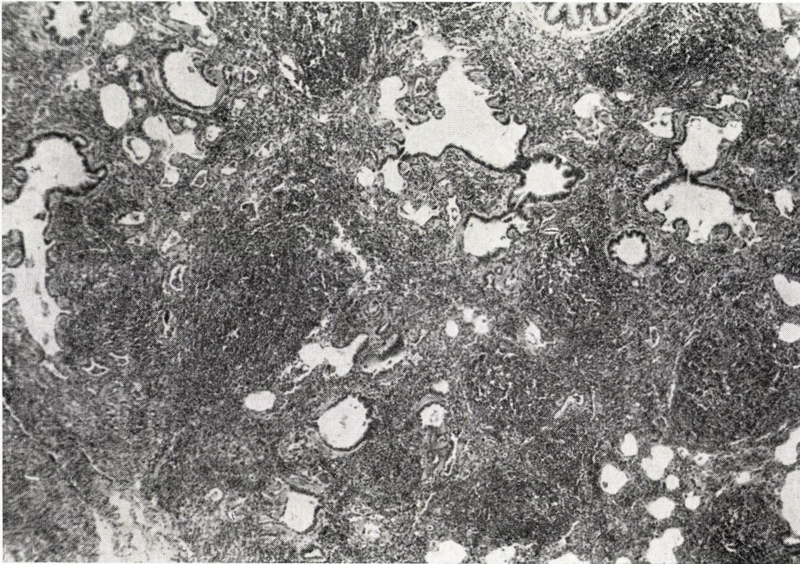

Fig. 5. Vigorous proliferation of the lymphoreticular tissue in the fibrotic lung. Haemalaun and eosin stain, ×45

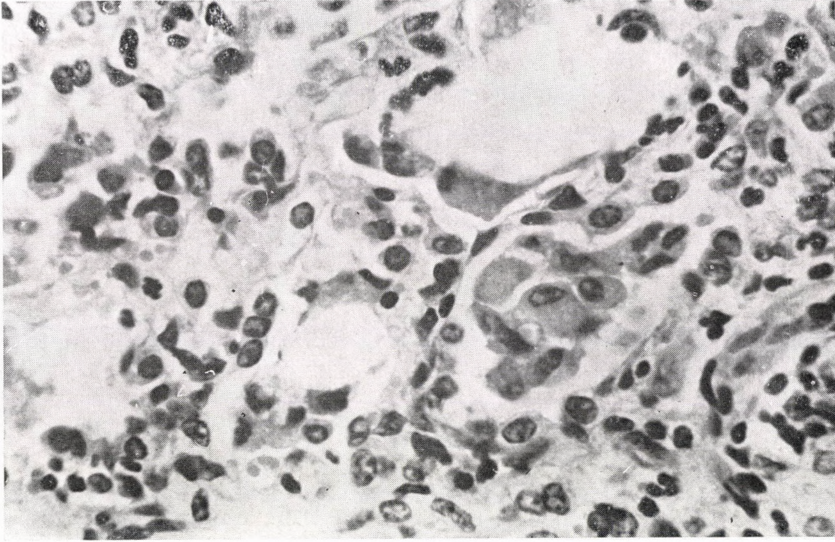

Fig. 6. Degenerated, desquamated alveolar epithelium and inflammatory cells in the alveolar lumen. Haemalaun and eosin stain, ×600

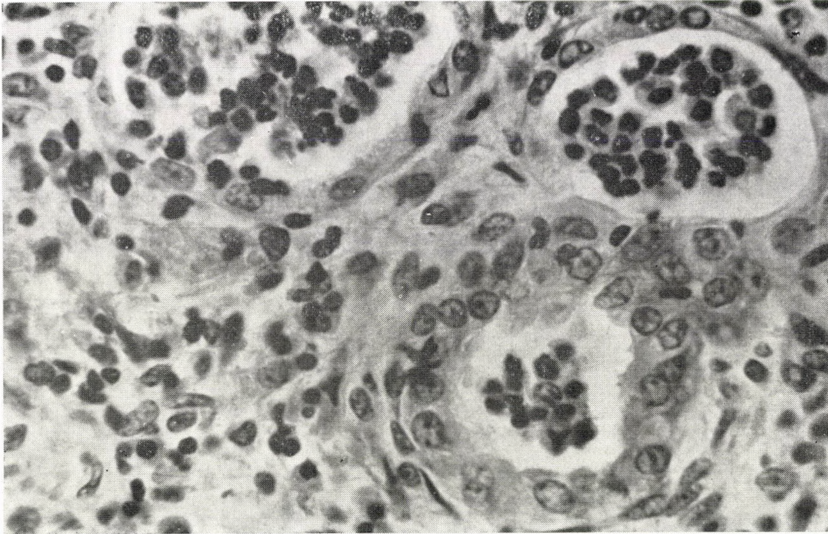

Fig. 7. The alveolar lumen inside the thickened walls is chiefly filled by inflammatory cells. Haemalaun and eosin stain, ×600

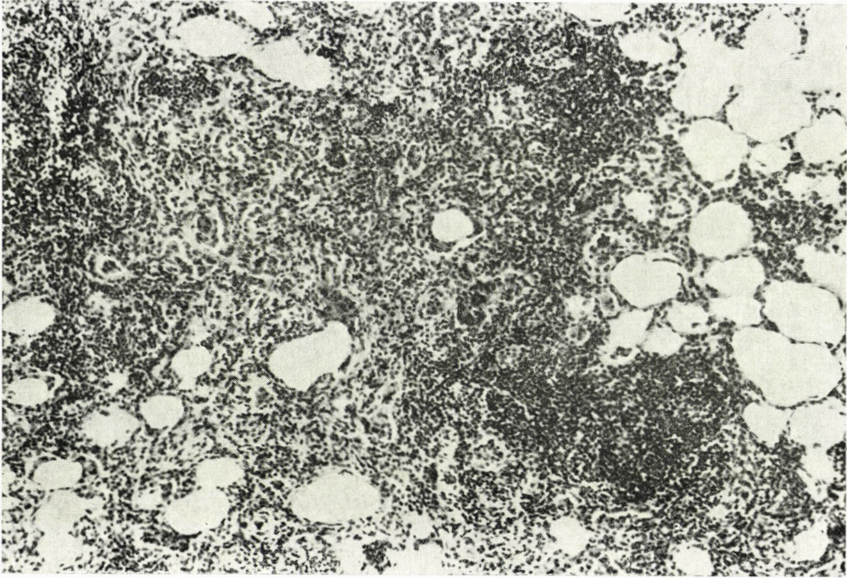

Fig. 8. Fibrotic detail of interstitial pneumonia. Haemalaun and eosin stain, ×95

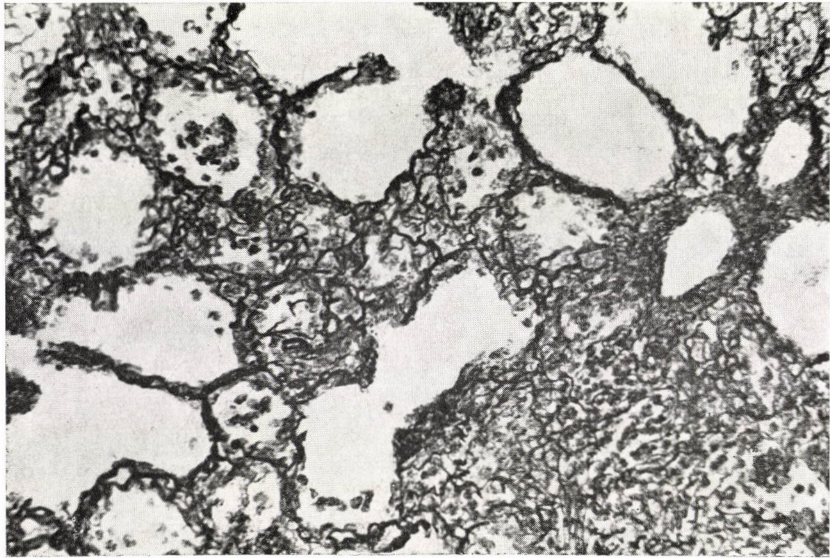

Fig. 9. Thickening and increase of argyrophilic fibres in the lung. Gömöri's silver impregnation technique, ×240

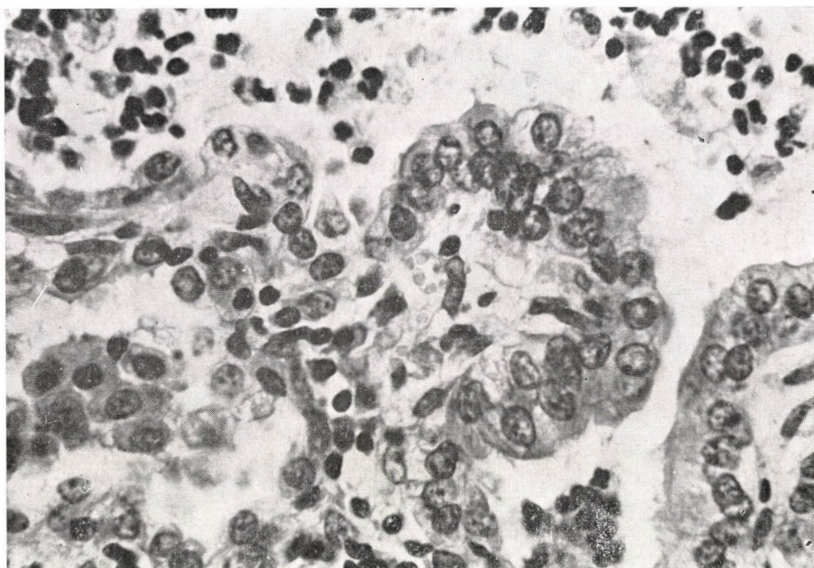

Fig. 10. Proliferation in the lumen of alveoli and bronchioli. Haemalaun and eosinstain $\times 600$

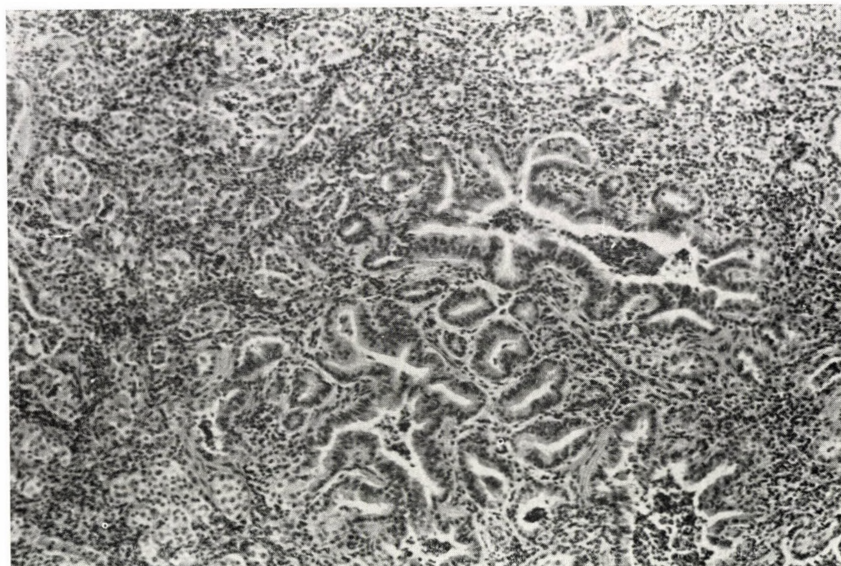

Fig. 11. Adenoma-like proliferation of minor airways. Haemalaun and eosin stain, $\times 95$

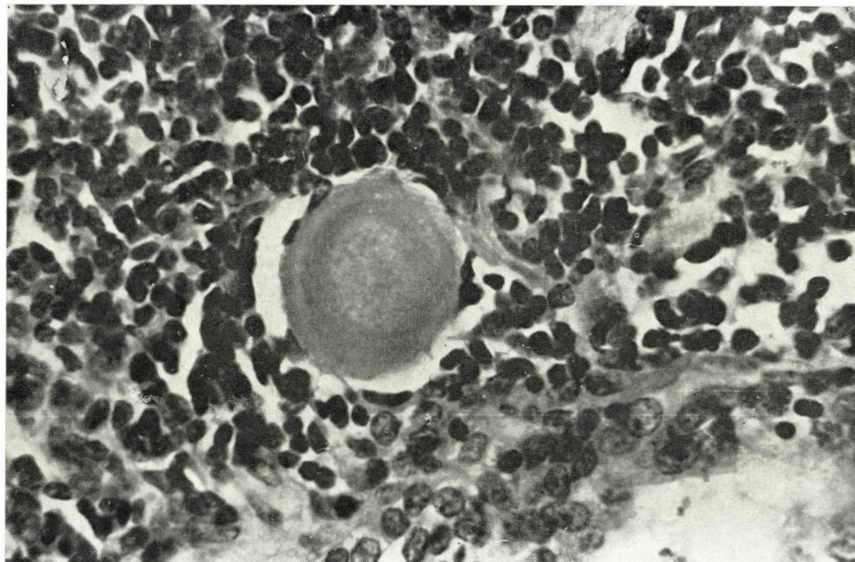

Fig. 12. Concentration-like body surrounded by inflammatory cells in the bronchiolar lumen. Haemalaun and eosin stain, ×600

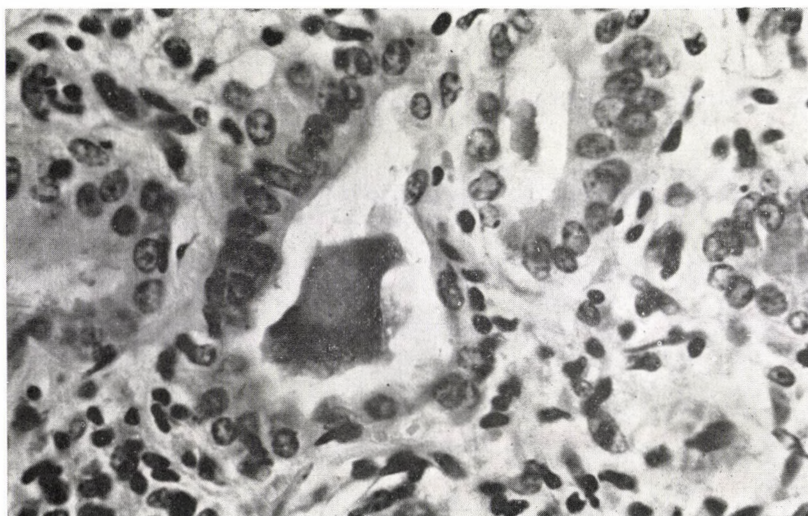

Fig. 13. Giant cell in the alveolar lumen. Haemalaun and eosin stain, ×600

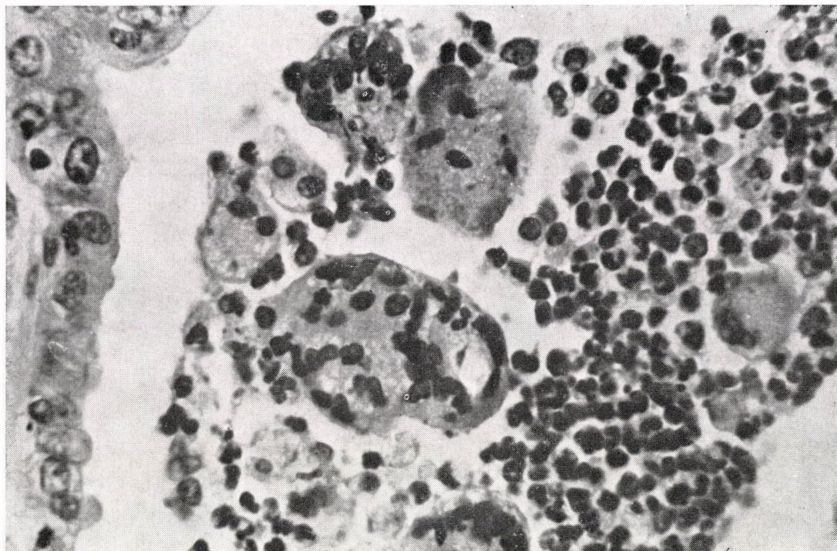

Fig. 14. Giant cells and inflammatory cells in the bronchiolar lumen. Haemalaun and eosin stain, ×600

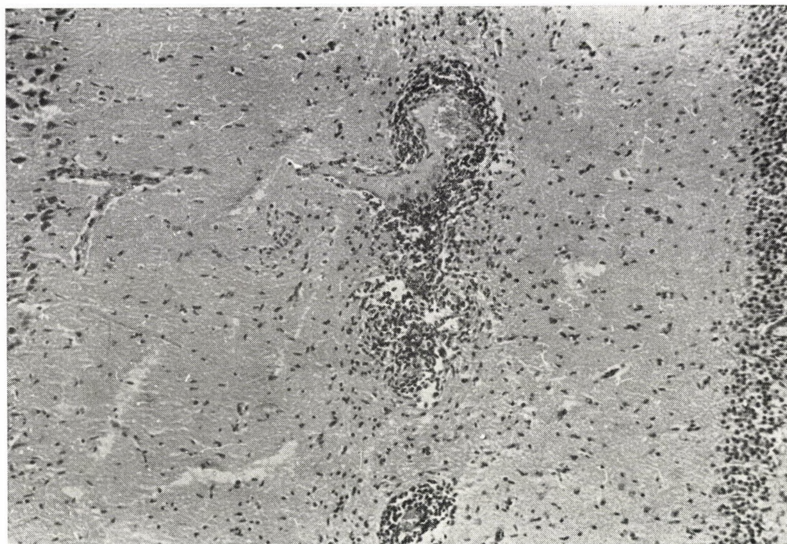

Fig. 15. Detail of Ammon's horn. Note the focal, mixed glia cell proliferation and the vascular lymphocyte coat. Haemalaun and eosin stain, ×95

Parasitological examinations of faecal samples and lungs revealed a very slight lung helminthosis, if any, which clearly had no causal relation with the pneumonia.

Discussion

We concluded from our examinations that the condition observed in sheep by us is identical with the disease described in other countries as Maedi, chronic progressive interstitial pneumonia or under other synonymous names.

We agree with other authors that the gross and microscopic lesions of the lungs are so characteristic that along with the observation of the clinical symptoms and course, a sure diagnosis can be established without isolation of the causative virus. Further studies are in progress to clarify whether the condition, showing all morphological characteristics of chronic interstitial sheep pneumonia, is etiologically also identical with the disease described abroad.

As far as we are informed, the disease did not previously occur in Hungary. Although there is not evidence, its introduction by imported breeder rams seems highly probable, because recently many such animals have been procured from several countries in which the disease occurs regularly. As the sperms of these rams were used for artificial insemination in nearly all large sheep flocks of the country, the incidence of chronic progressive interstitial pneumonia in additional flocks can well be expected.

Therapy is useless and attempts at protection by active immunization have also failed, at least as far as we know. In consideration of the contagious nature of the disease and the great economic losses caused by it, the following measures are proposed to prevent its spread. Breeder rams must not be procured from other than "clean" flocks either home or abroad. The veterinary hygienic control, ordered by the National Board for Animal Breeding, should be extended to this sheep disease. Sheep diseased in a pneumonia irresponsive to therapy should be regarded as suspect of Maedi and organs, especially the lungs of animals naturally died from, or emergency-slaughtered for, this condition, should be examined microscopically. If the incidence of the disease has been noted in a flock, all sick animals should be slaughtered as soon as possible.

SUMMARY

The viral disease Maedi (chronic progressive interstitial pneumonia) was observed in Hungary in three Merino-type sheep flocks early in 1972. As the condition had not previously been known in this country, its introduction by imported breeder rams seems probable. The main clinical symptoms are a severe progressive dyspnoea, increased respiration frequency and emaciation. The disease is irresponsive to therapy and always ends fatally after a course of 2—6 months. Grossly the lungs were found to have enlarged, weighing 1600—2000 g instead of the usual 600—800 g. There were extensive areas of atelectasis inside which grayish-pink miliary foci were seen and the peribronchial lymph nodes grew large with hyperplasia. The microscopic picture of the lung lesions corresponded to a chronic progressive interstitial

pneumonia, accompanied by lymph follicle formation, proliferation of the alveolar, bronchiolar and bronchial epithelium and adenoma-like changes. Among the inflammatory cells there were giant cells and a few concretion-like, basophilic bodies. The central nervous system was also involved; there was a non-purulent encephalitis, accompanied by gliosis. The disease is contagious and causes great economic losses. The Ministry for Agriculture and Food ordered that the infected breeds should be treated as closed flocks and issued several instructions for the prevention of further spread.

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OK SEROGROUPS AND LOOP-DILATING EFFECT OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM PIGS

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In *Escherichia coli* neonatal diarrhoea *E. coli*-enterotoxaemia and oedema disease of swine, the decisive causative role has generally been attributed to certain *E. coli* strains of well-defined serotypes.

Serological studies of isolates from diseased animals have shown that new serotypes may also appear and the incidence of the strains known as enteropathogenic may change with the country of occurrence and periods of time (SOJKA et al., 1960; SOJKA, 1965; GOSSLING and RHOADES, 1966, 1967; SZABÓ, 1965; MOON et al., 1966a, and others).

The observations that broth cultures of *E. coli* strains isolated from pigs suffering from *E. coli*-diarrhoea cause an accumulation of fluid in the ligated small-intestinal loop have been utilized as a new approach to the problem of the pathogenesis of *E. coli* infections in pigs (MOON et al., 1966b; SMITH and HALLS, 1967a; GYLES and BARNUM, 1967; NIELSEN and SAUTTER, 1968). The loop-dilating effect of the strains is related to their enterotoxin-producing capacity. On the basis of examinations along this line, those strains have been regarded as enteropathogenic which are capable of growing and producing enterotoxin(s) in swine small-intestine. Up to now SOJKA (1970) found 18 OK groups to meet this requirement.

Since the investigations of PESTI (1960) and SZABÓ (1965), no serological typing of swine *E. coli* strains has been reported in Hungary, neither have enteropathogenicity studies been described with the loop test. It seemed therefore worthwhile to examine (1) the OK groups of *E. coli* isolates from different diseases in various swine farms as well as (2) the relationship between the origin, loop-dilating effect and serogroup of the isolates.

Materials and methods

E. coli strains

A total of 92 strains* were isolated from swine herds of 58 farms, situated in different parts of the country, in the period 1969—1972. The outbreaks of *E. coli*-diseases were epizootologically unrelated to one another. Data of the isolation and maintenance of the strains have been described elsewhere (PESTI and SEMJÉN, 1973).

* One part of the strains was kindly supplied by dr. P. ÁLDÁSY and dr. T. SÜVEGES.

The sources of the examined 92 strains were the following: (1) 24 strains were isolated from suckling piglets 1 to 10 days of age, diseased in *E. coli*-diarrhoea; (2) 36 strains originated from pigs died of *E. coli*-enterotoxaemia. This group included piglets from 3 weeks to 3 months old, which had had a distinct diarrhoea but showed no signs of oedema disease at post mortem examination. (3) Twelve strains originated from cases diagnosed as oedema disease, among 3 weeks to 3 months old pigs. (4) A total of 20 strains, used as controls, originated from the following sources: 8 from the faeces of healthy animals, 7 from healthy animals in contact with diseased ones and 5 from the small intestine of animals died of diseases other than *E. coli* infection.

All isolates were examined for indole production, lactose, glucose, mannitol and inositol decomposing activity, haemolytic property and behaviour in the Voges-Proskauer, methyl red and Eikman's tests.

E. coli *antisera. Serological examinations*

O and OK antisera were prepared in rabbits with strains recognized as enteropathogenic, using the method of SOJKA (1965). The strains were kindly supplied by Dr. W. J. SOJKA (Central Veterinary Laboratory, Weybridge) and antisera were prepared to the following O serogroups: O8, O9, O10, O35, O45, O64, O108, O115, O116, O119, O138, O139, O141, O147, O149. The capsular antigen was determined by means of the K and OK sera 85b(B), 85c(B), 87(B), 89(B), 91(B), 88ab(L), 88c(L), and O45: K"E65", O116: K"V17", O138: K81(B), O139: K82(B), O9: K"P16"(A), O64: K"V142", O108: K"189", O10: K"V50", O35: K"V79", O115: K"V165", O119: K"V113".*

The O antigens were determined by tube agglutination, the K antigens by slide agglutination, according to the method of SOJKA (1965). Those strains whose O and K antigens could not be determined with any of the above sera, were classified as untypeable. Some strains were sent to the WHO International Escherichia Centre for O and K88 antigen determination; the work was done by I. ØRSKOV, to whom we are greatly indebted for the help. Her results were consistent with our findings. One of us (G. S.) typed several strains in Sojka's laboratory, using Dr. SOJKA's type sera. We followed SOJKA's scheme (1971) for the nomenclatural distinction of serogroup and serotype throughout.

Loop test

Of the 92 isolates 86 were studied for behaviour in the loop test. We used the method of SMITH and HALLS (1967), slightly modified by us (SEMJÉN and PESTI, 1973). The loop-dilating effect and enterotoxin-producing capacity of the strains will be described elsewhere.

* Certain K-antigens are designated in this paper with the symbols used in Weybridge (in quotation marks) for lack of an internationally approved designation.

Results

All examined strains produced indole, decomposed lactose, glucose, and mannitol, but did not split inositol, reacted positively in the methyl red and Eikman's tests, but negatively in the Voges-Proskauer test. Each of the 72 strains isolated from pigs diseased in *E. coli*-diarrhoea, *E. coli*-enterotoxaemia or oedema disease, had a haemolytic activity, but only one of the 8 strains originating from healthy animals possessed it. The majority of the isolates from healthy animals in contact with sick ones as well as from pigs diseased in a condition other than *E. coli* infection also displayed a haemolytic activity.

The results of serological examinations and behaviour in the loop test of strains originating from animals with *E. coli*-diarrhoea and *E. coli*-enterotoxaemia are shown in Table I. In this category, 68.3% of the strains belonged to the serogroups O147: K89, K88ac and O149: K91, K88ac. It seems remarkable that a strain belonging to the O139: K82 OK group was isolated from the carcases of two pigs died of *E. coli*-diarrhoea. The broth culture of one of these isolates was positive in the loop test. The overwhelming majority (87.5 and 94.2%, respectively) of the strains originating from the two diseases elicited a fluid accumulation in the ligated small intestinal loop.

The serological classification and behaviour in the loop test of strains originating from oedema disease and their controls are shown in Table II.

Table I

OK serogroups and loop dilating effect of *E. coli* strains isolated from pigs with *E. coli*-diarrhoea and *E. coli*-enterotoxaemia

Serogroups	*E. coli*-diarrhoea			*E. coli*-enterotoxaemia		
	No. of strains	Loop test		No. of strains	Loop test	
		positive	negative		positive	negative
O2*	—	—	—	1	—	1
O14 : K?, K88ac	1	1	—	—	—	—
O108 : K "V189"	—	—	—	3**	1	1
O115 : K "V165"	—	—	—	1	1	—
O138 : K81, K88ac	—	—	—	1	1	—
O139 : K82	2	1	1	—	—	—
O147 : K89, K88ac	9	9	—	12	12	—
O149 : K91, K88ac	8	8	—	13**	12	—
Untypeable	2	1	1	1	1	—
R-form	2	1	1	4	4	—
Totals	24	21	3	36	32	2

* Typed by I. ØRSKOV*.
** One strain was not examined by loop test.

Table II

OK serogroups and loop-dilating effect of isolates from oedema disease and of control strains

Serogroups	Oedema disease			Healthy animals			Control					
							Healthy animals in infected environment			Diseases unrelated to *E. coli*		
	No. of strains	Loop test		No. of strains	Loop test		No. of strains	Loop test		No. of strains	Loop test	
		pos.	neg.		pos.	neg.		pos.	neg.		pos.	neg.
O8 : K?, K88ac	—	—	—	—	—	—	—	—	—	1	1	—
O9 : K "P16" (A)	—	—	—	—	—	—	—	—	—	1	—**	—
O45 : K? (KE65)	—	—	—	—	—	—	1	—	1	—	—	—
O51*	—	—	—	1	—	1	—	—	—	—	—	—
O108 : K "V189"	—	—	—	—	—	—	1	1	—	—	—	—
O138 : K81	1	1	—	—	—	—	—	—	—	—	—	—
O139 : K—	1	—	1	—	—	—	—	—	—	—	—	—
O139 : K82	6	—	5**	—	—	—	—	—	—	—	—	—
O141 : K85ab	1	—	1	—	—	—	—	—	—	—	—	—
O141 : K85ac	1	—	1	—	—	—	—	—	—	—	—	—
O149 : K91, K88ac	—	—	—	—	—	—	3	3	—	—	—	—
Untypeable	1	—	1	7	1	4**	1	—	1	2	—	2
R-forms	1	—	1	—	—	—	1	—	1	1	—	1
Totals	12	1	10	8	1	5	7	4	3	5	1	3

* Typed by I. ØRSKOV.
** The missing strains were not tested.

Seven of the 12 isolates belonged to the O139 group. All strains, except one having the antigenic pattern O138: K81, failed to cause dilation of the ligated intestinal loop. The O and K antigens of the isolates originating from healthy animals could not be determined with the available sera and only one of 6 strains showed a positive loop test. Four of the 7 strains isolated from healthy animals kept in infected environment could be classified into the recognized enteropathogenic groups O149: K91, K88ac and O108: K"V189" and these were also loop-test positive. One of the remaining three strains showing a negative loop test belonged to the serogroup O45: K?(KE65); this OK group is common in oedema disease. Of the 5 strains originating from animals diseased in conditions aetiologically unrelated to *E. coli*, 2 were typeable for O and K antigens with the available sera. One of these strains (O8: K?, K88ac) elicited fluid accumulation in the ligated intestinal loop, the others were loop-test negative.

Discussion

In 1963, SOJKA and SWEENEY (Cit. SOJKA, 1965) made several isolations of a strain from pigs diseased in "enteritis"; they classified it into the group G1235 OK. ØRSKOV et al. (1964) recognized the strain as a new serotype, O147: K89, K88ac: H19. In the German Democratic Republic WITTIG (1963) and in Ireland SWEENEY (1964) frequently isolated a strain containing K88 antigen; the antigenic pattern of this strain was later identified by ØRSKOV et al. (1969) as O149: K91, K88ac: H10.

According to earlier studies in Hungary and abroad (SOJKA et al., 1957; EWING et al., 1958; CAMPBELL, 1959; SOJKA et al., 1960; SOJKA, 1965; SZABÓ, 1965; MOON et al., 1966, and others), the predominant O groups were O8, O9, O101, O138 and O141 in *E. coli*-diarrhoea; O138 and O141 in *E. coli*-enterotoxaemia and O139, O138 and O141 in oedema disease. Recent investigations have, however, disclosed other predominant serogroups. In Sweden, SÖDERLIND (1971) found the predominance of the O groups 8, 141 and 147 in *E. coli*-diseases of newborn piglets and the O group 141 in "weanling pigs with enteritis". In Canada, GYLES et al. (1971) identified 87% of the isolates from pigs with *E. coli*-diarrhoea as O8: K87, K88ac, O116: K"V17", K88ac, O147: K89, K88ac, O138: K81 and O45: K"E65" and failed to find strains of the OK groups 149: K91, K88ac, O138: K81, K88ac and O141: K85, although formerly KELEN et al. (1959) had presented evidence of the occurrence of the serotypes O141: Kx(B): NM and O138: K81: H8 (or H14). In the United States, the serotype O149: K91, K88ac: H10 was first isolated by GLANTZ and KRADEL (1971). In Australia, BEH (1971) found only the OK group O8: K87, K88ac, although he used the same antisera as we. WILLINGER and ILIADIS (1970) examined 200 haemolytic *E. coli* strains from pigs diseased in diarrhoea with the result that 30% belonged to the OK group O149: K91, K88ac and about 7% to O147: K89, K88ac. KNOX and DAM (1970) classified 96% of their isolates from piglets died of *E. coli*-diarrhoea into the OK group O149: K91. These are only a few examples; for details, the reader is referred to the review of SOJKA (1971) and SÖDERLIND (1971), from which it is clear that strains of the O149 and O147 groups have been isolated in several other countries as well.

The majority of the strains isolated in the course of this study from *E. coli*-diarrhoea and *E. coli*-enterotoxaemia cases belonged to the O groups O147 and O149, whereas the rest represented 6 different O groups, untypeable strains and R-forms. Our findings clearly indicate that recently the causative agents of *E. coli*-diarrhoea and *E. coli*-enterotoxaemia have chiefly belonged to the OK groups O147: K89, K88ac and O149: K91, K88ac in Hungary too.

The occurrence of two strains with O139: K82 antigenic pattern among the isolates from *E. coli*-diarrhoea is remarkable; one of them even caused accumulation of fluid in the ligated intestinal loop. Thus it seems probable

that the *E. coli* O139: K82, generally responsible for oedema disease, may occasionally cause an *E. coli*-diarrhoea in suckling piglets.

More than one half of the strains isolated from oedema disease belonged to the serogroup O139: K82. Apparently, strains possessing the O antigen O139 still play a decisive role in this condition. Except for a single strain of the O138: K81 serogroup, the isolates from oedema disease failed to elicit fluid accumulation in the ligated intestinal loop. This again favours the assumption that the clinically and pathologically different forms of *E. coli* infections are elicited by strains possessing various diseases-producing factors.

A substantial part of strains from *E. coli*-diarrhoea and *E. coli*-enterotoxaemia had a loop-dilating effect, *viz.* produced enterotoxin(s) (Smith and Halls, 1967b; Gyles and Barnum, 1969; Pesti and Semjén, 1973) thus the diarrhoeal symptoms seem to be closely relate dto the enterotoxin-producing capacity of the strain involved in the aetiology.

The OK groups of the strains isolated from healthy animals could not be determined with the available sera and all strains except one behaved negatively in the loop test. Strains belonging to serogroups recognized as enteropathogenic and giving a positive loop test were, however, often encountered among the isolates from healthy contacts of diseased swine. The fact that contact animals may carry enteropathogenic strains of *E. coli* indicates that the presence of factors other than the causative agent is also required for the establishment of an *E. coli*-infection.

In accordance with the observations of others, the present results suggest that as yet unidentified serotypes may appear along with, or instead of, the *E. coli* strains regularly involved in enteric *E. coli* swine diseases over certain periods. In this light, immunization attempts based on the serogroup or serotype seem to be of doubtful value. The majority of the strains from serogroups generally regarded as enteropathogenic gave a positive loop test (produced enterotoxin), while some strains of the same group *(e.g.* O108: K"V189") were lacking this capacity. Certain loop-test positive isolates from swine suffering from *E. coli*-diarrhoea could not be classified into any of Sojka's (1970) 18 serogroups. As, according to Smith and Halls (1968), the enterotoxin-producing capacity is transferable from one strain to the other, the loop-dilating effect of the loop-test positive, but untypeable strains may have originated from another strain of a recognized enteropathogenic serogroup.

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SUMMARY

A total of 92 *E. coli* strains from pigs were examined for OK serogroup and 86 of them were tested for loop-dilating effect. Of 60 strains isolated from animals diseased *in E. coli*-piglet diarrhoea and *E. coli*-enterotoxaemia, 42 (68.3%) belonged to the serogroups O147 :

K89, K88ac, and O149 : K91, K88ac, which had not previously been identified in Hungary. Of the remaining 18 strains 9 belonged to one of 6 further serogroups, the others were untypeable or R-forms. Ninety-one per cent of the strains isolated from the above two conditions had a loop-dilating effect.

Of the 12 strains originating from oedema disease 7 belonged to the serogroup O139 : K82 and all except one (O138 : K81) were loop-test negative. The majority of the isolates from healthy pigs and that of diseased in a condition unrelated to *E. coli* was untypeable with the available sera and the greater part of them failed to elicit accumulation of fluid in the ligated intestinal loop.

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STUDIES ON ENTEROPATHOGENICITY, LOOP DILATING EFFECT AND ENTEROTOXIN PRODUCING CAPACITY OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM ENTERIC DISEASES OF SWINE

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Escherichia coli is known to be always present in the normal intestinal flora. Certain *E. coli* species may, however, cause severe, sometimes even lethal diseases both in man and animals. The important aetiological role of *E. coli* in the diarrhoea of newborn piglets, calves and human infants has long been known, but several steps of the pathomechanism are still unclear.

Losses from gastro-enteritis due to *E. coli* are especially severe among intensively managed swine and cattle herds where the population density is high. This circumstance initiated closer investigations into the pathogenesis of *E. coli*-diseases.

American and English authors (Nielsen, 1963; Gyles, 1966; Smith and Halls, 1967; Gyles and Barnum, 1969; Smith and Gyles, 1970; Gyles, 1971, and others) showed that live cultures of *E. coli* strains isolated from diseases manifested by diarrhoea cause accumulation of fluid in the ligated swine intestinal loop and usually produce an exotoxin-like thermostable enterotoxin and a thermolabile enterotoxin associated with the bacterial cell. Enterotoxin production (1) is an important characteristic of the enteropathogenic *E. coli* strains, but no disease develops unless (2) multiplication of the enterotoxin-producing bacteria attain extremely high number in the upper segments of the small intestine. The loop test proved to be a reliable indicator of the enteropathogenic property and enterotoxin production of *E. coli* strains.

The aim of the present study was to obtain more information about the live cultures and enterotoxin action of *E. coli* strains isolated from pigs in different parts of Hungary. Isolates from outbreaks of *E. coli*-diseases were examined for loop-dilating effect and compared for behaviour in the loop test.

Thermostable and thermolabile enterotoxin preparations obtained from the cultures of the isolated strains were tested for loop-dilating effect and correlation was sought between their behaviour and that of the original live culture. Young suckling pigs were inoculated orally with thermostable and thermolabile enterotoxins to decide whether the bacterium-free enterotoxins are active in themselves.

Methods

A total of 86 *E. coli* strains were isolated from pigs of 52 herds in different parts of Hungary; 24, 34 and 11 strains originated from carcases of pigs died of *E. coli*-diarrhoea, *E. coli*-enterotoxaemia and oedema disease, respectively. The 12 control strains were isolated from healthy swine and swine diseased in conditions unrelated to *E. coli*. The outbreaks of *E. coli* disease in the different farms were epizootologically unlerated to one another. The isolations were made either from fresh carcases or from the small intestine (duodenum and jejunum) of emergency-slaughtered swine. Only a part of the control strains had originated from faecal samples. Samples of the small intestinal content were streaked directly on blood agar plates; only pure *E. coli* cultures of the first isolation were used for further study. The isolates were purified three times and transferred to broth medium for propagation; samples from each broth culture thus obtained were lyophilised in ampoules. All experiments were carried out with the freeze-dried bacteria. The isolations were performed in the period 1969—72.

The isolates were examined for the main biochemical and biological properties as well as for the serological (O and K) group; the results have been described elsewhere (Semjén and Pesti, 1973). The strains used in the loop test were incubated for 20 hours in nutrient broth and 1 ml of this culture was inoculated into the ligated loop. The germ count of the broth cultures usually ranged from 1×10^8 to 5×10^8 per ml.

Preparation of the thermostable (ST) enterotoxin. The method and medium described by Smith and Halls (1967) was used. The strains were cultured in a semi-liquid medium containing 0.3% agar (Proteose peptone, Difco, 10 g; NaCl, 5 g; beef extract (Oxoid), 10 g; glucose, 2 g in 1000 ml distilled water adjusted to pH 7.5) for 24 hours, then the cultures were filtered through gauze to remove the agar, centrifuged at 10.000 r.p.m. for 30 minutes and the supernatant, containing only few bacterial cells, was heated in a water bath of 65°C for 15 min. The enterotoxin preparations thus treated proved to be sterile. The preparations were stored at —20°C without addition of antibiotics. Fifteen ml ST enterotoxin was used for each loop test.

Preparation of the thermolabile (LT) enterotoxin. The method of Gyles and Barnum (1969) was used with the difference that the strains were cultured on a solid medium, a tryptose agar containing 0.2% glucose (Tryptose, Oxoid, 10 g; beef extract (Oxoid), 3 g; NaCl, 5 g; agar, 18 g; glucose, 2 g; in 1000 ml distilled water (pH 7.2) in Roux flasks for 24 hours at 37°C. The cultures were then washed off with distilled water, using 15 ml for each flask. The bacterium suspensions thus obtained were sonicated for 30 minutes in an MSE ultrasonic disintegrator, at 1.2 A. Prior to sonication, the *E. coli* counts of the suspension were determined on blood agar: it usually ranged from 1×10^{10} to 1×10^{11} per

ml. The sonicated suspension was centrifuged in a Backman ultracentrifuge (Spinco L2, 65B) for 30 minutes at 25,000 r.p.m. and the supernatant was passed through a G5 glass (Pyrex) filter to remove all bacteria. The LT preparations thus sterilized were stored at —20°C without addition of antibiotics. Of the LT toxin preparation 6 ml was used for each loop test.

Procedure and evaluation of the loop test. A total of 67 white bacon pigs, 8—12 weeks old, weighing on the average 10—15 kg, were used. All experimental animals were procured from one and the same herd and care was taken to use litter mates for each experimental series, as far as possible. The loop test was a slightly modified version of the procedure described by SMITH and HALLS (1967). After fasting for 24 hours, the animals were anaesthetized with a combination of Stresnil (R 1929) and Hypnodil(7315)(Jannsen Pharmaceutica); the former was administered intramuscularly, in a dose of 0.05 ml/1 kg body weight, the latter intraperitoneally, in a 0.22 ml dose. The pigs usually fell asleep 3—5 minutes after the anaesthetic treatment and remained practically motionless throughout the surgical intervention. The intestines were exposed through a left abdominal incision and the duodenum was ligated with silk thread at distances of 12 cm, beginning 80—100 cm away from the pylorus. The test materials were introduced into the ligated segments by means of a syringe. The animals were allowed to survive the operation for 24 hours, after which they were killed with an overdose of the anaesthetic and the results were read. Usually 25—35 ligated segments were prepared for the examination of broth cultures. The jejunal portions distal to the 35th segment are less suitable for the loop test, owing to their lower sensitivity, and only the first 15 segments are suitable for enterotoxin examinations. Thus, these were used throughout.

The loop-dilating effect of the live culture of each *E. coli* strain was tested on two animals, in two segments localized as distantly as possible from each other. Thus, four figures were obtained for the loop-dilating effect of the strain and the further calculations were made with the mean derived from these figures. Broth cultures were injected into four successive segments, between two segments left uninoculated; the segment after the distal empty one was inoculated with the broth culture of a reference strain with known loop-dilating activity. If the reference strain failed to dilate the loop, or fluid appeared in the uninoculated segments, the test was excluded from the evaluation. The enterotoxin tests were similarly performed, except that in addition to the live reference strain also standard enterotoxin (ST or LT) preparations of marked loop dilating activity were used as control.

The evaluation of the loop test was initially based on the determination of the amount of accumulated fluid in ml. The amount of fluid usually ranged from 20 to 100 ml in the positively reacting segments, whereas only 0.5—3 ml of a thick fluid was found in the negative segments.

As the preparation of equally long segments was impossible even with the utmost care, evaluation was later based on the weight of fluid related to 1 g intestinal wall (weight/weight ratio) and the figure thus obtained was regarded as dilatation index. The weight of the empty segment wall was subtracted from the total weight of the segment and the result was divided by the weight of the empty segment. Numerous comparative studies showed that index values of 1.00 or higher signify a positive reaction, whereas those less than 1.00 a negative reaction. On the basis of this finding, all results were further on expressed in index values.

Oral infection of suckling piglets. A 24-hour broth culture (30 ml) or the enterotoxin (30 ml) of the examined strains was administered to 24—72-hour old piglets by means of a gastric tube and the young animals were placed again with the sow. They were observed for 5 days, during which the appearance or absence of diarrhoea was recorded.

Results

Loop-dilating effect of live cultures. The examined 86 strains were divided into four groups according to origin. Twenty-four strains (group 1) were isolated from 1—10 days old suckling piglets with *E. coli*-diarrhoea. Thirty-four strains (group 2) originated from weanling 3—12 weeks old pigs with *E. coli*-enterotoxaemia. The main symptom of this disease was diarrhoea and no lesions indicative of oedema disease were found on post mortem examination. Eleven strains (group 3) were isolated from 3—12 weeks old pigs suffering from oedema disease. The strains used as controls originated from faecal samples or small intestine of healthy swine (6 strains), from healthy pigs kept in an infected environment (7 strains) and from the small intestine of pigs died of conditions other than *E. coli*-infection (4 strains). All isolates were examined by the loop test (Table I).

Table I

Enteral pathogenicity as assessed by the loop test for 86 *E. coli* strains isolated from swine of 47 herds in different parts of Hungary

		E. coli diarrhoea of suckling pigs 24 strains	*E. coli* entero-toxaemia 34 strains	Oedema disease 11 strains	Healthy animals 6 strains	Healthy animals in infected environment 7 strains	Diseases other than *E. coli*-infection 4 strains
Loop test positive	No.	21	32	1	1	4	1
strains	%	(87.5)	(94.1)	(9.0)			
Index* (w/w ratio)		4.28	3.26	5.02	2.31	3.49	3.83
No. of loop test negative strains		3	2	10	5	3	3
Index* (w/w ratio)		0.25	0.18	0.36	0.19	0.43	0.30

* Index figures for more than one strains are expressed as arithmetic means.

Twenty-one (87.5%) of the 24 strains from suckling piglets with *E. coli*-diarrhoea had a distinct loop-dilating effect and 32 (94.2%) of the 34 strains from post-weaning *E. coli*-diarrhoea behaved similarly. Ten of the 11 strains from oedema disease, which does not involve diarrhoeal symptoms had, however, no loop-dilating effect. Eleven control strains were loop-test negative and 6 were positive; 4 of the positive strains had been isolated from healthy contacts of pigs with *E. coli*-diarrhoea. Thus, the overwhelming majority (91.4%) of the isolates had a loop-dilating effect, showing that the *E. coli* strains originating from diarrhoeal diseases of swine in Hungary can be regarded as enteropathogenic, in accordance with observations made abroad. Table 1 also shows that the mean dilatation index ranged from 3.26 to 4.28; this means the presence of an average 32—42 g (ml) fluid in one segment. Consequently, positive strains had index values above 1.00 in all four segments of two animals and the four data from which the mean was calculated did not as a rule notably deviate. The degree of loop-dilating activity differed, of course, with the various strains; indices above 4.00 and ranging from 1.00 to 4.00 were roughly distributed half by half. It follows that under the given conditions (using as inoculum 1 ml of a broth culture containing 100—500 million germs/ml), the loop test is a reliable indicator of enteral pathogenicity.

Studies of the antigenic structure of the 86 isolates are reported in detail elsewhere; it should be, nevertheless, remarked here that the majority of the strains belonged to the serological group O147: K89, K88ac, or to O149: K91, K88ac

Serological typing of the isolates not possessing a loop-dilating effect showed that two strains (originating from *E. coli*-diarrhoea) of the O139 type (P27, P88) were also isolated. The O139 type strains have been known as typical causal agents of oedema disease but, according to our experimental results, they may exceptionally grow and cause diarrhoea in suckling pigs. Only two of the isolates from pigs with post-weaning diarrhoea were loop-test negative; these belonged to the serogroups O2 and O108, respectively. Ten of the 11 isolates from oedema disease failed to dilate the intestinal loop, whereas the remaining one caused a marked loop dilatation. The 11 strains were found to belong to the serogroups known to cause oedema disease (O138, O139, O141: K85ab). The single loop-test positive strain belonged to the serogroup O138, which is known to occur in diarrhoeal enteric diseases and in oedema disease as well and some strains of it have a loop-dilating effect. The fact that most oedema disease strains were loop-test negative clearly indicates that the enterotoxin-producing capacity plays no role in the pathogenesis of this condition.

Among the 10 strains originating from healthy swine and from swine with diseases unrelated to *E. coli*-infection, only 2 had a loop-dilating activity. It appears that enteropathogenic strains may occasionally occur in healthy animals. Among the 7 strains isolated from apparently healthy pigs kept in an

infected environment 4 were loop-test positive, indicating that enteropathogenic strains may be transferred from diseased to clinically healthy animals.

Testing of thermostable (ST) and thermolabile (LT) enterotoxins prepared from enteropathogenic E. coli *strains.* ST and LT enterotoxins, which may be held responsible for the loop-dilating effect, had been prepared from 37 serologically identified strains and examined in the loop test. The behaviour of both kinds of toxin was compared with that of the live culture of the toxin-producing strain. The results are shown in Table II.

Table II

Comparative studies by loop test of broth cultures and thermostable and thermolabile enterotoxins of 37 enteropathogenic *E. coli* strains

Origin of strains	Antigenic structure	No. of examined strains	Loop test with live culture		Loop test with LT and ST enterotoxins			
					thermolabile (LT)		thermostable (ST)	
			Effect	index $\bar{x}$	effect	index $\bar{x}$	effect	index $\bar{x}$
E. coli-diarrhoea	0147 : K89, K88ac	3	+	4.16	+	3.24	+	4.63
	0149 : K91, K88ac	5	+	3.97	+	5.78	+	5.30
	014 : K88ac	1	+	6.86	+	3.75	+	6.50
	0139	1	+	2.13	+	10.55	+	10.65
E. coli entero-toxaemia	0147 : K89, K88ac	9	+	4.16	+	6.79	+	4.47
	0149 : K91, K88ac	11	+	3.80	+	6.01	+	4.79
	0115	1	+	2.99	+	3.09	+	3.90
Oedema disease	0138	1	+	5.02	—	0.50	+	1.83
	0139	4	—	0.32	—	0.30	—	0.64
	0141, K85ab	1	—	0.84	—	0.25	—	0.33

All isolates from diarrhoeal diseases *(E. coli*-diarrhoea, *E. coli*-enterotoxaemia) were found to produce both thermostable and thermolabile enterotoxins, whereas all isolates from oedema disease except one, failed to produce either ST or LT. It seems therefore very probable that the strains responsible for diarrhoea produce both toxins. The interrelationship between ST and LT production was the same as observed by SMITH and GYLES (1970), *viz.* certain strains (0138) produced only ST, but no strain was found to produce LT alone. Two strains of the serogroups 0139 and 0115, respectively, had a very high LT and ST producing capacity. It should be mentioned here that strains of the 0115 group occur in diarrhoeal cases too.

Five loop-test negative strains from healthy swine were examined for ST and LT production, with negative results for both.

The great majority of isolates from diarrhoeal diseases contained K88 antigen. Two strains of this group had no K88 antigen, but they did produce LT enterotoxin. Thus, in our hands, K88 antigen production was not always associated to LT-producing capacity.

It should be mentioned that four O139 strains isolated from oedema disease were negative in all three tests, but an O139 strain from a piglet died of *E. coli*-diarrhoea had a marked loop-dilating effect and produced both ST and LT toxins.

Infection experiments with a live broth culture of strain O141: K85, K88 and with ST and LT enterotoxins prepared from it. The aim of this experiment was to clarify whether the ST and LT enterotoxins prepared *in vitro* can elicit diarrhoea on oral administration. The live broth culture of the original strain was similarly tested for comparison (Table III).

Table III

Treatment of 24—72 hours old suckling pigs with ST and LT enterotoxins and broth culture of the *E. coli* strain (O141 : K85ab, K88ac)

Treatment	Dose (ml)	No. of treated pigs	No. of pigs diseased in diarrhoea
O141 ST enterotoxin	30	3	3
O141 LT enterotoxin	30	3	3
O141 live broth culture (5×10^8 germs/ml)	30	6	5
Untreated animals (8 piglets)	—	—	2
Medium used for ST production ("blind" preparation)	30	2	—
Nutrient broth medium	30	2	—

Both the ST and LT type enterotoxin preparations caused a disease in the experimental animals. Both preparations had previously been shown to possess a marked loop-dilating effect. The control preparations not containing enterotoxin, did not elicit diarrhoea. The broth culture of the toxin-producing strain had a similar effect as the enterotoxins. The intercurrent disease of the two untreated control pigs was presumably unrelated to the *E. coli*-diarrhoea, because no haemolytic *E. coli* strains could be isolated from them.

Discussion

The overwhelming majority of the *E. coli* strains isolated from diarrhoea-associated *E. coli* diseases of swine in Hungary caused dilatation of the ligated swine intestinal loop. Thus the loop test seems to be a reliable indicator of the enteral pathogenicity of *E. coli* strains; its results were in good accordance

with the epidemiological observations and with the results of oral infection experiments as well.

It seems that the enterotoxin-producing "enteropathogenic" strains play a decisive role in piglet diarrhoea and in post-weaning *E. coli*-enterotoxaemia. This applies particularly to piglet diarrhoea, which may develop without predisposing factors, even if the mother sow is healthy. From this the possibility of carriership has been implied and loop-dilating *E. coli* strains have in fact been demonstrated in healthy swine and their environment by SOJKA (1971) and others, including ourselves.

The loop-dilating effect can be attributed to the enterotoxins. We consistently failed to obtain ST or LT enterotoxin preparations from strains not causing loop dilatation. But unlike TRUSZCZYNSKI and PILASZEK (1969), we were able to prepare loop-dilating enterotoxins in vitro from all strains that behaved positively in the loop test.

All isolates from *E. coli*-diarrhoea and *E. coli*-enterotoxaemia produced both types (ST and LT) of enterotoxin. This is in good accordance with the observation of GYLES and BARNUM (1969) that the overwhelming majority of the enteropathogenic *E. coli* strains produce both ST and LT enterotoxins.

No isolates from oedema disease except a single strain did produce a loop-dilating enterotoxin, thus, probably other not yet identified factors play the decisive role in the pathogenesis of oedema disease.

Little is known of the mechanism of ST and LT production. The ST preparations were not affected by treatment at 100°C but 121°C rendered them inactive. The LT preparations, however, lost loop-dilating activity after treatment at 65°C; this suggests that LT-enterotoxins are of protein nature. According to GYLES (1971), the LT-enterotoxin is a large-molecular substance, consisting at least in part of protein, it cannot pass through the G 200 Sephadex column. The molecular weight of the ST enterotoxin is presumably much lower.

The precise mechanism of the *E. coli*-disease is still far from being clear. Very probably the enterotoxins are responsible for the onset of diarrhoea, as was unequivocally shown by loop tests and oral infection experiments. But no ligated intestinal loops exist under natural conditions. Thus the enteropathogenic *E. coli*-bacteria have to multiply enormously in the small intestine to become capable of producing enterotoxin and to obviate the effects of peristalsis. At the onset of the disease, the peristalsis of the small intestine slows down for unknown reasons, resulting in extreme multiplication of the bacteria and mass production of enterotoxin.

It has been known since the fundamental investigations of DUBOS et al. (1965) and SAVAGE et al. (1968) that in the stomach and large intestine of rodents a layer consisting chiefly of bacteria, above all anaerobes *(B. fusiformis)*, develops in close association with the mucosa. Although no similar finding has as yet been reported in swine, mention should be made of AR-

BUCKLE's (1971) observation that the enteropathogenic swine *E. coli*-strains adhere to, or enter into, the mucopolysaccharide layer covering the mucosa. STALEY (1969) observed a similar phenomenon. According to the microbial genetic examinations of SMITH and LINGGOOD (1971), the antigen K88 may play a role in the phenomenon. Although all except two of our enteropathogenic isolates possessed antigen K88, the precise role of this antigen is still far from being clear. It seems certain, that the enterotoxin-producing capacity is not closely related with the presence of K88 (SMITH and GYLES, 1970), but the enterotoxin is indispensable for the onset of the symptoms. The pathogenicity of the *E. coli* strains is clearly unrelated with their haemolytic activity (SMITH and LINGGOOD, 1971), whence the reason why the great majority of the enteropathogenic *E. coli* strains produce haemolysis, remains to be clarified. It should be mentioned that our isolates from *E. coli*-diarrhoea, *E. coli*-enterotoxaemia and oedema disease all showed a haemolytic activity.

The fine mechanism of the diarrhoea-inducing capacity of enterotoxin is also unclear. AL-AWQUATI et al. (1972) advanced the theory that *E. coli*-enterotoxins act like *V. cholerae*-enterotoxins via the stimulation of adenylcyclase enzyme production resulting in an accumulation of cyclic adenosine-3,5-monophosphate (cAMP) in the mucosal cells, and this upsets the mechanism of mucosal cell transport. Another possibility to be considered is that the enterotoxin may affect the permeability of the capillary vessel systems in the mucosal, submucosal and mesenteric areas. At all events, the accumulation of large amounts of fluid in the intestinal segments suggests a severe disorder of the transport mechanism.

The fact that the enteropathogenic *E. coli* strains produce enterotoxin(s) is a circumstance to be considered in the establishment of control measures. Observations along this line will be reported elsewhere.

It was described earlier (SEMJÉN and PESTI, 1971) that neither the extracellular ST-enterotoxin, nor the intracellular LT-enterotoxin is identical with the *E. coli*-endotoxin. Authors generally agree on this point.

According to recent observations, the strains isolated from enteric *E. coli*-diseases of calves, lambs and human infants are also capable of enterotoxin production. This and the results reported in this paper throw an entirely new light on the prevailing concept of enteric *E. coli*-diseases. Now it seems fairly certain that enterotoxins rather than endotoxin play a role in the diarrhoea-associated forms of these conditions. As far as we are informed, diarrhoea has never been produced by orally administered *E. coli* endotoxin.

SUMMARY

Out of 58 *E. coli* strains isolated from pigs diseased in *E. coli*-diarrhoea or *E. coli*-enterotoxaemia, 91.4% caused dilatation of the ligated swine intestinal loop. Thus, the loop test is suitable for the demonstration of the enteral pathogenicity of *E. coli* isolates; the

loop test results were in good accordance with the epidemiological observations and with the results of oral infection experiments as well. An extracellular thermostable (ST) and an intracellular thermolabile (LT) enterotoxin could be prepared by an *in vitro* method from all isolates from both types of diarrhoeal disease. All except one of the 11 strains isolated from oedema disease failed to dilate the ligated swine intestinal loop and produced neither ST nor LT enterotoxin.

Certain hypotheses on the pathomechanism of diarrhoea are discussed.

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COMPARATIVE STUDIES ON COMPLEMENT PRESERVATION

By

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The preservation of complement for the complement-fixation (CF) test has long been a problem of serologists. A variety of methods have been proposed for this purpose.

FLOSDORF (1949) recommended sodium acetate and boric acid as preserving agents and combined them with NaCl for use as a stabilizer for freeze-drying. SHERWOOD (1951) described as safe measures of preservation storage at +4 to +9°C for 24 hr, deep freezing for 3—4 days, and dilution in an equal volume of aqueous solution of sodium acetate and boric acid for long storage in dry ice. ORLOV (1954) gave several methods in his textbook, based on the use of (1) boric acid plus sodium sulphate, (2) boric acid, sodium sulphate plus sodium acetate, (3) boric acid and lithium acetate, (4) boric acid and NaCl, (5) NaCl alone, (6) magnesium sulphate plus boric acid, (7) boric acid plus sodium acetate, (8) boric acid plus sodium citrate, each of which is to be added to the guinea-pig serum at variable levels and proportions. ORLOV also described complement stabilization by freeze-drying in magnesium sulphate solution. According to CARPENTER (1956), the complement activity was retained in a lyophilised or frozen state for a long time and at —15°C for several months. HALLMANN (1955) described, apart from storage in dry ice and freeze-drying, a wide range of stabilizers, including (1) various combinations of boric acid and NaCl, mixtures of (2) sodium acetate and boric acid, and (3) sodium acetate in saline, added to the guinea-pig serum at appropriate concentrations. The preparation of choice for mass demands is, nevertheless, the freeze-dried complement. In Hungary the use of lyophilised complement containing no stabilizer was approved in 1958. However, this preparation lost much of its activity during lyophilization and storage. Attempts have therefore been made to improve the activity of the preparation.

The experiments performed in this laboratory were based on the working hypothesis that the activation of complement requires Ca^{++} and Mg^{++} (ALFÖLDY et al., 1967). According to MELNICK et al. (1968) and MAYER et al. (1954), Ca^{++} ions are required in the first phase, whereas Mg^{++} ions in the second phase of the CF reaction. The Ca^{++} ions activate certain enzymes and stabilize the colloid system, while the Mg^{++} ions activate the phosphatase in the course of haemolysis (MORROS SARDA, 1968).

In the present studies, magnesium sulphate and calcium chloride as well as their mixture were tested at the usual concentrations for effect on the titre of freeze-dried complement.

Materials and methods

Complement. Fresh guinea-pig serum was stored (1) without stabilizer; (2) with 0.5 g $MgSO_4$ and 2.5 g sucrose in 100 ml; (3) with 0.5 g $CaCl_2$ and 2.5 g sucrose in 100 ml; (4) with a stabilizer preparation (SIKOS, 1970) according to

instructions by the producer (HUMAN, Budapest); (5) with 0.25 g $CaCl_2$, 0.25 g $MgSO_4$ and 2.5 g sucrose in 100 ml.

From each test material, 1.1 ml amounts were distributed in ampoules with a Cornwall pipette to form in the ampoules a layer about 1 cm in thickness. After freezing at —30°C for 60 min, lyophilization was conducted for about 24 hr, then the ampoules were filled with sterile filtered air and sealed with an oxygen equipment. All preparations were, of course, Tesla-negative. The freeze-dried preparations containing active complement were pale pink, the inactive ones were white. The residual moisture was 1.9 and 2.3 as determined according to Flosdorf and Fischer, respectively.

The various complement preparations were titrated with our usual method, using the following dilutions: 4, 3.6, 2.8, 2.4, 2, 1.6, 0.8 and 0.4%. The FAO/WHO instructions (Alton and Jones, 1967) for complement titration were observed throughout. The degree of haemolysis was scored by one, two, three and four crosses.

With each titration series, fresh guinea-pig serum was set up as control, using the dilutions specified above.

1 : 20,000-diluted haemolysin and a 2% suspension of three times washed sheep erythrocytes were used in all tests.

Results

The effect of the different stabilizers was of variable degree (Table I). The mixture of $MgSO_4$ and $CaCl_2$ proved to be the most efficient and the stabilizer preparation of the HUMAN Institute was practically equivalent.

Table I

Time and temperature of storage		$MgSO_4$ 5 g sucrose	$CaCl_2$ 5 g sucrose	"HUMAN" stabilizer	$MgSO_4$ 2.5 g $CaCl_2$ 2.5 g sucrose 25 g	Without stabilizer
		Highest dilution per cent producing full haemolysis				
1 day	25°C	2.0	2.0	2.0	1.6	2.4
2 weeks	37°C	2.4	2.4	2.4	2.0	2.4
2 weeks	25°C	2.0	2.4	2.0	2.0	2.4
2 weeks	4°C	2.0	2.4	2.0	1.6	2.4
1 month	4°C	2.0	2.4	2.0	1.6	2.4
2 months	4°C	2.4	2.4	2.0	1.6	2.8
6 months	4°C	2.8	2.8	2.4	2.0	4.0
11 months	4°C	2.8	4.0	2.4	2.4	not measurable*
1 year	4°C	3.2	4.0	2.8	2.4	not measurable*

* Explanation: "Not measurable" means >4.0%. In our opinion complement preparations for routine use must not exceed a titre of 3% even after one year.

$MgSO_4$ and $CaCl_2$ were less effective when applied in themselves. The complement preparation freeze-dried without stabilizer lost half of its activity in 6 months.

Ten batches of complement subsequently prepared with the $MgSO_4$ + $CaCl_2$ mixture for routine purposes were still active in 1.6—2.4% dilutions after observation periods from 6 months to one year. The $MgSO_4$ + $CaCl_2$ stabilizer was therefore accepted as a preservative in industrial-scale production of freeze-dried complement.

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SUMMARY

The effect of various stabilizers on the activity of freeze-dried complement was tested by comparison with a lyophilised preparation containing no stabilizer. The $MgSO_4$ + $CaCl_2$ stabilizer had the greatest preserving effect; the complement stored with it was still active in a 2.4% dilution after storage for one year. Ten batches of freeze-dried complement subsequently prepared with $MgSO_4$ + $CaCl_2$ for routine purposes proved to be similarly active in due course.

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MINERAL CONTENTS OF THE HORN OF THE FOOT OF SWINE OF DIFFERENT BREEDS AND AGES

By

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In an earlier study (A. B. Kovács and M. Szilágyi, 1973) several mineral components (Ca, P, Mg, Na, Fe, Zn, Cu) of the horny part of the foot of cattle, sheep and swine were determined quantitatively and compared.

This paper is a report of more detailed studies on swine along the same line. The nails of adult Cornwall and KAHYB swine were analysed for the concentrations of the above elements in the wall, sole and frog regions and similar measurements were carried out on nail specimens from Cornwall, Duroc and Herceghalom-hybrid piglets.

The differentiation of anatomical regions, including younger (wall) and older parts (sole) was important not only for comparison, but also in a clinical respect. It is known that the toe of the wall plays an important role in the development of the entire horny casing and the sole is responsible for the strength and resistance of the whole horny structure, carrying besides in a certain sense also the weight of the body.

Materials and methods

These were the same as described in the previous publication of this series (B. Kovács and Szilágyi, 1973).

Specimens of nail were obtained from 30 Cornwall and 31 KAHYB swine, and from a total of 106 piglets (31 Cornwall, 23 Duroc and 52 Herceghalom-hybrid). The adult animals were given the usual fattener diet, the piglets a common rearing feed formula.

The concentrations of the elements were expressed as $\mu g/g$ dry material (ppm). Mean values (M) and standard error of the mean (S.E.M.) were calculated for each series. The concentration differences between anatomical regions were analysed by Student's t-test.

The results are summarized in five Tables.

Table I

Ca, P, Mg, Na, Fe, Zn and Cu concentrations (ppm) in different anatomical regions of the horny part of the foot of adult Cornwall and KAHYB swine

Elements	Breed	WALL (TOE)		SOLE		FROG	
		n	M ± S.E.M. ppm	*n*	M ± S.E.M. ppm	*n*	M ± S.E.M. ppm
Ca	Cornwall	26	981 ±42	27	1141 ±34	27	668 ±23
	KAHYB	27	775 ±35	31	821 ±26	27	842 ±46
P	Cornwall	24	1196 ±32	27	1002 ±32	26	1335 ±46
	KAHYB	26	1185 ±40	26	1090 ±39	27	1448 ±31
Mg	Cornwall	26	217 ±14	30	234 ±13	23	491 ±29
	KAHYB	27	204 ±15	31	219 ± 9	27	292 ±13
Na	Cornwall	21	381 ±14	30	309 ±10	25	745 ±46
	KAHYB	22	314 ±15	26	361 ±13	19	640 ±39
Fe	Cornwall	22	125 ±12.7	29	61 ± 5.3	24	71 ± 5.6
	KAHYB	26	116 ±11.7	31	123 ±12.6	26	127 ±13.1
Zn	Cornwall	24	162 ± 5.4	26	92 ± 2.2	27	33 ± 1.0
	KAHYB	25	147 ± 4.3	31	116 ± 2.9	24	29 ± 1.2
Cu	Cornwall	26	9.5± 0.4	30	5.1± 0.2	25	4.1± 0.4
	KAHYB	27	12.7± 1.1	29	9.3± 0.6	27	7.5± 0.7

Results and conclusions

As can be seen from Table I, the mineral matter composition of the nail was different in the Cornwall and KAHYB breeds. The former have light-coloured nails, the latter have pigmented, dark ones. In accordance with the observations of Zacherl and Weiser (1963) and Anke (1965) on white and pigmented bovine hair, the pigmented horny matter was found to contain more calcium and magnesium than the colourless one. The copper contents differed to a lesser degree, being only slightly higher in the KAHYB breed. The proportions of zinc and iron changed with the anatomical region in both breeds, the zinc content being the higher in the toe part of the wall.

Table I also shows that the distribution of the examined elements in the different parts of the nail is non-uniform. The frog contains much more phosphorus, sodium and magnesium, but much less copper and zinc than the toe and sole. A further difference was observed between the earlier-formed (older) solar region and the later-formed (younger) toe part, the latter containing considerably more zinc and copper than the former in both breeds.

Table II

Comparison of the concentrations of certain elements in the walln (toe) and sole of the horny part of the foot of adult Cornwall and KAHYB swine

Elements	Breed	WALL (TOE)		SOLE		t	P
		n	M ± S.E.M. ppm	n	M ± S.E.M. ppm		
Ca	Cornwall	26	981 ±42	27	1141 ±34	2.95	<0.01
	KAHYB	27	775 ±35	31	821 ±26	—	n. s.*
P	Cornwall	24	1196 ±32	27	1002 ±32	4.24	<0.001
	KAHYB	26	1185 ±40	26	1090 ±39	2.12	<0.05
Mg	Cornwall	26	217 ±14	30	234 ±13	—	n. s.
	KAHYB	27	204 ±15	31	219 ± 9	—	n. s.
Na	Cornwall	21	381 ±14	30	309 ±10	4.24	<0.001
	KAHYB	22	314 ±15	26	361 ±13	2.41	<0.05
Fe	Cornwall	22	125 ±12.7	29	61 ± 5.3	5.09	<0.001
	KAHYB	26	116 ±11.7	31	123 ±12.6	—	n. s.
Zn	Cornwall	24	162 ± 5.4	26	92 ± 2.2	12.24	<0.001
	KAHYB	25	147 ± 4.3	31	116 ± 2.9	6.24	<0.001
Cu	Cornwall	26	9.5± 0.4	30	5.1± 0.2	8.98	<0.001
	KAHYB	27	12.7± 1.1	29	9.3± 0.6	2.84	<0.01

* Not significant.

The results of a mathematical-statistical analysis of differences between the mineral matter composition of anatomical regions are shown in Tables II, III and IV. Variance was greater in the Cornwall than in the KAHYB breed.

The highest phosphorus concentration was found in the frog, which is the softest part and it was the lowest in the toe, which is the hardest. The significance of this concentration difference was $P < 0.001$, $P < 0.05$.

In both breeds, the frog contained much more magnesium and sodium than the sole and toe ($P < 0.001$); the latter two regions had almost identical sodium contents. Calcium concentration, however, differed greatly between the breeds, being nearly uniformly distributed in the three anatomical regions of the nail of KAHYB, but showing significant concentration differences in Cornwall ($P < 0.01$, $P < 0.001$), in which the sole contained the maximum amount and the softer frog the minimum.

Similar variations of mineral matter composition were reported by Kerk (1970) for cattle nail, by Weiser et al. (1965) for horse hoof and by Anke (1965) for bovine hair.

Table III

Comparison of the concentrations of certain elements in the wall (toe) and frog of the horny part of the foot of adult Cornwall and KAHYB swine

Elements	Breed	WALL (TOE)		FROG		t	P
		n	M ± S.E.M. ppm	n	M ± S.E.M. ppm		
Ca	Cornwall	26	981 ±42	27	668 ±23	6.60	<0.001
	KAHYB	27	775 ±35	27	843 ±46	—	n. s.*
P	Cornwall	24	1196 ±32	26	1335 ±46	2.44	<0.05
	KAHYB	26	1185 ±40	27	1448 ±31	5.19	<0.001
Mg	Cornwall	26	217 ±14	23	491 ±29	8.73	<0.001
	KAHYB	27	204 ±15	27	292 ±13	4.38	<0.001
Na	Cornwall	21	381 ±14	25	745 ±46	6.94	<0.001
	KAHYB	22	314 ±15	19	640 ±39	8.60	<0.001
Fe	Cornwall	22	125 ±12.7	24	71 ± 5.6	4.00	<0.001
	KAHYB	26	116 ±11.7	26	127 ±13.1	—	n. s.
Zn	Cornwall	24	162 ± 5.4	27	33 ± 1.0	24,60	<0.001
	KAHYB	25	147 ± 4.3	24	29 ± 1.2	26.07	<0.001
Cu	Cornwall	26	9.5 ± 0.4	25	4.1 ± 0.4	8.97	<0.001
	KAHYB	27	12.7 ± 1.1	27	7.5 ± 0.7	4.13	<0.001

* Not significant.

Zinc and copper contents were much higher in the toe (younger part) than in the solar region. KERK (1970) observed the same in cattle. ZACHERL and WEISER (1963) and ANKE (1965) reported a similar distribution of zinc in bovine hair, but ANKE determined nearly identical copper contents in its proximal and distal parts. According to WEISER et al. (1965), zinc and copper do not notably vary with the anatomical regions of the horse's hoof.

As to calcium and phosphorus, it is known that the latter predominates in the soft tissues of the body, whereas the former in the bones (POWELLEIT, 1966). The horny wall contains nearly identical amounts (1 : 1) of these elements, whereas the softer frog contains almost twice as much phosphorus as calcium.

According to practical observations, the nail of KAHYB swine is less resistant than that of Cornwall swine. The experimental observation that in the former breed, the calcium/phosphorus quotient is lower than one even for the toe region, weighs in favour of this opinion. The Ca/P ratio may, among other factors, influence the strength of the nail. Further experiments are in progress to substantiate this assumption.

Table IV

Comparison of the concentrations of certain elements in the sole and frog of the horny part of the foot of adult Cornwall and KAHYB swine

Elements	Breed	SOLE		FROG		t	P
		n	M ± S.E.M. ppm	n	M ± S.E.M. ppm		
Ca	Cornwall	27	1141 ±34	27	668 ±23	11.42	<0.001
	KAHYB	31	821 ±26	27	843 ±46	—	n. s.*
P	Cornwall	27	1002 ±32	26	1335 ±46	5.98	<0.001
	KAHYB	26	1090 ±39	27	1448 ±31	9.46	<0.001
Mg	Cornwall	30	234 ±13	23	491 ±29	7.60	<0.001
	KAHYB	31	219 ± 9	27	292 ±13	4.60	<0.001
Na	Cornwall	30	309 ±10	25	745 ±46	9.91	<0.001
	KAHYB	26	361 ±13	19	640 ±39	7.55	<0.001
Fe	Cornwall	29	61 ± 5.3	24	71 ± 5.6	—	n. s.
	KAHYB	31	123 ±12.6	26	127 ±13.1	—	n. s.
Zn	Cornwall	26	92 ± 2.2	27	33 ± 1.0	23.92	<0.001
	KAHYB	31	116 ± 2.9	24	29 ± 1.2	24.64	<0.001
Cu	Cornwall	30	5.1± 0.2	25	4.1± 0.4	2.36	<0.05
	KAHYB	29	9.3± 0.6	27	7.5± 0.7	1.86	<0.1

* Not significant.

Data of examinations of the horny part of the foot of piglets (Cornwall, Duroc, Herceghalom-hybrid) are summarized in Table V. The mineral con-

Table V

Ca, P, Mg, Na, Fe, Zn and Cu concentrations (ppm) in the horn of the foot of Cornwall, Duroc and Herceghalom-hybrid piglets

Elements	CORNWALL		DUROC		HERCEGHALOM-HYBRID	
	n	M ± S.E.M. ppm	n	M ± S.E.M. ppm	n	M ± S.E.M. ppm
Ca	22	1276 ±87	21	1494 ±80	39	1425 ±105
P	24	1153 ±54	23	2097 ±91	41	1674 ±102
Mg	21	219 ±16	21	308 ±13	48	385 ± 19
Na	28	896 ±40	23	951 ±39	47	630 ± 27
Fe	19	163 ±17.8	20	189 ±13.2	50	170 ± 12.7
Zn	31	123 ± 4.5	23	94 ± 2.7	52	112 ± 2.5
Cu	20	6.9± 0.4	23	14.8± 0.8	50	15.7 ± 0.9

tents, especially the concentrations of P, Na, Mg and Cu, differed between the examined three breeds.

The highest concentrations of the elements determined, especially of phosphorus, were found in the nail of Duroc piglets. Cornwall piglets had very low copper levels. As we were not able to determine the mineral constituents of the piglet diet, the probable relationship between the dietary mineral supply and the mineral components of the horny part of the foot remains to be clarified.

The nails of piglets are richer in mineral matter compared to adult swine. Especially the sodium concentration was high in all three breeds, but zinc levels were slightly lower than in adults. The zinc content of cattle hair changes in a similar manner with age (VAN KOETSVELD, 1966; MARTIN et al., 1969), whereas lambs have much less zinc in their hair than adult sheep (GROPPEL and HENNIG, 1971).

It should be noted that the mineral composition of the nails of Cornwall piglets bore a closer resemblance to the toe (younger part) of adult Cornwall swine than to the nails of any young or adult animals from the other breeds.

SUMMARY

Examinations of the horny part of the foot of adult Cornwall and KAHYB swine as well as of Cornwall, Duroc and Herceghalom-hybrid piglets, on a total 167 animals, for Ca, P, Mg, Na, Fe, Zn and Cu contents, have shown that the concentrations of these elements differ between breeds.

The distribution of the elements was dissimilar in the anatomically and functionally different parts (wall, sole, frog) of the nail. Concentration differences between the anatomical regions were found to be greater than the inter-breed differences between identical regions.

The nails of piglets contained a much greater amount of mineral matter than those of adult swine.

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THE PHYSIOLOGICAL ROLE OF COLOSTRAL TRYPSIN INHIBITOR: EXPERIMENTS WITH PIGLETS AND KITTENS

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The colostrum of certain mammalian species contains a trypsin inhibitor (CTI) of polypeptide nature. The relative concentration of this inhibitor in the colostrum of sows (LASKOWSKI et al., 1957), cows (LASKOWSKI and LASKOWSKI, 1951), and women (LASKOWSKI and LASKOWSKI, 1951; HYÁNEK et al., 1965) was found to be 67 : 10 : 1 (LASKOWSKI et al., 1957). Except in the case of human colostrum, in which the amount of inhibitor is very small, it is suggested that CTI has the function of protecting the colostral antibodies from digestion and thus ensuring that they are absorbed in a biologically active form (LASKOWSKI et al., 1958). In a number of experiments (LASKOWSKI et al., 1958; NORDBRING and OLSSON, 1958a, b; D'ADDABBO et al., 1967) it has been shown that the absorption of proteins can be increased by simultaneous peroral administration of trypsin inhibitor, although CHAMBERLAIN et al. (1965) were unable to prolong the period of protein absorption by this means.

The stomach of new-born animals contains little or no pepsin, and no free HCl at all (HILL, 1956; WALKER, 1959; LEWIS et al., 1957; and others). At the same time the trypsin inhibitor of the sow's colostrum is completely, that of bovine colostrum partially, resistant to pepsin digestion (KASSELL and LASKOWSKI, 1956).

HARDY (1969a) demonstrated that the greater part of a saline solution of labelled IgG administered to new-born piglets was split in the intestines and that after absorption the fragments were excreted by the kidneys. Colostrum, the trypsin inhibitor preparation Trasylol, and carrier proteins given simultaneously exerted a protective effect on the IgG. Similar results were obtained by HARDY (1969b) in experiments with calves. However, the mechanism by which colostral antibodies are protected have remained obscure.

The present work deals with the interrelationships of CTI, protein digestion and protein absorption in piglets and kittens reared under conventional conditions. A report on digestion in suckling rats has already been published (BAINTNER and JUHÁSZ, 1971).

One of the things that had to be kept in mind during these investigations is the change in the ability of enterocytes to absorb proteins. In earlier studies it was shown that both non-selective (BAINTNER and VERESS, 1967) and selective protein absorption (BAINTNER and VERESS, 1970) can be related to definite cell types covering the intestinal villi. Under some unknown effect cells unable to absorb proteins replace the earlier ones (BAINTNER and VERESS, 1967, 1970). The experiments of CLARKE and HARDY (1969, 1970, 1971a, b) have demonstrated that this epithelial change does not occur along the whole length of the intestine at the same time, but the ability to take up macromolecules becomes gradually limited to the more distal parts of the small intestine, before disappearing completely.

Methods

The investigations were carried out with piglets, 2 hours to 9 days of age, and with kittens, 2 hours to 21 days of age. In all cases the animals were allowed to suckle up to the time of slaughter. The animals were stunned and then bled by cutting the throat. After the abdomen had been opened the small intestine

was taken out and the mesentery removed. Any blood leaking from the intestinal vessels was carefully mopped up to prevent contamination of the intestinal contents with trypsin inhibitors of blood origin. The intestinal contents of the piglets were squeezed out into a test tube and diluted 1 : 10 before tested. In the kittens, because of the smaller volumes of intestinal contents, the small intestine was flushed with physiological saline and the washings collected in a test tube. The volume of saline used for flushing was 5 ml, irrespective of the age or weight of the animal.

The diluted intestinal contents and washings were centrifuged and filtered until optically clear. The samples were stored at —20°C until tested. Some precipitation usually reappeared after freezing and was removed by centrifugation. No attempt was made to assess the quantity of enzymes lost during purification.

The intestine was imagined as being divided into 100 parts, so that the site of each histological specimen could be expressed in terms of a percentage, beginning from the pylorus. In this way specimens were taken from the jejunum (45—50%) and from the ileum (75—80%).

Activity assays. The activities given for piglets were calculated on the basis of ml undiluted intestinal contents; those for kittens in terms of ml washings.

1. N-α-benzoyl-L-arginine ethyl ester (BAEE) and acetyl-L-tyrosine ethyl ester (ATEE) splitting activities were determined by the method of SCHWERT and TAKENAKA (1955) making direct spectrophotometric readings at room temperature on a Spektromom 201 apparatus. BAEE-splitting was measured at 253 nm in 0.1 *M* pH 8 Tris-HCl buffer, ATEE-splitting at 237 nm in 0.1 *M* pH 7 Tris-HCl buffer. The background UV absorption was compensated by widening of the slit. Any changes in non-specific absorption were checked by setting up controls without substrate.

Activities determined in the above manner were resolved into sensitive and resistant activities by the addition of excess soybean trypsin inhibitor (STI, 0.2 mg/3 ml final concentration):

Substrate	Soybean trypsin inhibitor	
	sensitive	resistant
BAEE	trypsin	"kallikrein"
ATEE	"chymotrypsin B" (chymotrypsin A)	chymotrypsin-like activity

The fraction denoted as "kallikrein" was shown to be composed partly or completely of pancreatic kallikrein on the basis of the following properties: It was produced by the pancreas as a zymogen; it split BAEE, but not N-α-benzoyl-L-arginine-p-nitroanilide (BAPA); it was resistant to STI, but in-

hibited by Trasylol; it was irreversibly inactivated by N/4 sulphuric acid, as used in the trypsin preparation method of KUNITZ (1939). The STI-sensitive ATEE-splitting activity had the characteristics of chymotrypsin B, being CTI-resistant (WU and LASKOWSKI, 1960).

The choice of expressing the trypsin and chymotrypsin activities in terms of μg enzyme was somewhat arbitrary. The calculations were based on the highest specific activities, measured by similar methods, that could be found in biochemicals catalogues:

12,000 BAEE units/min = 1 mg trypsin

14,000 ATEE units/min = 1 mg chymotrypsin

The "kallikrein" and chymotrypsin-like activities were calculated in the same way, assuming similar molecular weights and specific activities.

2. Determination of colostral trypsin inhibitor (CTI) in intestinal contents and colostrum. It was determined how much of the solution under study had to be added to reduce the activity of a known trypsin solution to 40—60%. The measurement is inaccurate at lower inhibition, while for inhibition approaching 100%, due to dissociation of the trypsin-trypsin inhibitor complex, the inhibition does not change parallel with inhibitor concentration. The uninhibited (sample volume zero) and inhibited activity values were plotted as a function of the volumes of sample added and the points joined with a straight line. Elongation of this line to the abscissa gave an intercept which represented the theoretical amount of solution required to produce 100% inhibition. From this it was possible to calculate the amount of trypsin inhibited by 1 ml solution. The concentration of trypsin inhibitor was expressed as μg trypsin inhibited by 1 ml of intestinal contents or washings. If the solution under study contained "kallikrein" activity the trypsin inhibition values were corrected accordingly.

The colostrum used in these measurements was prepared in the following way: ½ ml colostrum was diluted with ½ ml distilled water and, after shaking, 4 ml 5% trichloroacetic acid was added. Fat and precipitated proteins were removed by centrifugation and filtration. The CTI of the clear filtrate was determined in the above manner. It was usually much more difficult to achieve optical clarity with colostrum than with intestinal contents.

3. Esteroproteolytic enzyme was determined at room temperature with caproyl salicylate as substrate, in 0.1 *M* pH 8 Tris-HCl buffer at 300 nm (GJESSING et al., 1959; MCCONNELL and GJESSING, 1966).

4. Determination of chymotrypsinogen. Intestinal samples with high CTI content were activated by excess chymotrypsin-free trypsin (CALBIOCHEM) and the ATEE-splitting activity was measured by the method described above. The procedure was suitable for detection of chymotrypsin-like proenzyme and of chymotrypsinogen B, but not of the zymogen of α-chymotrypsin. The reason for this is that CTI and STI have separate binding sites for trypsin and

chymotrypsin (Čechová et al., 1969), and thus the trypsin-CTI complex formed after the addition of trypsin is still able to bind α-chymotrypsin at the ratio 1 : 1 : 1 (Krahn and Stevens, 1971).

5. Lipase activity was determined at 37°C with tributyrin substrate by continuous titration with 0.01 N NaOH of the butyric acid released. One unit of activity was equal to 1 $\mu mol \times min^{-1}$ butyric acid released by 1 ml of sample.

6. Amylase activity was determined by measuring the rate of disappearance of the iodine-starch colour, following the method of Somogyi (1955).

Histological examinations were carried out on formalin-fixed specimens embedded in paraffin and stained with haematoxylin and eosin.

Milk was obtained from the mother cats by collecting milk droplets in a micropipette by capillary effect.

Results

1. Experiments with piglets. Eight of the 14 piglets farrowed by one sow referred to as the "large litter" and seven of nine piglets of a second sow ("small litter") were sacrificed. The activity of caproyl salicylate-splitting enzyme was determined from another 15 piglets.

The intestinal contents taken from the piglets were yellow. On standing, the samples from 1- to 2-day-old piglets turned green on the surface in contact with the air. Samples from older animals remained yellow.

The CTI content of colostrum from the sow with the large litter declined rapidly, inhibiting 2130 μg trypsin per ml at parturition, 22 μg 2 days later, and only trace amounts on the third day after parturition. No colostrum samples could be taken from the sow with the small litter.

The declining CTI concentrations of intestinal contents from piglets of the two litters (Fig. 1) formed a roughly exponential curve. In both litters the concentrations fell rapidly as a function of age, although the decrease was significantly more protracted for the small litter.

In no sample was it possible to detect the simultaneous occurrence of CTI and trypsin, because of formation of inactive complex between the two. However, it was possible to detect "kallikrein" and "chymotrypsin B" activities in the presence of CTI (Figs 2, 3). In the majority of samples from the youngest piglets no proteolytic activity was demonstrable, except for the caproyl salicylate-splitting enzyme. Attempts made to detect chymotrypsinogen in samples taken from three piglets a few hours after birth failed in all cases. The ATEE-splitting activities obtained after activation corresponded to the very low ATEE-splitting activity of the added trypsin itself.

"Chymotrypsin B" activity generally appeared somewhat earlier than "kallikrein", while trypsin and chymotrypsin-like activities appeared when

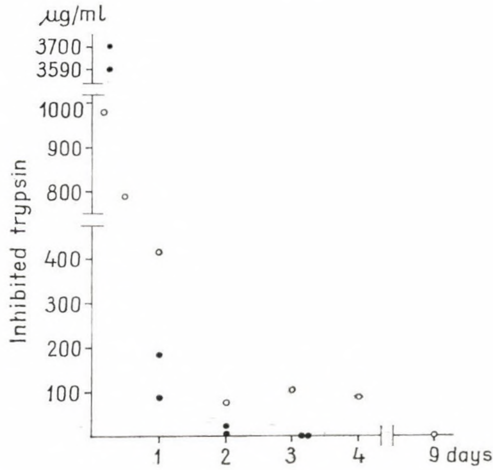

Fig 1. Trypsin inhibitor concentrations in the intestinal contents of baby pigs. ○ Small litter; ● large litter

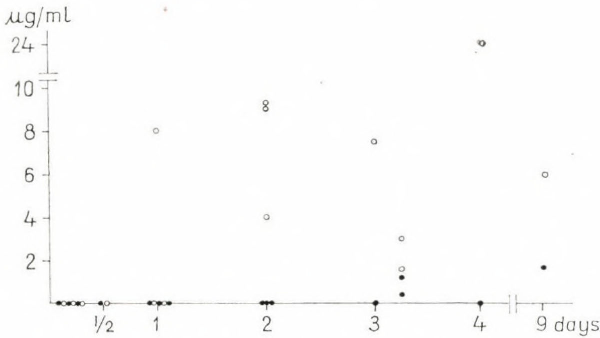

Fig. 2. Trypsin (●) and "kallikrein" (○) activities in the intestinal contents of piglets

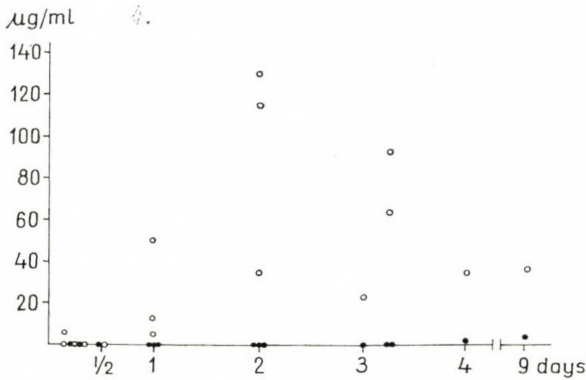

Fig. 3. "Chymotrypsin B" (○) and chymotrypsin-like (●)activities in the intestinal contents of piglets

piglets were several days old. Proteolytic activities tended to increase with advancing age.

Intestinal contents contained low, but distinct amylase and lipase activities in the first hours of life, and both activities tended to increase with age. Amylase values ranged between 10 and 1000 Smogyi units/ml, those of lipase between 0.7 and 42 units/ml.

Trypsin inhibitor concentrations of urine samples corresponded to values published before (BAINTNER, 1970b).

Histological examinations revealed the presence of relatively small eosinophilic droplets soon after first sucking (Fig. 8), and later these droplets filled the greater part of enterocytes (Fig. 9). No eosinophilic droplets were visible in the jejunum after two days of age. In the piglets of the small litter the ileum was also examined histologically and eosinophilic droplets were observed in the ileal enterocytes until the fourth postnatal day. After the disappearance of the eosinophilic droplets unvacuolized cells were found in the jejunum and mostly "empty" vacuoles in the ileum. The small, sporadically occurring, eosinophilic inclusion bodies never filled the vacuoles completely. The brownish colour of the ileum with predominantly vacuolized enterocytes contrasted with the greyish colour of the jejunum. The histological findings were largely uniform among litter-mates of the same age.

2. Experiments with kittens. Colostrum or milk was taken from eight dams between ½—6 days after parturition. CTI was detected in every sample.

Nineteen kittens were used for measurements of enzyme activities and 37 for histological studies; of these 14 were used in both studies. The results varied substantially between litters, but values for litter-mates of the same

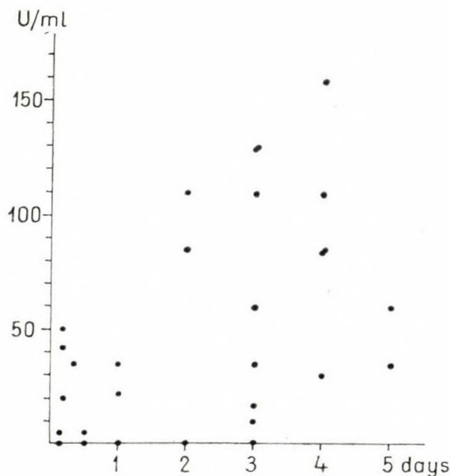

Fig. 4. Caproyl salicylate-splitting activity in the intestinal contents of piglets

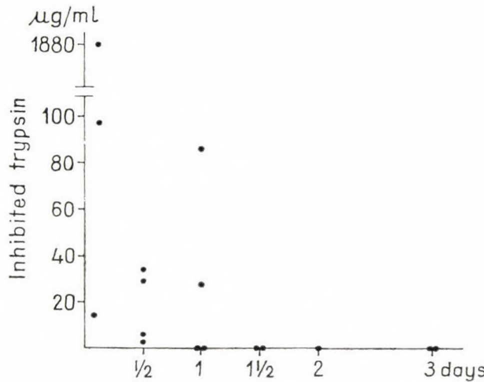

Fig. 5. Trypsin inhibitor concentrations in the intestinal washings of kittens

age were similar. Variations due to litter size were not investigated.

The CTI concentration of intestinal contents decreased rapidly with advancing age (Fig. 5). In some animals "kallikrein" (Fig. 6) and "chymotrypsin B" (Fig. 7) activities were present a few hours after first sucking. Trypsin activity appeared at 1—1½ days of age, simultaneously with the disappearance of CTI. Chymotrypsin-like activity was not detectable even at three days. Amylase values ranged between 0 and 13 Somogyi units/ml, those of lipase between 0 and 4.2 units/ml.

Urine samples of kittens inhibited trypsin between 160 and 840 μg/ml in the first one and a half days of life (7 animals). In the same samples protein was detected by precipitation with trichloroacetic acid. In contrast, only low trypsin inhibitor values, between 2.3 and 23 μg/ml, occurred from 2½ days to

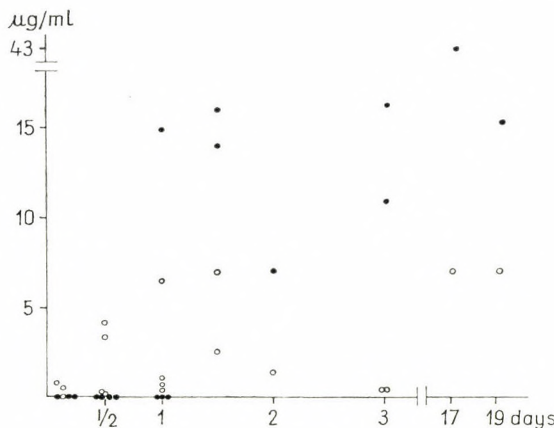

Fig. 6. Trypsin (●) and "kallikrein" (○) activities in the intestinal washings of kittens

1 week (10 animals).

The histological finding varied considerably between the different litters. Eosinophilic droplets predominated in the enterocytes on the first day of life. After one and a half days of age no eosinophilic droplets were found, though "empty" vacuoles were visible in the ileum.

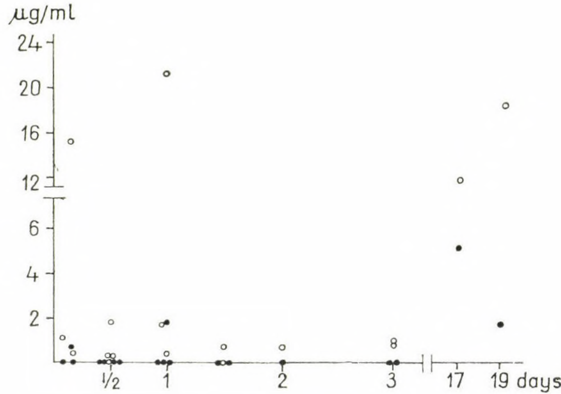

Fig 7 "Chymotrypsin B" (○) and chymotrypsin-like (●) activities in the intestinal washings of kittens

Discussion

The small intestinal contents held extraordinarily large amounts of CTI for a number of hours after the first sucking, especially in piglets. The CTI concentration in the intestinal contents (Figs 1 and 5) diminished rapidly with age, followed, with some delay, by the level in the urine (BAINTNER, 1970b). Literary data (LASKOWSKI et al., 1957) and determinations of samples from the sow with the large litter demonstrate a similar decrease of CTI in colostrum, parallel to the change of colostrum to normal milk. The CTI concentrations of colostrum, intestinal contents and urine in analogous phases of the changes were of the same order of magnitude. However, trypsin inhibitor does not completely disappear from the milk and the urine, but it falls to a negligible level.

The main sources of error in comparing the CTI concentrations of colostrum and intestinal contents were that generally the less suckled teats were succesfully sampled and that some of the CTI was lost during precipitation of the colostral proteins (PEDERSEN et al., 1971). A further source of error with colostrum from the cats was the small volume of the samples. Therefore, numerical evaluation of these latter determinations were considered questionable. Nevertheless, determinations clearly showed that cat colostrum con-

tained significant quantities of CTI.

The CTI level declined more slowly in the small intestine of piglets from the small litter than of those from the large litter (Fig. 1). The sow with the large litter was sucked most of the time by twice as many piglets as that with the small litter, so clearly more rapid sucking is attended by faster depletion of colostrum. Consequently, in investigating the fall in colostral CTI content it is not sufficient just to take account of the time elapsed after parturition, as did LASKOWSKI et al. (1957), but the rate of sucking, which is most conveniently expressed by litter size, must also be considered.

Based on the present experiment, the development of protein digestion can be divided into 3 successive periods. In the first period, which lasts about half a day after birth in piglets, proteolytic enzymes are as a rule not detectable (Figs 2, 3). An exception is the esteroproteolytic enzyme (Fig. 4), which in general appears already after the first sucking. In the same period the highest CTI values are found. Thus, in piglets there is a period when intestinal proteolysis is insignificant or lacking. This period did not occur consistently in kittens (Figs 6, 7). The present experiments give no answer as to whether the pancreas is delayed in secreting proteases in this period, or the secreted proteases cannot be activated. Whatever is the case, chymotrypsinogen could not be detected in the samples examined.

The second period lasts roughly from half a day to 2 days of age in piglets and from birth to one or one and a half days in kittens. In this period the intestine still contains CTI, but the CTI-resistant pancreatic proteases already make their appearance (Figs 2, 3, 6, 7). The apparent lack of trypsin is very likely due to the presence of CTI.

The third period begins with the disappearance of CTI from the intestinal contents. At the same time trypsin appears in the small intestine and later a chymotrypsin-like activity as well. In this way the complement of proteolytic enzymes is filled out to the pattern characteristic of the suckling animal. Further changes are likely later on, during the physiological preparation for weaning (GORRILL and FRIEND, 1970; CORRING and AUMAITRE, 1971). Thus, only a part of the variations in proteolytic activities can be explained by the effect of CTI.

The α-chymotrypsin was not determined separately in these experiments; while the intestine still contains CTI, it cannot occur in a free state. If it appeared later, it was measured together with the "chymotrypsin B" activity.

The absolute values of the results for piglets and kittens cannot be easily compared, due to the differences in sample taking (intestinal content and washing fluid) and in body size. However, it seems that less trypsin is produced by piglet (omnivorous species) than by kittens (carnivorous species), in contrast, more CTI and amylase are present in piglets.

Simultaneously with the appearance of a marked trypsin activity,

amylase, lipase and "chymotrypsin B" activities showed a transient decrease in the kittens, probably as a result of their inactivation by trypsin. In the older animals these activities were higher again.

In the histological examinations attention was primarily focused on the eosinophilic droplets in enterocytes on the villi. These were first described by

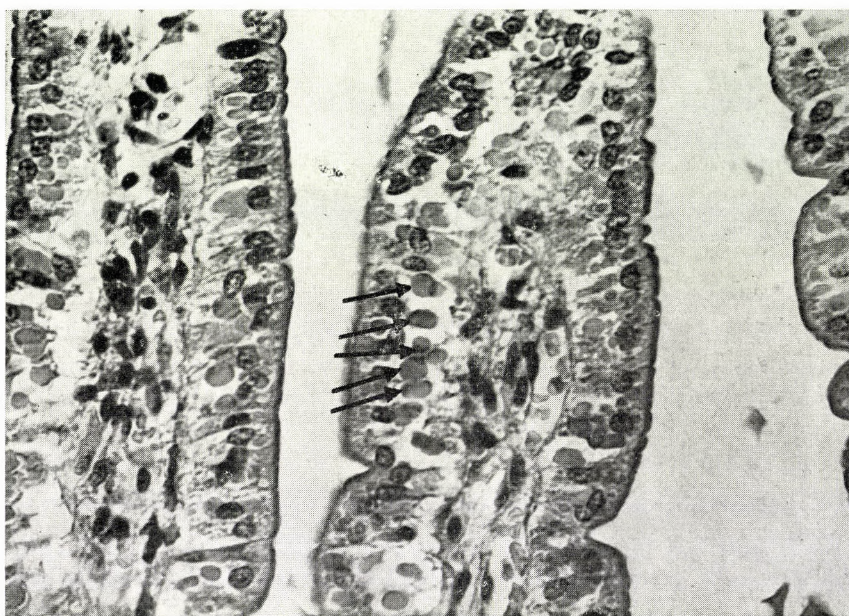

Fig.8 . Jejunum from a new-born piglet 3 hours after the first sucking. Eosinophilic droplets of medium size can be seen at the base of the enterocytes (arrows), small droplets in the apical region. At this time there was a large excess of trypsin inhibitor in the intestinal content and no demonstrable proteolytic activity. Haematoxylin-eosin (H—E) staining

Parat (1924) and were later related to protein absorption by Comeline et al. (1951). Baintner and Veress (1967) distinguished them from the morphological signs of the ability to absorb proteins. Nevertheless, it was presumed that they represent signs of actual protein absorption in animals of the non-selective protein absorption type, to which piglets and kittens belong.

Eosinophilic droplets were indeed observed in the enterocytes of the jejunum and ileum in piglets and kittens after first sucking (Figs 8, 9). Their formation and transcellular passage corresponded to earlier descriptions (Baintner and Veress, 1967; Veress and Baintner, 1970). This is the period when piglets display the most pronounced protein absorption and when the proteolytic enzymes are generally lacking. In the second phase of the development of proteolysis no change could be detected in either the form or the staining of the eosinophilic droplets. In this period the full complement of

proteases was still not present. It seems that the function of active proteases was restricted to splitting of definite peptide-bonds and proteolysis stopped after rough fragmentation of the proteins, these fragments being absorbed in the same way as intact proteins. The appearance of such protein fragments has in fact been demonstrated by HARDY (1969a, b).

In two piglets of the large litter sacrificed at 2 days of age the droplets

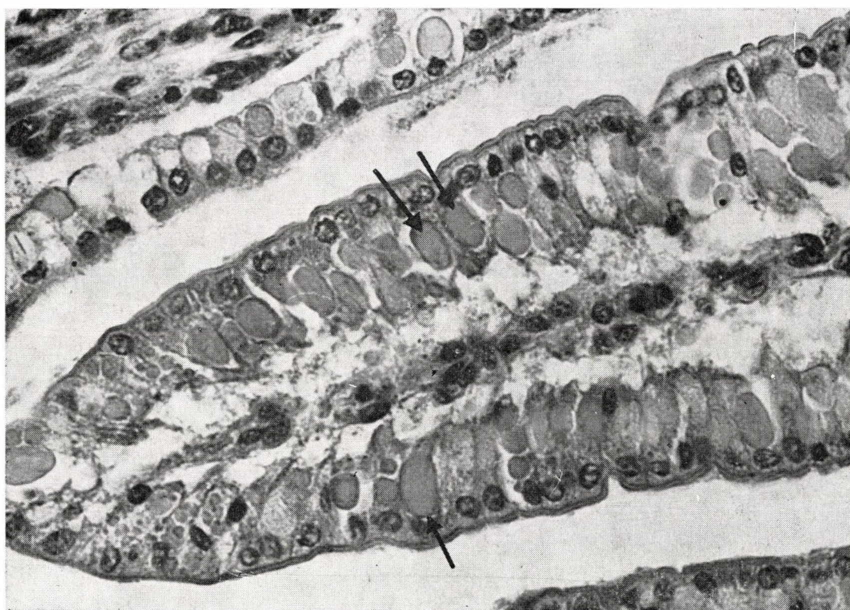

Fig. 9. Jejunum of a 1-day-old piglet. Large eosinophilic droplets are visible in the entero cytes (arrows). Trypsin inhibitor and proteases insenstive to colostral trypsin inhibitor were present in the intestinal content. H—E staining

occurred only scattered in a few cells (Fig. 10), probably in those ones which lagged behind with the secretion of colostral proteins into the intercellular space. New, developing eosinophilic droplets were not observed. This is regarded as the state just before the disappearance of the eosinophilic droplets. In the corresponding intestinal contents there was scarcely any CTI, and if a non-uniform distribution of the inhibitor is assumed, it is possible that there was no CTI at all at the jejunal site from where the specimen was taken.

After CTI had vanished and trypsin had made its appearance, eosinophilic droplets could not be demonstrated in any case. Accordingly, these droplets could be found later in piglets from the small litter than in those from the large one. In the third period of the development of proteolysis, when the proteolytic pattern has been supplemented with trypsin, it appears that proteolysis becomes practically complete, and morphological signs of protein absorption cannot be demonstrated.

In kittens, the disappearance of CTI and the appearance of trypsin took place earlier than in piglets (Figs 5, 6) and accordingly the eosinophilic droplets vanished sooner. The morphological and biochemical findings were in complete agreement.

The present results are in accordance with the assumption that CTI exerts a protective effect on colostral proteins during their absorption. Besides CTI, probably also other factors have a role in this protective effect. In kittens,

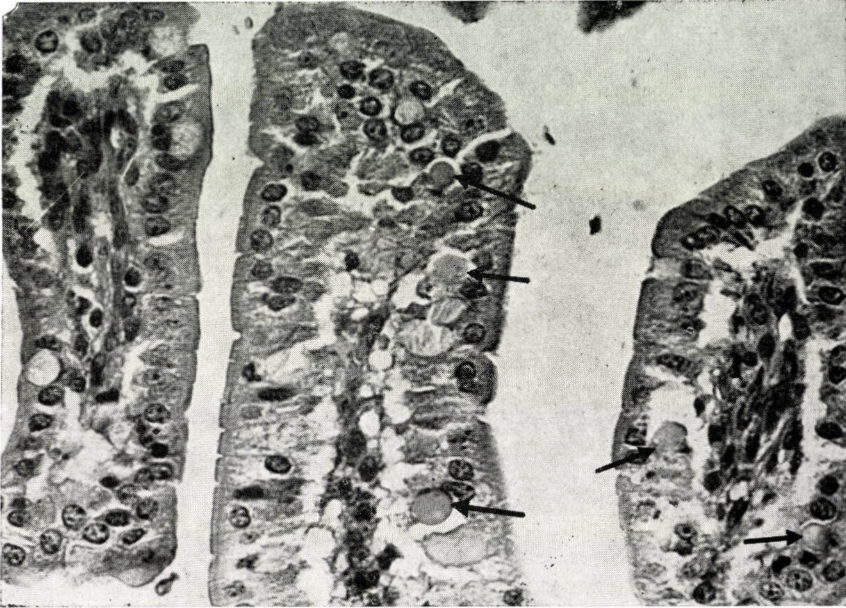

Fig. 10. Jejunum of a 2-day-old piglet at the time when protein absorption ceases. The intestinal villi on the right and in the centre contain a few large eosinophilic droplets (arrows), the villus on the left has none. No small eosinophilic droplets were observed. By this time there was scarcely any trypsin inhibitor in the intestinal content, but trypsin was still not detectable. H—E staining

which obtain maternal antibodies both prenatally and postnatally, the protection of colostral proteins was not so full as in piglets, which display only postnatal immune transfer (BRAMBELL, 1958). The protection of antibodies during absorption in suckling rats cannot be explained in the same way as that in piglets and kittens (BAINTNER and JUHÁSZ, 1971).

The present results also indicate that in piglets and kittens the actual cessation of protein absorption is due not so much to the loss of ability to absorb proteins, or to the regression of this ability to more distal parts of the intestine, but to the start of proteolysis. That is to say that two processes of a

more or less switch-over nature take place in the intestine of the suckling animals:

1. Switch-over from inhibition of protein digestion to full protein digestion, and in consequence, from absorption of proteins to absorption of amino acids. The timing of this switch-over is determined, in addition to the pancreatic function, by factors independent of the suckling animal, such as the amount of maternal colostrum and its CTI content, or the litter size.

2. Switch-over from the protein absorptive enterocytes to the cells of the adult type, due to a change in the 'direction' of differentiation of the growing enterocytes. With advancing age this process takes place more and more in the distal portions of the intestine; its timing is probably determined genetically.

In the selectively absorbing rat the cessation of protein absorption is concurrent with the change in the intestinal epithelium (Clarke and Hardy, 1969; Baintner and Veress, 1970). However, in animals of the non-selective type the term 'closure' must be recognized as covering two distinct processes. It is proposed that the designation true closure be applied to the cessation of the ability to absorb proteins, and the designation apparent closure to denote the cessation of protein absorption that is related to the start of digestion. I shall publish the relevant morphological evidence in the accompanying paper.

Between the two events mentioned there is a period when proteins are already being absorbed in the form of amino acids, although at least a portion of the intestinal epithelium is still capable of absorbing proteins. As regards the physiological significance of this period, it is supposed that the animal uses this 'reserve' epithelium to buffer the temporal fluctuations of the colostrum-feeding period and to protect itself against the harmful effects of the colostrum. For the colostrum fed after true closure exerts a changed effect: in the absence of capacity to absorb protein, bacteria grow on the undigested proteins, and the animal reacts with diarrhoea (Baintner, 1970a).

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I am grateful to Dr. Franz Fiedler for his help in the identification of enzymes; to Gábor Doleschall for the synthesis of caproyl salicylate; to Dr. András Bálint for the use of the spectrophotometer; and to Mihály Sajgó for a sample of chymotrypsin-free trypsin.

SUMMARY

Digestion in the intestinal lumen and the histology of the intestinal epithelium were studied in two non-selective protein-absorbing species; new-born piglets and kittens.

It was found that in the intestines of piglets in the period directly after first sucking there was a large excess of trypsin inhibitor and it was generally impossible to demonstrate the presence of proteolytic enzymes of pancreatic origin. The trypsin inhibitor concentration in the colostrum and intestinal contents decreased at a rate depending on litter size. In the intestines of kittens there was less trypsin inhibitor and digestion started earlier than in piglets.

As the trypsin inhibitor concentration declined the pattern of proteases characteristic of suckling animals gradually developed. The first proteases to appear were those which are unaffected by colostral trypsin inhibitor. Trypsin appeared simultaneously with the disappearance of trypsin inhibitor from the intestine. At the same time proteinuria ceased and eosinophilic droplets vanished from the enterocytes.

Some age-dependent quantitative and qualitative changes also occurred in the proteolytic activity independently of the effect of colostral trypsin inhibitor.

The protection of maternal antibodies during non-selective protein absorption can be satisfactorily explained by the presence of trypsin inhibitor. In piglets and kittens the cessation of protein absorption is due not so much to the loss of ability to absorb proteins (true closure) as to the start of protein digestion (apparent closure).

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ТОМ 23 — ВЫП. 3

РЕЗЮМЕ

ДАННЫЕ К МИНЕРАЛЬНОМУ СОСТАВУ КОПЫТНОГО РОГА КРУПНОГО РОГАТОГО СКОТА, ОВЦЫ И СВИНЬИ

А. Б. КОВАЧ и М. СИЛАДИ

Определялись содержание **Ca**, **P**, **Mg**, **Na**, **Fe**, **Zn** и **Cu** в здоровом копытном рогу крупного рогатого скота, овцы и свиньи. Данные собраны в таблицах и сравнены с данными других авторов. Полученные значения считаются характерными для вида и породы и будут использованы в последующих исследованиях, как исходные таковые, когда авторы будут изучать минеральный состав копытных рогов животных разных пород в разных условиях кормления и содержания и рогов, показывающих патологические изменения.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ПОВЕДЕНИЯ БИОПЛАСТИЧЕСКИХ И МЕТАЛЛИЧЕСКИХ ИМПЛАНТАТОВ КОСТНОМОЗГОВОЙ ПОЛОСТИ

К. ШОМОДЬВАРИ и Н. ХОРВАТ

При экспериментальных переломах бедренной кости морской свинки и собаки для фиксирования концов перелома авторами применялся биопласт, способный к рассасыванию. Обнаружено, что химически соответственно обработанные биопластовые винты в костномозговой полости ведут себя тканедружески и в зависимости от предварительной обработки после данного времени рассасываются. Описываются сопровождающие рассасывание тканевые явления и констатируется, что последние более благоприятны, чем явления при употреблении металлических винтов. Согласно наблюдениям биопласт благоприятно действует на регенерацию кости и костномозговой полости и образование костной мозоли. Ведется работа на получение биопласта нужной прочности и замену металлических винтов биопластом.

ИЗБИРАТЕЛЬНАЯ ПИТАТЕЛЬНАЯ СРЕДА ДЛЯ ИЗОЛЯЦИИ CORYNEBACTERIUM PYOGENES

ДЬ. СЕМЕРЕДИ

Докладывается об избирательной питательной среде для изоляции **Corinebacterium pyogenes**, содержащей 1230 МЕ/мл Полимиксина Б и 0,01% теллурита калия. Теллурит калия тормозит размножение полимиксинрезистентных штаммов протея.

Среди изученных микроорганизмов размножение рожи свиней и энтерококков не удается полностью задержать, но поскольку последние при температуре 39 °С образуют видимые черноватые колонии уже после одних суток, их можно легко отдифференцировать от колоний **C. pyogenes**, которые становятся видимыми после 2—3 суток и обладают коричневатым цветом.

Описанная избирательная среда удобна для диагностической изоляции **C. pyogenes** из загрязненного микробами экссудата и гнойного материала.

БОЛЕЗНЬ МЭДИ (ХРОНИЧЕСКАЯ ПРОГРЕССИВНАЯ ИНТЕРСТИЦИАЛЬНАЯ ПНЕВМОНИЯ) СРЕДИ ОВЕЦ ВЕНГРИИ

Т. ШЮВЕГЕШ и А. СЕКИ

Вирусное заболевание «мэди» (хроническая прогрессивная интерстициальная пневмония) обнаружена в трех поголовьях мериносных овец весной 1972 г. Поскольку заболевание в стране до сих пор не регистрировали, кажется вероятным, что оно сюда

попало с импортными животными. Наиболее заметными клиническими симптомами явились тяжелая прогредирующая одышка, учащение дыхания и сильное исхудание. Болезнь не поддается лечению и всегда заканчивается летальным исходом после 2—6 месяцев. При вскрытии бросается в глаза увеличение легких при весе 1600—2000 г, вместо 600—800 г. В них видны обширные ателектиатические территории с милиарными серовато-розовыми очагами и увеличение перибронхиальных лимфоузлов с гиперплазией. Микроскопическая картина изменений легких совпадала с таковой хронической прогрессивной интерстициальной пневмонии, сопровождающейся формированием лимфоузлов, пролиферацией альвеолярного бронхиолярного и бронхического эпителия и возникновением аденоматозных изменений. Центральная нервная система была тоже поражена: налицевствовали негнойной энкефалит и сопровождающий его глиоз. Болезнь является сильно инфекционной и вызывает большой экономический ущерб. Министерством сельского хозяйства и пищевой промышленности издано распоряжение, согласно которому пораженные поголовья должны быть декларированы закрытыми и изданы инструкции на предупреждение распространения заразы.

ОК СЕРОГРУППЫ И КИШЕЧНУЮ ПЕТЛЮ РАСШИРЯЮЩИЙ ЭФФЕКТ КОЛИБАЦИЛЛЕЗНЫХ ШТАММОВ, ИЗОЛИРОВАННЫХ ИЗ СВИНЬИ

Г. ШЕМЙЕН и Л. ПЕШТИ

Всего 92 колибациллезных штамма от свиней изучено на принадлежность к серологической группе ОК и среди них 86 штаммов проверено на кишечную петлю расширяющее действие. Среди 60 штаммов, изолированных из поросят, страдающих колибациллезным поносом и колибациллезной энтеротоксимией, 42 (68,3%) принадлежало к серогруппам O147 : K88ac и O149 : K91, K88ac, которые до сих пор не были обнаружены в Венгрии. Среди остальных 18 штаммов 9 принадлежали к некоторой из 6 дальнейших серогрупп, остаток штаммов неподдавался типизации или представляли собой форму R. 91% штаммов, изолированных из болеющих упомянутыми выше болезнями животных, обладали кишечную петлю расширяющим действием.

Среди 12 штаммов из животных с отечной болезнью 7 принадлежали к серотипу O139 : K82 и за исключением одного (O138 : K81) они не обладали кишечную петлю расширяющим действием. Большинство изолятов из здоровых животных и таковых от больных, но не колибациллезом, не поддавались типизации имеющимися в распоряжении сыворотками и не вызывали накопления жидкости в изолированной лигатурами кишечной петле.

ИЗУЧЕНИЕ ЭНТЕРОПАТОГЕННОСТИ, КИШЕЧНУЮ ПЕТЛЮ РАСШИРЯЮЩИЙ ЭФФЕКТ И ЭНТЕРОТОКСИН ПРОДУЦИРУЮЩЕЙ СПОСОБНОСТИ ШТАММОВ ESCHERICHIA COLI, ИЗОЛИРОВАННЫХ ИЗ БОЛЬНЫХ ЭНТЕРИТАМИ СВИНЕЙ

Л. ПЕШТИ и Г. ШЕМЙЕН

Среди 58 штаммов **E. coli**, изолированных из больных колибациллезной дизентерией или колибациллезной энтеротоксимией свиней, 91,4% вызывало расширение изолированной лигатурами свиной кишечной петли. Таким образом проба с петлей является удобным способом доказательства энтеральной патогенности колибациллезных изолятов. Результаты пробы с кишечной петлей согласовались с эпизоотологическими наблюдениями и результатами экспериментов заражения через рот. Из всех изолятов обоих типов болезни **in vitro** методом удалось получить один экстрацелюлярный термостабильный и один термолабильный интрацелюлярный энтеротоксин. За исключением одного среди 11 штаммов, изолированных при отечной болезни, ни один не расширял изолированную петлю и не продуцировал ни термолабильного, ни термостабильного токсина.

Дискутируются гипотезы патомеханизма диареи.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ РАЗНЫХ СПОСОБОВ КОНСЕРВИРОВАНИЯ КОМПЛЕМЕНТА

Г. НАДЬ и Д. ФОДОР

Изучен эффект разных стабилизаторов активности холодом высушенного комплемента путем сравнения с лиофилизированным комплементом без стабилизатора. Стабилизатор $MgSO_4 + CaCl_2$ обладал наилучшим консервирующим эффектом: консервированный

им комплемент был активным даже в разведении 2,4% после хранения один год. Десять партий лиофилизированного комплемента, приготовленного использованием $MgSO_4$ + + $CaCl_2$ для рутинного использования, оказались подобным образом активными. Поэтому авторы для стабилизации лиофилизированного комплемента рекомендуют смесь $MgSO_4$ и $CaCl_2$.

МИНЕРАЛЬНЫЙ СОСТАВ КОПЫТНОГО РОГА СВИНЕЙ РАЗНЫХ ПОРОД И ВОЗРАСТА

А. Б. КОВАЧ и М. СИЛАДИ

Изучено содержание фосфора, натрия, магния, кальция, меди, олова и железа копытного рога свиней пород корнвал и КА—ХИБ, дальше, поросят пород корнвал, дюрок и герцегхаломского гибрида (всего 167 животных). Обнаружено, что перечисленные элементы в копытном роге корнвал и КА—ХИБ имеются в неодинаковой концентрации.

Образцы для исследования брались от трех разных, анатомически и функционально типичных частей (венчик, роговой край подошвы и стрелка) и разное распределение перечисленных элементов было консеквентным. Разница в концентрации элементов в отдельных анатомически отличимых участках рога была больше, чем между теми же участками разных пород. В копытном роге поросят перечисленные элементы были в высшей концентрации, чем в роге взрослых свиней.

ФИЗИОЛОГИЧЕСКАЯ РОЛЬ КОЛОСТРАЛЬНОГО ИНГИБИТОРА ТРИПСИНА: ЭКСПЕРИМЕНТЫ НА ПОРОСЯТАХ И КОТЯТАХ

К. БАНИТНЕР

Изучен кишечный просвет и гистология кишечного эпителия у двух видов, неселективно абсорбирующих белок: новорожденных поросят и котят.

Обнаружено, что в кишечнике поросят непосредственно после первого сосания имеется большое количество ингибитора трипсина и, таким образом, обычно невозможно доказать наличие протеолитических знзимов панкреатического происхождения. Концентрация ингибитора трипсина в молозиве и кишечном содержимом соразмерно с величиной помета понижается. В кишечнике котят имеется меньше ингибитора трипсина и пищеварение начинается раньше, чем у поросят.

По мере понижения концентрации ингибитора трипсина постепенно развивается характерный для сосунов состав протеаз. Первыми появляются протеазы, на которых ингибитор трипсина не действует. Трипсин появляется симультанно с исчезновением ингибитора трипсина из кишечника. К тому же времени прекращается протеинурия и эозинофильные капли исчезают из эритроцитов.

Наблюдаются и возрастом обусловленные количественные и качественные изменения в протеолитической активности, независимо от действия колострального ингибитора трипсина.

Защиту материнскими антителами в период неселективной абсорбции протеина можно достаточно объяснить наличием ингибитора трипсина. Остановка всасывания протеина у поросят и котят является не так потерей этой способности, а началом переваривания белка.

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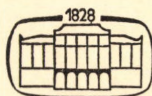

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OCCURRENCE OF SALMONELLAE IN FOOD PRODUCTS OF ANIMAL ORIGIN IN CALENDAR YEAR 1971*

By

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Central Laboratory of Veterinary Meat Control Service (Head: J. TAKÁCS)

(Received May 28, 1972)

The Central Laboratory of the Veterinary Meat Control Service has been concerned for more than 25 years with the identification of Salmonella strains dispatched from different veterinary fields, especially those occurring in foodstuffs and with the collection and publication of data on this work (TAKÁCS and NAGY, 1969). A further aspect of these activities is making comparison of salmonellae derived from medical fields and human sources with the salmonellae isolated from animals or animal origined foods. This comparative work has often take notice of or call attention to links in the salmonella infective pattern that might be important in diminishing the amount of salmonella contaminations.

Within the narrower scope of the veterinary health service proper work is concerned with detecting and, eventually eradicating sources of infection, and with ensuring that condemned materials of animal origin do not reach the market.

The most arduous task is without doubt that of eliminating the infective link from one animal to the next in the contagious chain. Because the main sources of S. infection among livestock, particularly under intensive rearing conditions, are contaminated feed and the environment, the difficulties here are acute. Hungary is unable to produce sufficient animal protein feed to meet the demands of its intensive units so the country is forced to rely, in part, on imports of meat-, blood-, fish-, and bone-meal from abroad, mainly from South America. The extent to which these feeds are contaminated has been investigated by NYIREDY, TAKÁCS and NAGY (1966).

KAMPELMACHER et al. (1965) have ascertained that animal feeds need to be heat-treated for at least 30 minutes at 80–85° to safely kill salmonellae without significant deterioration in the quality of the feed. This is the method of choice for the production of pelleted and granulated feeds, for overall

* Lecture delivered at Siófok, Hungary 5th May, 1972 to mark the 15th Jubilee Days of Public Health.

security should ideally be achieved by heat-treatment and not rest on microbiological control.

Another major source of mass S. infection in intensive farming is the formation of new animal groups according to weight or stage of development. (This method is no longer used in Hungary.) With each new group formed the proportion of symptomless carriers and excretors increases, and if the animals are further subjected to some deficiency in feeding or other kind of stress, the number of latent infections in a susceptible herd doubly exposed to infection, can mount very rapidly. Improper handling of animals during transport to the slaughterhouse is again a debilitating factor which can lead to a significant increase in the number of contaminated animals just prior to slaughter (TAKÁCS, 1971).

Through symptomless carriers and excretors, S. contaminations may have get further opportunity to spread during the processing of carcases, especially where the hygienic conditions are inadequate.

In Hungary little food of animal origin is eaten raw, why not the number of epidemic food poisonings but first of all an increasing in the number of symptomless carrier persons occurs.

Meat inspection is intended to select the meat of the animals in which clinical symptoms or pathological changes indicate the presence of primary paratyphoid infection. The traditional macroscopic methods of meat control however, are unsuitable for the meat of those animals whose latent contamination can be attributed to symptomless S. carrier (TAKÁCS and SIMONFFY, 1970).

Therefore we would like to organize an information service between the farm and the meat inspector at the abattoir. This aims at the separate slaughtering of S. infected animalgroups and at the special care in the limited and regulated use of the meat of these animals. This regulation is still in an early stage, though of immense importance where pigs and poultry are concerned.

Processing poultry at slaughter-houses the problem is the cooling method using ice-water because if the number of S. carrier is high entire bath of carcases may cause a surface contamination with Salmonellae. There are experiments for cooling with shower and there are countries where the ice-water cooling is allowed only for a definite period.

It can be diminished only by a concerted effort in the field of husbandry, industrial processing and marketing as well for eliminating the infective sources as far as possible. In present circumstances the number of S. contaminations can be diminished but completely it couldn't be eliminated in countries with developed hygienic either, where the control against Salmonellae has old traditions. The aim is to reduce the natural S. infections to a minimum.

The diminishing of the number of S. infections must be planned and

coordinated with the resources and must embrace all links of the infective chain.

A part of this falls on the veterinary health service, there is a common field of veterinary and public health but the task of agricultural field isn't less either.

The informative work is of great importance. This must cover hygiene in breeding and feeding, environmental hygiene, transport, processing at slaughter and in the meat industry, storage, preservation and food-sale.

Results

The data for 1971 on S. isolations in this laboratory from samples of animal origin is presented below in Tables I—VII.

The number of S. strains isolated from the individual samples is summarized in Table I.

Table I

Distribution of Salmonella species isolated from various test materials in 1971

Sample		No. of contamined samples	Percentage of all isolations (1.427)
Group	Species		
Complementary bacteriological meat examination	pig	541	37.91
	cattle	23	1.61
	calf	3	0.21
	horse	1	0.07
	goose	97	6.79
	duck	3	0.21
	chicken	1	0.07
Meat products and meat-industry control	salted, smoked and dried sausages	77	5.39
	semi-prepared meat products	177	12.40
	ready-for marketing meat products	63	4.41
	samples for hygienic control	296	20.72
Raw materials for meat products	liver	35	2.45
	meat	70	4.90
	lard	1	0.07
	gut	8	0.56
	plasma	2	0.14
Animal protein feeds	meat meal, fish meal	24	1.68
Miscellaneous materials of animal and other origin		5	0.35

The distribution of these species is shown in Table II.

Table II

Distribution of Salmonella species in samples examined in 1971

	Salmonella species	No. of isolations	Percentage of all isolations (1.427)
More than 100 cases	*S. anatum*	452	31.67
	S. cholerae-suis	367	25.71
	S. typhi-murium	122	8.54
10–100 cases	*S. derby*	98	6.86
	S. meleagridis	67	4.69
	S. manhattan	38	2.66
	S. reading	34	2.38
	S. kapemba	29	2.03
	S. senftenberg	26	1.82
	S. bareilly	21	1.47
	S. infantis	20	1.40
	S. enteritidis	16	1.12
	S. panama	15	1.05
	S. brandenburg	15	1.05
	S. heidelberg	12	0.84
	S. give	12	0.84
	S. london	11	0.77
3–10 cases	*S. abony*	7	0.49
	S. bradford	5	0.35
	S. chester	5	0.35
	S. mission	5	0.35
	S. taksony	5	0.35
	S. abony var. haifa	4	0.28
	S. bredeney	4	0.28
	S. isangi	4	0.28
	S. epicrates	4	0.28
	S. stanley	3	0.21
	S. worthington	3	0.21
	S. minnesota	3	0.21
2 cases	*S. kilwa*	2	0.14
	S. paratyphi-B	2	0.14
	S. livingstone	2	0.14
	S. menston	2	0.14
	S. senftenberg var. newcastle	2	0.14
	S. havanna	2	0.14
1 case	*S. coeln*	1	0.07
	S. montevideo	1	0.07
	S. thompson	1	0.07
	S. typhi-suis	1	0.07
	S. kottbus	1	0.07
	S. newport	1	0.07
	S. dublin	1	0.07
	S. westhampton	1	0.07

Table III gives the distribution by host species and serotypes of the S. species found in the course of meat inspections.

Table III

Salmonella special isolated at the complementary bacteriological meat examination of meat animals in 1971

Salmonella species and serotypes		Pig				Cattle			Calf	Horse	Goose			Duck	Chicken
		>10	3 – 10	2	1	3 – 10	2	1	1	1	>10	3 – 10	1	3 – 10	1
		cases													
B	*S. abony*		3												
	S. abony var. *haifa*								1						
	S. brandenburg			2											
	S. coeln				1										
	S. derby	15					2								
	S. heidelberg		4							1					
	S. reading		3												
	S. typhi-murium	13				9			1		70			3	1
C_1	*S. bareilly*										18				
	S. cholerae-suis	358					2		1						
	S. isangi				1										
	S. mission		3					1							
	S. typhi-suis				1										
C_2	*S. kottbus*				1										
	S. manhattan		8												
D_1	*S. dublin*							1							
	S. enteritidis		7			4									
	S. kapemba				1										
E_1	*S. anatum*	102					2					8			
	S. london				1										
	S. meleagridis	12													
	S. westhampton												1		
E_4	*S. senftenberg*		5				2								
No. of isolates		5	7	1	6	2	4	2	3	1	2	1	1	1	1
Total		19 species				8 species			3 species	1 species	4 species			1 species	1 species

Table IV

Salmonella species isolated from meat industrial raw materials in 1971

	Salmonella species and serotypes	Liver				Meat				Lard	Gut		Plasma
		>10	3–10	2	1	>10	3–10	2	1	1	3–10	1	2
		cases											
B	*S. brandenburg*				1		9						
	S. bredeney			2									
	S. derby		5				5						
	S. heidelberg				1		3					1	
	S. reading			2			3				6		
	S. typhi-murium							2					
C_1	*S. bareilly*								1				
	S. cholerae-suis												2
	S. infantis			2				2					
	S. isangi								1				
	S. montevideo				1								
	S. thompson								1				
C_2	*S. newport*				1								
D_1	*S. enteritidis*											1	
	S. panama				1				1				
E_1	*S. anatum*	19				31				1			
	S. epicrates								1				
	S. london						5						
	S. meleagridis								1				
E_4	*S. senftenberg*						3						
	S. taksony								1				
No of isolates		1	1	3	5	1	6	2	7	1	1	2	1
Total		10 species				16 species				1 species	3 species		1 species

Table IV details the S. species (and their serotype) that were cultured from raw materials in the meat industry.

The comparable data are given in Table V for S. species cultivated from meat products, and in Table VI for species isolated from samples for hygienic control, animal feeds and other samples.

The distribution by serotypes of all isolated Salmonellae is summed up by Table VII.

Table V

Salmonella species isolated from meat products in 1971

Salmonella species and serotypes		Salted, smoked and dried sausages				Semi-prepared meat products				Ready for marketing meat products			
		>10	3–10	2	1	>10	3–10	2	1	>10	3–10	2	1
		cases											
B	*S. abony*								1				
	S. bradford				1								
	S. brandenburg				1				1				1
	S. chester				1								
	S. derby	17					7					2	
	S. heidelberg												1
	S. paratyphi-B			2									
	S. reading									20			
	S. stanley												1
	S. typhi-murium		4					2			6		
C_1	*S. bareilly*								1				
	S. cholerae-suis							2					
	S. infantis	12							1			2	
	S. isangi												1
C_2	*S. manhattan*		3				8						
D_1	*S. kapemba*		5										
	S. panama		6										1
E_1	*S. anatum*	11				139				26			
	S. epicrates												1
	S. give				1								
	S. london								1				
	S. meleagridis	12				12							1
E_4	*S. senftenberg*								1				
L	*S. minnesota*				1				1				
No of isolates		4	4	1	5	2	2	2	7	2	1	2	7
Total		14 species				13 species				12 species			

Table VI

Salmonella species isolated at the hygienic conditions detecting examinations and from animal protein feeds and miscellaneous materials of animal and other origin in 1971

Salmonella species and serotypes		Samples or hygienic conditions detecting examinations				Animal protein feeds: meat-meal fish-meal			Miscellaneous materials of animal and other origin	
		>10	3–10	2	1	3–10	2	1	3–10	1
		cases								
B	*S. abony*		3							
	S. abony var. haifa		3							
	S. bradford				1	3				
	S. bredeney						2			
	S. chester		4							
	S. derby	44						1		
	S. heidelberg				1					
	S. kilwa			2						
	S. stanley						2			
	S. typhi-murium	11								
C_1	*S. bareilly*							1		
	S. cholerae-suis			2						
	S. infantis				1					
	S. isangi				1					
	S. livingstone						2			
	S. menston						2			
	S. mission				1					
C_2	*S. manhattan*	19								
D_1	*S. enteritidis*								4	
	S. kapemba	23								
	S. panama		4				2			
E_1	*S. anatum*	108				4				1
	S. epicrates			2						
	S. give	11								
	S. london		4							
	S. meleagridis	29								
E_4	*S. senftenberg*	15								
	S. senftenberg var. newcastle						2			

Salmonella speciej and serotypes	Samples or hygienic conditions detecting examinations				Animal protein feeds: meat-meal fish-meal			Miscellaneous materials of animal and other origin	
	>10	3—10	2	1	3—10	2	1	3—10	1
	casns								
G_2 *S. taksony*		4							
S. havana			2						
S. worthington					3				
L *S. minnesota*				1					
No. of isolates	8	6	4	6	3	6	2	1	1
Total	24 species				11 species			2 species	

Discussion

During 1971 a total of 1427 S. strains of 43 species were identified in this laboratory. The most frequently occurring species were *S. anatum*, *S. cholerae-suis* and *S. typhi-murium*, each of which was cultured in more than 100 cases. Species isolated for the first time in Hungary during the year were: *S. mission*, in 5 cases (from pigs, cattle, and a sample for hygienic control); *S. abony var. haifa* in 4 cases (from cattle and a sample for hygienic control); *S. epicrates* in 4 cases (from meat imported from West Germany, sausage and a sample for hygienic control); *S. kilwa* in 2 cases (from samples for hygienic control); *S. menston* in 2 cases (from meat-meal of French origin); *S. havanna* in 2 cases (from samples for hygienic control); and *S. coeln* in 1 case (from a pig).

With regard to frequency of occurence in the individual host species, the commonest salmonellas in pig were *S. cholerae-suis* and *S. anatum*, and altogether 19 S. species were isolated. In cattle *S. typi-murium* and *S. enteritidis* predominated, and altogether 8 species were isolated. No particular strain can be recorded for calves because only 3 species were isolated, each in a single case. The situation is similar with the horse, from which one species was isolated again in a single case only. *S. typhi-murium* and a long way back *S. bareilly* were the species found most frequently in geese; altogether 4 species were isolated. In ducks as well as in chicken only *S. typhi-murium* was found.

The liver and meat samples were taken mostly from imported, frozen lots. Fourteen species occured in preserved meat products (i.e. salted, cold smoked and dried) under Hungarian Standard 5857-70. Investigation of raw, smoked sausage for the presence of salmonella is compulsory.

Sampling of semi-cooked products during manufacture revealed 13 S. strains, while samples of ready-cooked meat products yielded 12 strains.

Table VII

Distribution of Salmonella serological O-groups in 1971

O-antigens	Complementary bacteriological meat examination of meat animals							Control of meat products and in the meat industrial plant					Animal protein feeds	Miscellaneous samples of animal and other origin
	pig	cattle	calf	horse	goose	duck	chicken	Salted, smoked and dried sausages	Semipre-pared meat products	Ready for marketing meat products	Hygienic control	Raw materials for meat industry		
B	41	11	2	1	70	3	1	26	11	31	69	40	8	
C_1	363	3	1		18			12	4	3	5	10	5	
C_2	9							3	8		19	1		
D_1	8	5						11		1	27	3	2	4
E_1	115	2			9			24	152	28	154	58	4	1
E_4	5	2							1		19	4	2	
G_2											2		3	
L								1	1		1			
Total	541	23	3	1	97	3	1	77	177	63	296	118	24	5

Total: 1.427 Salmonella species

Legends: [solid box] the most frequently occurring serotype; [dotted box] the second most frequently occurring serotype

In the latter class the most frequently contaminated foodstuff was a special raw, smoked sausage that is mostly prepared in agricultural cooperatives, using a technology differing from that of industrially produced "Gyulai" sausage, Vienna sausage, "Bácskai" sausage, greaves and cooked smoked ham.

The extent of contamination of samples sent in for hygienic control underlines the need for constant, daily disinfection in slaughterhouses and the meat industry in the prevention of food poisoning.

Animal protein feeds constituted the major source of new species not previously isolated in the country before. This was the route by which *S. worthington, senftenberg var. newcastle, manilla, tennessee* and *stanleyville* entered Hungary in previous years and *S. menston* in 1971. *S. stanley* was the only strain imported with animal feeds whose incidence increased significantly, though it must be noted that earlier experiences show that situation is not necessarily as encouraging as the figures might suggest. Thus, although spreading or *S. cubana* first isolated in 1968—69 has been noticed since in the veterinary health field, this salmonella was the cause of several outbreaks of food-poisoning in nursery schools in 1971 through human carriers.

There is also a danger of introduction of infection with other imported materials of animal origin. In 1970, for example, a S. strain with the 4.12 d antigenic structure and not fitting into the Kauffmann—White scheme was isolated in 7 cases from pork imported from England. Hobbs (1969/70) has often isolated this strain in England in samples of symptomless salmonella excreting swine and poultry. It is likewise fairly certain that *S. epicrates* was introduced with beef imported from the GFR.

Imported frozen liver and meat too are often carriers of S. strains that have never, or only rarely, occurred before in our samples. A significant curbing of S. contamination in feedstuff of animal origin is attainable only through the regulated use of these substances.

The very frequent occurrence of *S. anatum* in almost every investigated animal group is another matter for concern. As this strain is not adapted to swine, it often produces a symptomless carrier and excreting state in this species; in fact, if poultry are disregarded, *S. anatum* occurred almost exclusively in swine at slaughtering.

According to these 1971 data, most of the isolated S. belong to serotype B, though type E_1 were very common as well.

SUMMARY

In 1971 1427 S. strains, isolated from samples of animal origin, were identified. The most frequently isolated strains were of *S. anatum, cholerae-suis* and *typhi-murium*, while *epicrates, kilwa, menston, havanna* and *coeln* were found for the first time in Hungary. Among meat animals, pig, and among poultry, duck and goose, proved the host animals, these contaminations beeing attributable primarily to animal protein feeds. A further source of S. infection was the imported frozen meat and liver.

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COMPARISON OF HAEMOLYSIN PREPARATIONS OBTAINED BY DIFFERENT METHODS

By

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To prepare haemolysin in rabbits for the complement fixation (CF) test, sheep erythrocytes are administered either alone or in combination with anti-reticulocyte serum or sheep serum. Marshall (1947) injected rabbits intravenously with 5 ml of a 10% sheep erythrocyte suspension on 5 or 6 occasions at 5-day intervals. Orlov (1954, 1963) used rising intravenous doses of sheep erythrocytes on 5 occasions at 3-day intervals: the initial dose was 0.5 ml and each subsequent dose was risen by 0.25 ml, diluted in 2.5 ml saline throughout. Hallmann (1955) gave three intravenous doses — 2.0, 1.5 and 1.0 ml — at 5- to 6-day intervals. Manninger (1960) recommended three intravenous doses of 3—5 ml, at 2- to 4-day intervals.

Bogomolec (1954) sensitized rabbits in addition to three 1.5 ml-doses of a 50% sheep erythrocyte suspension with three intravenous doses, 1 ml each, of anti-reticulocyte serum (titre, 1 : 20). McArthur and Kolmer (1955) employed a more complicated scheme of sensitization, lasting for four weeks. The animals were given 0.5 ml sheep serum on the first day of the week, 1.0 ml on the second day and 0.2 ml on each subsequent day except for the sixth, from which onwards 1.0, 2.0, 3.0 and 4.0 ml of sheep erythrocyte suspension were administered intravenously. Injections of the erythrocytes and serum were separated by 30 minutes from each other. Ruge (1960) used a similar technology, which is described in detail under Materials and Methods.

In this laboratory haemolysin preparations obtained by nine different methods were compared.

Materials and Methods

(see Table)

Preparation of haemolysin

I. Orlov's method (1954, 1963). Three times washed sheep erythrocytes were administered to rabbits in doses of 0.5, 0.75, 1.0, 1.25 and 1.5 ml each diluted in 2.5 ml saline at intervals of 2 or 3 days. The total dose of packed erythrocytes was 5 ml for each rabbit. The animals were exsanguinated seven days after the last inoculation.

II. Ruge's schedule (1960) without administering serum. A 10% sheep erythrocyte suspension was used for sensitization, in doses of 1, 2, 3 and 4 ml, administered at 2- or 3-day intervals. The rabbits were bled 7 days after the last inoculation. For further details see paragraph IV.

Table I

Method No.	Inoculum	Total volume in ml	Schedule of sensitization												Titres. Highest serum dilution still producing complete haemolysis	Mean titre
			week													
			1.		2.			3.		4.				5.		
			day													
			1.	3.	6.	8.	10.	13.	15.	17.	20.	22.	24.	27.		
I.	Sheep erythrocytes	5.0	0.5	0.75	1.0	1.25	1.5			ch.					2 000 5 000 6 000 8 000 8 000	1 : 5800
II.	Sheep erythrocytes	1.0	1.0	2.0	3.0	4.0			ch.						< 100 1 000 2 000 4 000 5 000	1 : 2 420
III.	Sheep erythrocytes	3.0	1.0	2.0	3.0	4.0	5.0			ch.					< 100 3 000 8 000 10 000 15 000	1 : 7 220
IV.	Sheep serum + sheep erythrocytes from different donors	8.1 1.0	0.5	1.0	0.2 1.3	0.2 1.8	0.2 2.3		1.0	0.2 2.0	0.2 3.0	0.2 4.0		ch.	10 000 10 000 25 000 25 000 25 000	1 : 19 000
V.	Sheep serum + sheep erythrocytes from one and the same donor	8.1 1.0	0.5	1.0	0.2 1.3	0.2 1.8	0.2 2.8		1.0	0.2 2.0	0.2 3.0	0.2 4.0		ch.	10 000 10 000 10 000 15 000 20 000	1 : 13 000

VI.	Bovine serum + sheep erythrocytes from different donors	8.1 1.0	0.5	1.0	0.2 1.3	0.2 1.8	0.2 2.3		1.0	0.2 2.0	0.2 3.0	0.2 4.0		ch.	< 100 < 100 1 000 5 000 6 000	1 : 2 450
VII.	Sheep serum from different donors	8.1	0.5	1.0	0.2 1.3	0.2 1.8	0.2 2.3			0.2	0.2	0.2		ch.	< 100 1 000 1 000 1 000 2 000	1 : 1 020
VIII.	Sheep serum from one and the same donor	8.1	0.5	1.0	0.2 1.3	0.2 1.8	0.2 2.3			0.2	0.2	0.2		ch.	< 100 < 100 < 100 1 000 3 000	1 : 860
IX.	Bovine serum	8.1	0.5	1.0	0.2 1.3	0.2 1.8	0.2 2.3			0.2	0.2	0.2		ch.	— — < 100 < 100 < 100	—

III. Our own method. A 20% suspension of three times washed sheep erythrocytes was administered intravenously in doses of 1, 2, 3, 4 and 5 ml, at intervals of 2 or 3 days. The total dose of packed sheep erythrocytes was 3 ml for each rabbit. The animals were exsanguinated 7 days after the last injection.

IV. RUGE's original method (1960). Sensitization of rabbits was carried out over a period of four weeks; sheep serum was given on 11 occasions, 10% sheep erythrocyte suspension on four occasions, by intravenous route, on the schedule shown in Table I. The working-principle of the method is that each inoculation is made with serum or erythrocytes from different donors, even when given on one and the same day. Two inoculations given on the same day, whether serum + serum or serum + erythrocyte suspension, were separated by a 30-minute interval to prevent anaphylaxis. Under this schedule, each rabbit was treated with a total dose of 8.1 ml serum and 1 ml packed erythrocyte suspension. The animals were exsanguinated 5 days after the last inoculation.

V. RUGE's schedule, using serum and erythrocytes from one and the same donor. The schedule of sensitization and bleeding was as in paragraph IV.

VI. RUGE's schedule with bovine serum instead of sheep serum.

VII. RUGE's schedule with sera from different donors without erythrocyte suspension.

VIII. RUGE's schedule with serum from the same donor without erythrocytes.

IX. RUGE's schedule with bovine serum instead of sheep serum, no erythrocytes.

X. Control. The rabbits of this group were not treated. Five rabbits were in each of the ten groups. The rabbit sera were titrated individually, according to the FAO/WHO prescriptions (ALTON and JONES, 1967). They were inactivated for 30 minutes at 56°C and no glycerol or other preservative was added. A control series was set up with each titration, using a standard haemolysin preparation diluted 1 : 2000 for use and a freeze-dried complement preparation (PHYLAXIA, Budapest).

Stock suspensions of sheep erythrocytes were prepared by centrifugation of sheep blood at 2000 r.p.m. for 30 minutes and three washes with saline at the same r.p.m.

Results

(see Table I)

I. The four-fold deviations between the titres of the preparations were due to individual variations in the response of the rabbits. Arithmetic mean of reciprocal titres (AMRT): 5800.

II. The low titres can, to a certain degree, be explained by the small total dose of erythrocytes. AMRT: 2400.

III. Titre values obtained by this technically simple method were relatively high (AMRT: 7200) although the total dose of packed erythrocytes was intermediate between the dose levels used for the procedures I and II.

IV. Very high titres were obtained. Three of the five rabbits had a serum titre of 1 : 25,000. AMRT: 19,000.

V. None of the five rabbits inoculated with serum from one and the same donor had a titre level below 1 : 10,000. AMRT: 13,000.

VI. The titre values were not superior to those obtained in group II, in which the same erythrocyte dose was used. AMRT: 2400.

VII. Inoculations with sera from different donors resulted in a considerable haemolysin production. AMRT: 1020.

VIII. Serial inoculations with serum from one and the same donor also resulted in some haemolysin production.

IX. No anti-sheep haemolysin production took place after treatment with bovine serum.

X. Serum samples from the untreated control rabbits were negative.

Discussion

It follows from the results that the quantity of sheep erythrocytes plays a substantial role if exclusively erythrocytes are administered (groups I, II and III). If in addition, serum is injected (groups IV, V and VI), the sheep serum seems to have a specific stimulating effect (groups IV and V); bovine serum (group VI) failed to stimulate haemolysin production.

It is remarkable, that sheep serum was in itself capable of inducing haemolysin production, probably because traces of red cells are always present in the serum.

As the standard prescriptions valid in Hungary require a haemolysin preparation with a working-titre ranging from 1 : 2000 to 1 : 2500, it was worth while to seek after a method which would result in a haemolysin titre of at least 1 : 8000 (the working-titre is one quarter of this value). According to the present study, procedures III, IV and V meet this requirement. RUGE's original method proved to be the best, but the use of serum and erythrocytes from one and the same donor was nearly as efficient. It should be mentioned that procedure III has the advantage of being much simpler than RUGE's method.

Further examinations are required to obtain more information about the effect of sheep serum stimulating haemolysin production. Due to this

effect high haemolysin titres were obtained even if the erythrocyte dose was small.

Repeated administrations of bovine serum caused paralytic symptoms; no such phenomena were observed with sheep serum.

SUMMARY

Rabbits were sensitized to obtain haemolysin preparations for comparative purposes: groups of rabbits were treated either with: sheep erythrocytes, sheep serum, bovine serum, or sheep or bovine serum plus sheep erythrocytes. The doses of erythrocytes were varied.

The sera of the sensitized rabbits were titrated for haemolysin individually, according to the FAO/WHO prescriptions. The highest haemolysin titres were obtained by combined treatment with sheep erythrocytes and sheep serum from different or identical donors. The haemolysin titres of rabbits treated with erythrocytes alone depended on the dose.

Measurable haemolysin was found in the sera of rabbits treated with sheep serum alone.

Inoculation of sheep serum both from a specific or different donors prior to and simultaneously with, the administration of the sheep erythrocytes markedly stimulated the production of haemolysin. Bovine serum failed to exert such a stimulating effect.

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PROLONGATION OF THE PERIOD OF PROTEIN ABSORPTION: EXPERIMENTS WITH KITTENS AND PIGLETS

By

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The suckling rat is capable of selective absorption of IgG up to the end of the 3rd week of life (Brambell, 1958), even though a high proteolytic activity is demonstrable in its small intestine (Baintner and Juhász, 1971). In the piglet, lamb and calf, on the other hand, there is practically no selection of absorbed proteins (Payne and Marsh, 1962; Karlsson, 1966) and protein uptake persists only over the first 1 1/2 to 2 days (Mason et al., 1930: McCarthy and McDougall, 1949; Möhring and Strunz, 19C8). At the same time, eosinophilic droplets are visible in the enterocytes lining the small intestinal villi (Comline et al., 1951) and low-molecular-weight proteins — including colostral trypsin inhibitor (CTI) — can be detected in the urine (Pierce, 1959; Baintner, 1970).

The new-born kitten has been found to exhibit all the typical features of non-selective protein uptake; the concomitant presence of CTI, eosinophilic droplets and proteinuria being demonstrable for 1 to 1 1/2 days (Baintner, 1973). The same investigations also revealed that in both kittens and piglets active protein absorption, — as indicated by the presence of eosinophilic droplets, — ceases when CTI vanishes from the small intestine and the pattern of proteolytic enzymes is completed by the appearance of trypsin, *i.e.* when protein digestion becomes practically perfect.

In so far as it can be assumed that the full onset of protein digestion is responsible for this loss of protein absorption, the question arises as to whether the small intestine of the kitten and piglet remains potentially capable of further protein uptake. Clarke and Hardy (1970, 1971) have established that ileal enterocytes of the piglet are able to take up indigestible polyvinylpyrrolidone (PVP) up to the 12th—15th day of life, and those of the kitten up to about the 14th day (with considerable individual variation), but that, except in very young animals, this PVP is not transmitted into the circulation. By this time the ileal enterocytes carry seemingly empty vacuoles (Clarke and Hardy, 1970; Veress and Baintner, unpublished).

The experiments reported herein aimed to establish whether the small intestine of kittens more than 1 to 1 1/2 days old is able to take up colostral proteins in circumstances where protein digestion is inhibited.

Materials and Methods

I. Kittens

Altogether 24 kittens (18 experimental, 6 control) were employed. Of these, six were 4 1/2 days old, four 6 days old, three 7 days old, two 8 1/2 days old, and nine 12—14 days old. One animal in each litter was set aside

as control except in the preliminary experiments, which were uncontrolled. The kittens were allowed to suck until the day of the experiment. On the morning of that day they were removed from the dam and fasted for 1 to 3 hours, depending on the fullness of the stomach. Each experimental animal then received by stomach tube, depending on body weight, 5 to 7 ml colostrum of body-temperature, and 2 to 3 hours later a further 2 to 4 ml. Cow's colostrum was obtained from the first milking; it inhibited approximately 370 μg trypsin per ml. To intensify the inhibitory effect 0.5 ml duck's egg white (which contains trypsin and chymotrypsin inhibitors) and 2000 kallikrein-inhibiting units of Trasylol (BAYER, trypsin and kallikrein inhibitor) were added to each 10 ml of colostrum. In preliminary trials, 3 mg/ml soy-bean trypsin inhibitor was added to the cow's colostrum fed to two kittens, while another three kittens received sow's colostrum. Control animals were fed every two hours on commercial cow's milk of body-temperature, administered by pipette *ad libitum.*

As kitten only discharges urine on stimulation by the dam's tongue, much urine accumulated, especially in the controls. To evoke the mictural reflex a cottonwool pad, wetted with a little lukewarm water, was applied from time to time, the drops of urine being soaked up with cotton-wool.

Throughout the experiment, all animals were kept in a dark, warm place until sacrificed, 5—6 hours after the first feeding. Urine was pressed from the bladder into a test tube. The small intestine was flushed through with 5 ml saline, and the washings collected likewise.

The total proteolytic activity of intestinal washings was determined by measuring the splitting of benzoyl-arginine ethyl ester (BAEE) substrate as described by SCHWERT and TAKENAKA (1955). This activity was then segregated into two components, *viz.* the trypsin component that was suppressed by soy-bean trypsin inhibitor and the "kallikrein" component that was not. Activities were expressed in terms of spectrophotometric units per min. per 0.1 ml intestinal washings.

The trypsin inhibitor activity of the urine samples was assayed by measuring the extent to which the BAEE-splitting activity of a known amount of trypsin was reduced. To overcome the non-specific change in U.V. absorption that frequently took place after alkalization, the samples were left in pH-8 buffer for 15 min before determination. The presence of urinary protein was tested by precipitation with 10% trichloroacetic acid.

Expressing intestinal location in per cent of the distance from the pylorus to the caecum, samples for histological examinations were taken from the jejunum (45—50%) and the ileum (75—80%). The samples were fixed in formalin, embedded in paraffin, and stained with haematoxylin and eosin.

II. Piglets

Two 4-day-old and two 4 1/2-day-old piglets were transferred to a recently farrowed foster-sow. Within about 2 hours they had accustomed themselves to their new place and began sucking. A further 2—3 hours later the animals were killed. One piglet in each litter which had been left with the dam was sacrificed as a control. In addition, two 11-day-old control piglets were also sacrificed.

Sample-taking and assays followed the same scheme as with the kittens except: 1. proteolytic activities were measured in 10-fold diluted intestinal contents; 2. trypsin inhibitor activity was determined in the intestinal contents, but not in urine; 3. staining for histological examinations was carried out with alcian blue combined with the periodic acid-Schiff reaction, in addition to haematoxylin-eosin staining.

Results

I. Kittens

A mean trypsin activity of 46 ± 19* units (range: 22—64 units) was measured in the intestinal washings collected from control animals, significantly greater ($p < 0.01$) than the mean activity of 4 ± 2.4 units (range: 1.2—11.5 units) found with the colostrum-fed animals. The mean "kallikrein" activities of 5.6 ± 3 units for the controls and 5.4 ± 3 units for the experimental kittens did not differ at the level of significance.

No protein was demonstrable in the urine from the control kittens (five samples tested), whereas on addition of trichloroacetic acid samples from four experimental animals became opalescent, in two samples even some flocculent precipitate was formed; in a further four samples the reaction was doubtful, and in only the remaining two was it definitely negative. Urine from experimental animals also inhibited significantly ($p < 0.05$) more trypsin (mean: 33 ± 21 μg/ml) than samples from controls (8.6 ± 3 μg/ml). The highest trypsin inhibitor concentrations were associated with the urine samples shown to contain protein.

In the histological examinations, no eosinophilic droplets were observed in the small intestine of control kittens; the findings were completely negative in the jejunum of all six, and also in the ileum of two animals, while the ileum of the remaining four displayed "empty" vacuoles.

The findings were likewise negative in the jejunum of all experimental animals and further in the ileum of two. Typical "empty" vacuoles never occurred in the ileum; all vacuoles that were present contained greater or

* $\pm$ standard deviation

lesser amounts of eosinophilic material, which was regarded as a positive reaction. In the ileal enterocytes of eight animals pronounced, and in a further two poorly-defined, eosinophilic droplets were visible.

The enterocytes of the two 14-day-old kittens used in the preliminary experiment and fed colostrum supplemented with soy-bean trypsin inhibitor enclosed only a few minute droplets. Among the three 4 1/2-day-old kittens fed sow's colostrum, the findings were negative in one, but in the other two, large eosinophilic droplets, almost entirely filling the ileal cells, were observed.

II. Piglets

Trypsin-inhibitor and enzyme activities were assayed only with three piglets of one of the litters. Intestinal contents of the fostered piglets inhibited respectively 2160 and 1700 μg trypsin per ml, and contained no trypsin activity. No CTI was detected in the intestinal contents of the control animal, but a trypsin activity corresponding to 1.2 μg/ml was measured. "Kallikrein" activity was 5 and 8 μg/ml for the fostered piglets and 9 μg/ml for the control.

There was no difference in the histological picture between the intestine of control and experimental animals. The findings were negative in the jejunum, while in the ileum large vacuoles enclosing excentrically situated, generally 1—2 μm (sometimes 4—6 μm), PAS-positive, eosinophilic inclusion bodies were observed.

Discussion

I. Kittens

The trypsin and "kallikrein" activities of the intestinal washings were determined in order to check the *in vivo* trypsin-inhibiting effect of the ingested colostrum. Of the materials administered, trypsin is inhibited, besides by the colostrum, by both duck's egg white and Trasylol, whereas "kallikrein" is inhibited only by Trasylol. As a result of the treatment the trypsin activity was strongly reduced in the experimental animals, though it did not disappear entirely; the "kallikrein" activity was not significantly modified.

The fact that a slight trypsin activity remained, in spite of the huge amount of inhibitor administered, is explicable in several ways:

i) the pancreas may respond to the inhibition of digestion by enhancing its enzyme production;

ii) the stomach releases chyme only in small driblets;

iii) a large surplus of inhibitor may be required to achieve 100% inhibition, owing to dissociation of the trypsin-CTI complex.

In a previous study (Baintner, 1973) no eosinophilic droplets were noticed in the small intestine of kittens older than 1 1/2 days. In the ileum

the droplets had been replaced by "empty" vacuoles, then, at an age depending very much on the individual and the litter, these, too, vanished. The kittens investigated in the present experiment were therefore of an age (4 1/2—14 days) at which, under normal conditions, eosinophilic droplets could not be expected to occur. Nevertheless, a control animal which did not receive any trypsin inhibitor, was set aside from each litter.

Eosinophilic droplets indeed did not appear in these control kittens and failed to be induced even in the jejunum of the experimental animals; but in most experimental animals this proved possible in the ileum. Negative findings or weak reactions were encountered mainly among the oldest animals, and in these cases the ileum appeared similar to the jejunum (Fig. 1). Nevertheless, no completely "empty" vacuoles were seen in the experimental kittens, indicating that the vacuoles observed in the controls (Fig. 2) must have become filled with protein and so transformed to eosinophilic droplets in the course of the experiment. Individual animals exhibited various stages in this process; in kittens sacrificed 2 hours after the first administration of colostrum, the eosinophilic droplets were small and located mainly in the apical region of the enterocytes; in other animals the vacuoles were already partially filled with eosinophilic material (Figs 3 and 5); and finally, in a few animals eosinophilic droplets were seen to fill the enterocytes completely

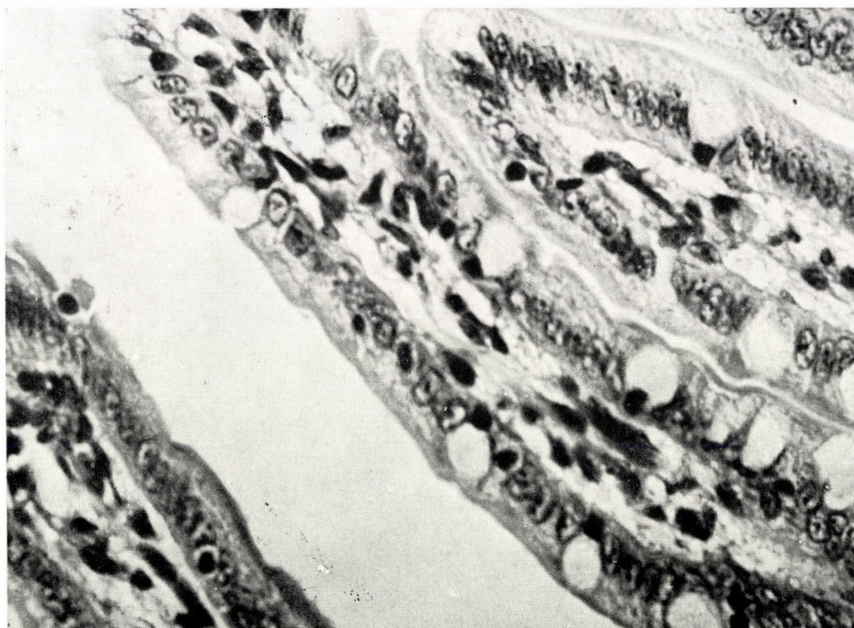

Fig. 1. Jejunum of a 7-day-old kitten fed sow's colostrum. No vacuoles or eosinophilic droplets are visible. Haematoxylin-eosin (H—E) stain

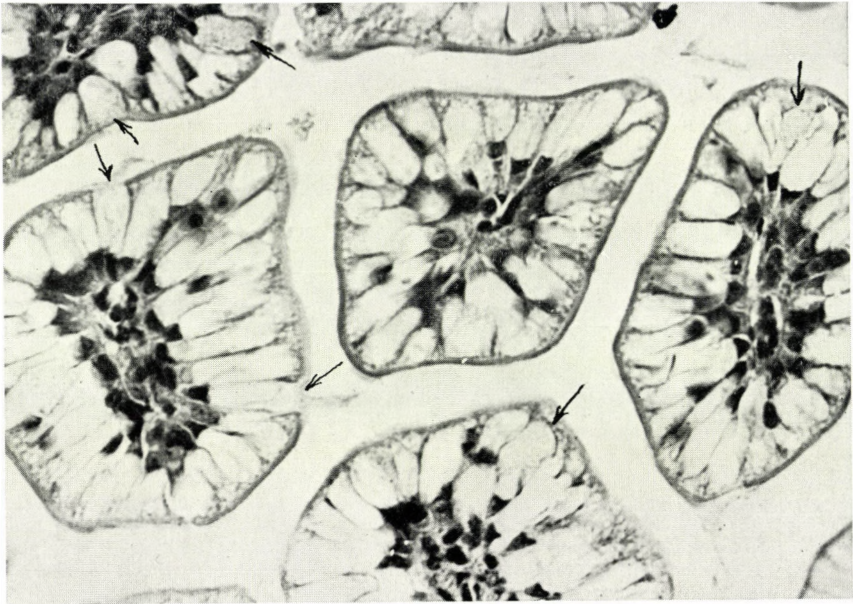

Fig. 2. Ileum of a 2-day-old control kitten. Each enterocyte contains a large "empty" vacuole in supranuclear location. The arrows point to sporadically present goblet cells. H—E stain

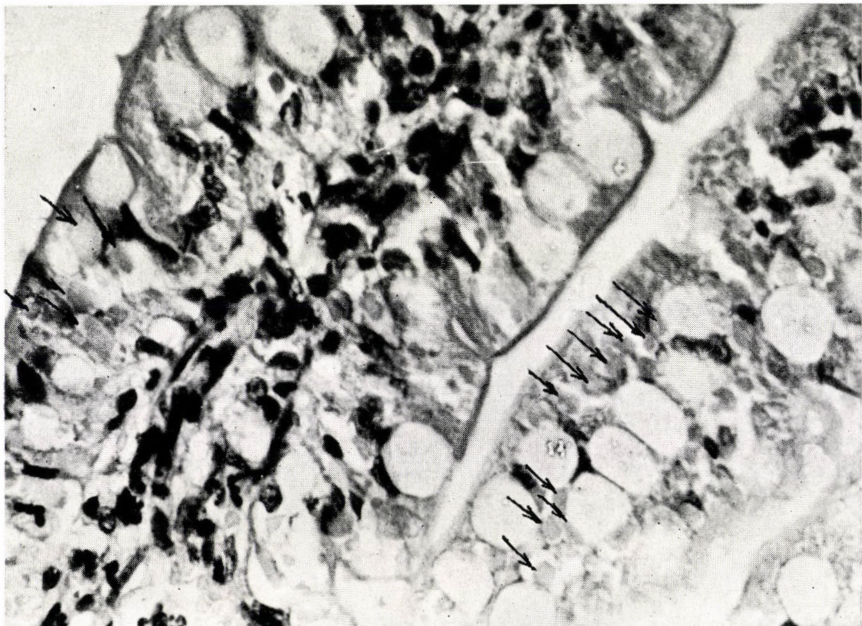

Fig. 3. Ileum of a 13 1/2-day-old kitten fed cow's colostrum. The arrows point to medium-sized eosinophilic droplets. H—E stain

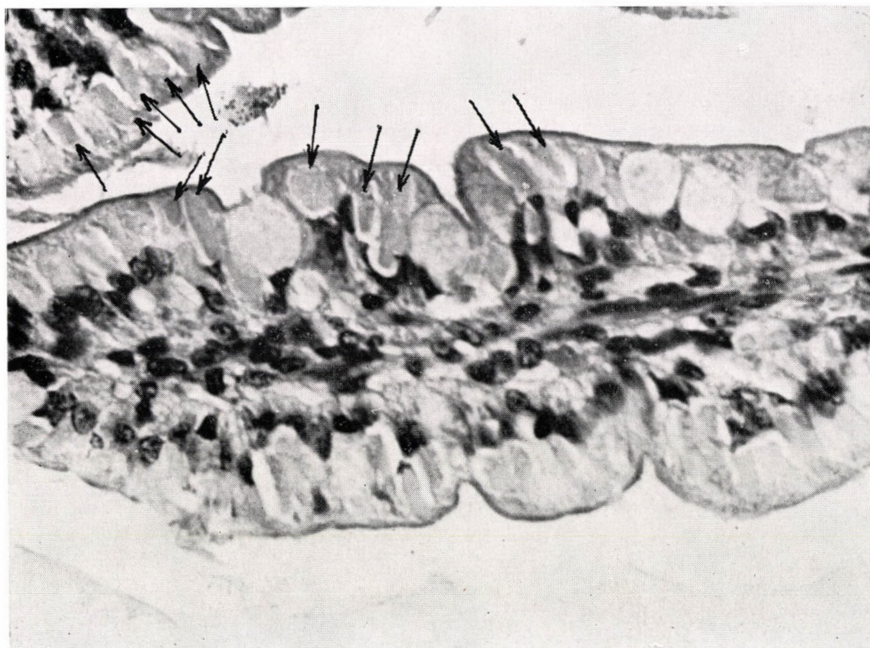

Fig. 4. Ileum of a 4 1/2-day-old kitten fed cow's colostrum. The arrows point to large eosinophilic droplets. H—E stain

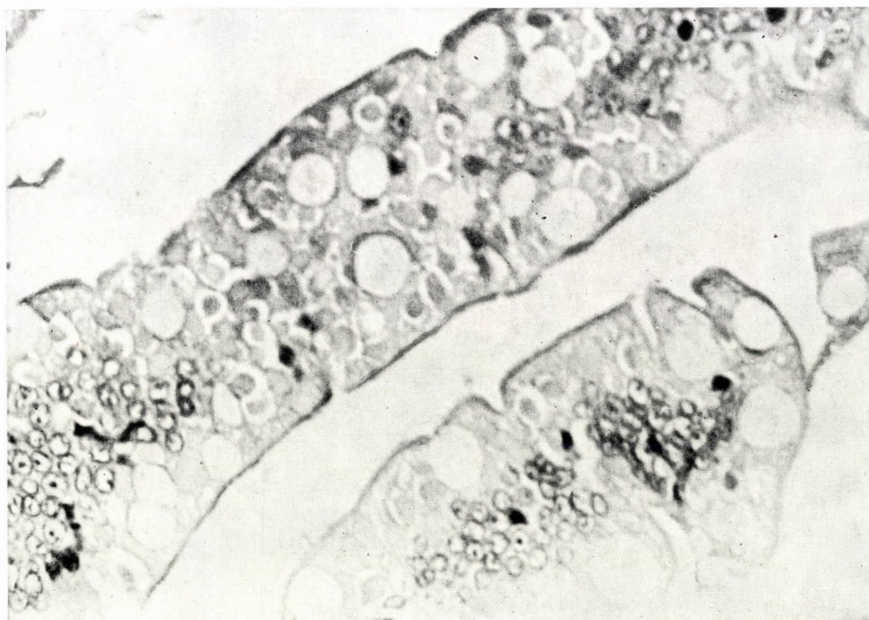

Fig. 5. Ileum of a 5-day-old kitten fed cow's colostrum, in tangential section. The enterocytes contain eosinophilic droplets. H—E stain

(Fig. 4). The latter sections resembled in all respects those prepared from 1-day-old kittens.

These observations suggest that the ileal enterocytes of the kitten can retain their competency to take up protein from the intestinal lumen after the colostrum-sucking period up to a definite age, provided digestion is inhibited; and furthermore, those cells which retain this competency after digestion has started are marked by "empty" vacuoles. It must be emphasized, nevertheless, that part of the reason for the neutralization of digestive enzymes and the ensuing induction of eosinophilic droplets is that colostrum is much thicker than milk, and also the kittens were given large doses of colostrum.

The experiments of CLARKE and HARDY (1970, 1971) have demonstrated that although PVP is taken up by the ileal enterocytes of piglets and kittens older than 2 days, it is not discharged into the circulation. As an indirect way of detecting the absorption of proteins, the present investigations made use of proteinuria of alimentary origin (BAINTNER, 1970). Protein was detected in the urine of half the experimental animals, and in a number of others the reaction was at least doubtful; in addition, the trypsin-inhibitor content of the urine rose significantly above the control level. Both observations indicate that some portion of the ingested colostral protein must have reached the circulation. However, since the incubation times were too short for such investigations, and since only the low-molecular-weight proteins and protein fragments having not been resorbed in the renal proximal tubules appear in the urine, it will be necessary to conduct experiments of different design before any final conclusions can be drawn on this point.

As mentioned above, the presence of eosinophilic droplets in the enterocytes can be regarded as an indicator of protein absorption, and their vacuolization as an indicator of protein-absorbing ability. In the jejunum, however, where no "empty" vacuoles are found once the eosinophilic droplets have disappeared, it seems that vacuolization is not applicable as a criterion of protein-absorbing ability. Whatever is the case, the failure to evoke eosinophilic droplets indicate that the jejunum had already lost its competence to absorb proteins from the intestinal lumen in the more than 4-day-old kittens used in these experiments. With its greyish hue, it was, indeed, easily distinguished from the brownish ileum, *i.e.* these two functionally discrete segments were discriminable even to the unaided eye.

The present experiments suggest the possibility that the withdrawal of protein-absorbing competence towards the more distal intestinal segments is not a continuous process, but occurs stepwise. Thus it may be that the ability first vanishes from the jejunum as a whole (or from both duodenum and jejunum in certain species), and subsequently from the ileum.

The vacuoles observed in the kitten ileum contrast with those of the rat ileum in several respects. In the rat (a non-selective protein absorber),

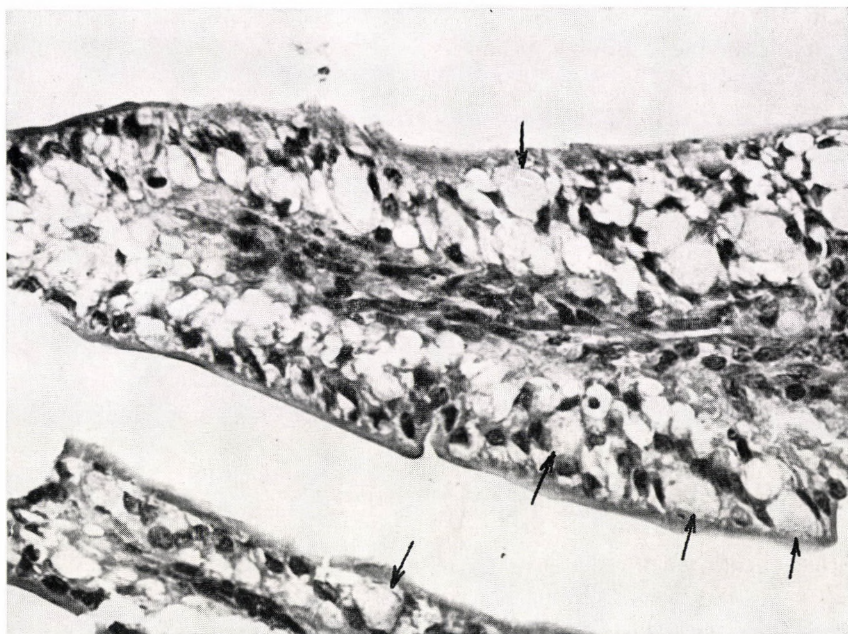

Fig. 6. Ileum of a 3-day-old control kitten. The "empty" enterocytic vacuoles lie predominantly in subnuclear locations. The arrows point to goblet cells. H—E stain

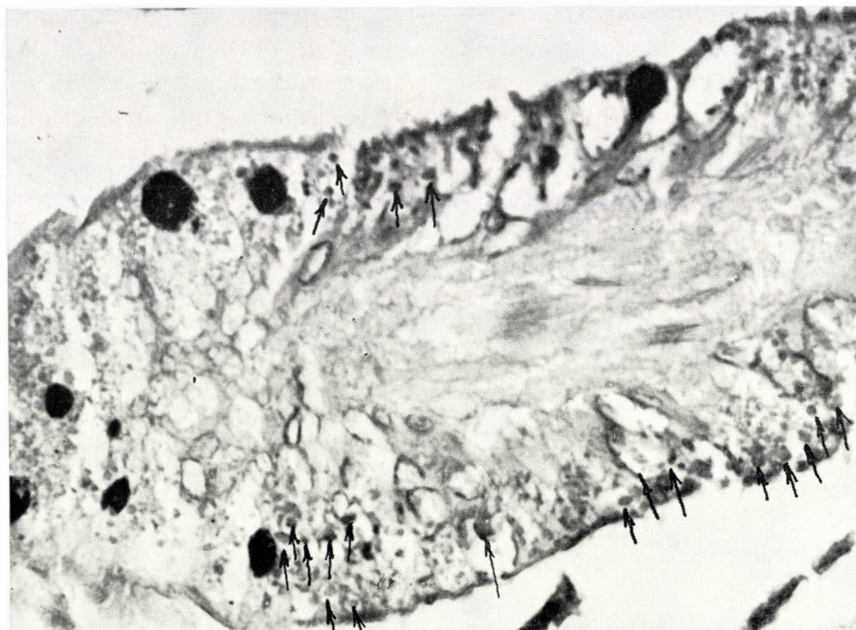

Fig. 7. Ileum of an 11-day-old control piglet. Note the numerous small, PAS-positive eosinophilic inclusion bodies (arrows) in the vacuoles. The intensely PAS-positive (dark) patches are goblet cells. (Periodic acid-Schiff reaction, alcian blue stain)

they are well-defined, always situated supranuclearly, and generally contain eosinophilic inclusion bodies, whereas in the kitten they are generally less distinct, can lie either above or below the nucleus, and contain no inclusion bodies(Figs 2 and 6). This points to their having an intracellular mobility similar to that apparently possessed by eosinophilic droplets. Since the ileum presumably cannot contain much protein that could be absorbed once protein digestion has started (BAINTNER, 1973), it may be that ileal enterocytes utilize their non-selective protein-uptake mechanism to transport digested materials as well. The possible physiological significance of the late disappearance of the protein-absorbing cell type is discussed elsewhere (BAINTNER, 1973).

II. Piglets

So far, attempts to restore eosinophilic droplets in piglets have proved unsuccessful. The reason for this may lie in the discrepancies of the conditions from those in the kitten experiments, particularly the small colostral doses and the early sacrifice. Compared with that in the kitten, the trypsin activity of the piglet's intestine is low (BAINTNER, 1973). The first colostrum of the foster-sow inhibited completely this small quantity of trypsin and even produced a massive surplus of CTI.

A similar difference was found between the jejunum and ileum of the control piglets as in the kittens. The vacuoles of the ileal enterocytes resembled those seen in the kitten in their variable intracellular location ("mobile vacuoles"), but on the other hand they contained eosinophilic inclusion bodies. There were usually much smaller than the inclusion bodies reported in the rat intestine (BAINTNER and VERESS, 1967) and showed up particularly well with PAS-alcian blue stain (Fig. 7). It is conceivable that in piglets part of the dietary protein reaches the intestine in undigested form and taken up by enterocytes, manifests itself in the form of inclusion bodies.

*

Acknowledgements: I would like to thank Dr Ferenc BRITTIG, Miss Mária KEREKES and Mrs. Emőke KÁDÁR for preparing the histological sections and making the photographs, Mrs. Said MAHMOUD for her help with the enzyme assays, and the management of the Komárom State Farm for putting the piglets at my disposal.

SUMMARY

An attempt was made to restore the characteristic histological picture of the neonatal kitten and piglet intestine in animals older than 4 days.

By administering a large dose of cow's colostrum to kittens 4 1/2—14 days of age, we succeeded in transforming the vacuoles of ileal enterocytes into the eosinophilic droplets

characteristic of active protein absorption. This provides further evidence that ileal enterocytes retain their ability to take up protein for some time after protein digestion has started. Direct proof that proteins taken up under such circumstances are also released into the circulation awaits further experimentation.

Eosinophilic droplets were not induced in the enterocytes of 4—4 1/2 days old piglets that had been removed from the dam to a newly-farrowed foster-sow, but there were demonstrable morphological signs of a residual protein absorption, in the form of minute eosinophilic inclusion bodies, in ileal enterocytes of both experimental and control animals.

In control animals of both species vacuolization appeared only in ileal enterocytes, but never in the jejunum. The variable location of the vacuoles in relation to the cell nucleus suggests that they have intracellular mobility.

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FREQUENCY AND CLINICOPATHOLOGICAL IMPORTANCE OF ORGANIC LESIONS IN CERTAIN TYPES OF INFECTIOUS BOVINE MASTITIS

By

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Pathological examinations of infectious bovine mastitis are focused almost exclusively on the udder. Few case reports have dealt with the changes caused by the agent(s) of mastitis in other organs. Chiefly the supramammary lymph nodes were studied, owing to their close anatomical and functional relationship with the udder (HAMMER and ENGLERT, 1958; POUNDEN et al., 1952; RENK, 1956, 1957, 1961), but lesions apparently resulting from septikaemia or dissemination of the mastitis agent were also observed in the spleen, liver, kidneys, lungs, adrenals, serous membranes, *etc.*

Similar changes observed in certain organs of cows with infectious mastitis stimulated systematic investigations into the nature and frequency of organic lesions caused by mastitis agents and into the diagnostic value of post-mortem findings in such cases. The results of gross, microscopic and bacteriological examinations are summarized in this report.

Materials and Methods

One hundred consecutive cows, sent to the abattoir or to the emergency slaughterhouse of the town Pécs for acute or chronic mastitis were examined during the period 1971—1972. Thus the number of cases in which one or another type of change was encountered, reflects the percentage occurrence. The lesions observed in the different organs were tabulated according to the causal agent.

Of the 100 cows 89 had been emergency-slaughtered for clinical mastitis, the remaining 11 cases were detected at the routine meat-hygienic inspection in the abattoir.

In every case, detailed post-mortem examinations were carried out on all organs and on both halves of the body. Specimens for microscopic examination were taken from the udder, supramammary lymph nodes, spleen, liver, kidneys and changed parts of organs, fixed in 10% formalin, embedded in paraffin or frozen, and the sections were stained with haematoxylin and eosin or. if required, with other dyes as well.

Complementary bacteriological examinations were also performed in each case, with the methods described earlier (SZÁZADOS and KÁDAS, 1972). Apart from the routinely examined organs (spleen, liver, kidney, two muscle specimens, praescapular, popliteal and supramammary lymph nodes as well as the udder), isolation was attempted from all parts which appeared affected (internal iliac lymph nodes, lungs, joints, *etc.).*

Results

Bacteriological examinations

Pseudomonas aeruginosa was isolated from the udder in 33 cases, *Klebsiella pneumoniae* in 25 cases, *Corynebacterium pyogenes* in 14 cases, *Escherichia coli* in 10 cases and *Staphylococcus aureus* in 9 cases. Mixed infection with *Staph. aureus* and *C. pyogenes* occurred in two cases and with *Staph. aureus* and *E. coli* in one case. Five animals had mixed saprophytic udder flora and in one case the udder was bacteriologically sterile.

As to the type of mastitis, the 33 cases caused by *Ps. aeruginosa* were all chronic and proved to be histologically a process of actinomycosis character. Of the 25 cases due to *K. pneumoniae,* 15 were acute and 10 chronic, with necroses. *C. pyogenes* caused in two cases mastitis acuta gravis and in 12 cases chronic purulent mastitis with abscess formation. Of the cases due to *E. coli,* seven were acute grave mastitis, two semi-acute and necrotic and one was chronic. *Staph. aureus* caused acute grave mastitis in one case, chronic purulent mastitis in six cases and actinomycosis-like process in two cases. Among the three mixed infections, the single bacteriologically sterile case and the five cases in which there was a mixed saprophytic udder flora, chronic mastitis was found in 4 cases and a semi-acute process in one case.

In 52 cases, the causal agent was isolated only from the udder and supramammary lymph nodes, in 47 cases also from some of the other organs. One case was bacteriologically sterile.

The dissemination of the pathogenic bacteria appeared to be unrelated to the acute or chronic nature of mastitis. In part of the cases, dissemination occurred in both acute grave and chronic forms, whereas in the other part all organs except the udder and supramammary lymph nodes were bacteriologically sterile, regardless of the nature of the process.

Gross lesions

Lymph nodes

Next to the udder, lesions were most frequently found in the supramammary lymph nodes, which were involved in 72 cases. The most frequent

Table I

Gross lesions according to organ and type of change	Frequency of gross lesions according to causal agent								Number of lesions in different organs
	Ps. aeruginosa	*K. pneumoniae*	*C. pyogenes*	*E. coli*	*Staph. aureus*	Mixed saprophytic microflora	Bacteriologically sterile	Mixed infection	
Swelling of spleen	13	10	6	5	6	2	—	1	43
Infarctions in spleen	2	6	—	—	—	—	—	—	8
Swelling of supramammary lymph nodes	20	8	6	4	6	3	—	3	50
Purulent inflammation of supramammal lymph nodes	1	4	5	1	1	—	—	—	12
Necrosis in supramammary lymph nodes	—	6	—	1	—	—	—	—	7
Actinomycosis-type metastases in supramammary lymph nodes	3	—	—	—	—	—	—	—	3
Swelling of regional lymph nodes	4	3	2	—	2	—	—	1	12
Necrosis in regional lymph nodes	—	4	—	—	1	—	—	—	5
Fatty degeneration in liver	3	3	4	2	2	1	—	1	16
Necrotic-inflammatory foci in liver	6	12	—	—	—	—	—	2	20
Fatty degeneration + necrotic-inflammatory foci in liver	1	2	—	1	—	—	—	—	4
Fresh haemorrhagic infarction in liver	1	—	—	—	—	—	—	—	1
Icterus	2	1	1	—	—	—	—	—	4
Swelling of gall-bladder	3	4	—	—	1	—	—	—	8
Cholecystitis	—	1	—	1	—	—	—	—	2
Fatty degeneration in kidneys	1	—	—	—	1	—	—	—	2
Purulent nephritis due to metastasis	—	1	—	1	—	—	—	—	2
Infarctions in kidneys	—	5	—	1	2	—	—	—	8
Focal interstitial nephritis	16	—	—	—	—	—	—	1	17
Lung processes due to metastasis	1	1	1	—	1	—	—	—	4
Arthritis	8	3	4	1	—	1	—	1	18
Distribution of 100 cases of mastitis according to the causal agent	33	25	14	10	9	5	1	3	

change was a simple acute inflammation, occurring in 50 cases out of 72. The swollen, enlarged lymph nodes showed microscopically follicular hyperplasia and sinus catarrh.

Purulent inflammation of the lymph nodes was relatively rare (12 cases), and grossly inapparent; it was usually detected on microscopic examination. Although a purulent lymphodenitis occurred with all agents studied, *C. pyogenes* (5 cases out of 14) and *K. pneumoniae* (4 of 25 cases) were most frequently responsible for it.

Fresh or chronic septic infarction of the supramammal lymph nodes (Fig. 1) was found in seven cases, of which six were due to *K. pneumoniae* and one was due to *E. coli*.

Microscopic metastases to the supramammal lymph nodes were observed in three cases of actinomycosis-like *Ps. aeruginosa* mastitis, whereas no metastases occurred in two similar processes caused by *Staph. aureus*.

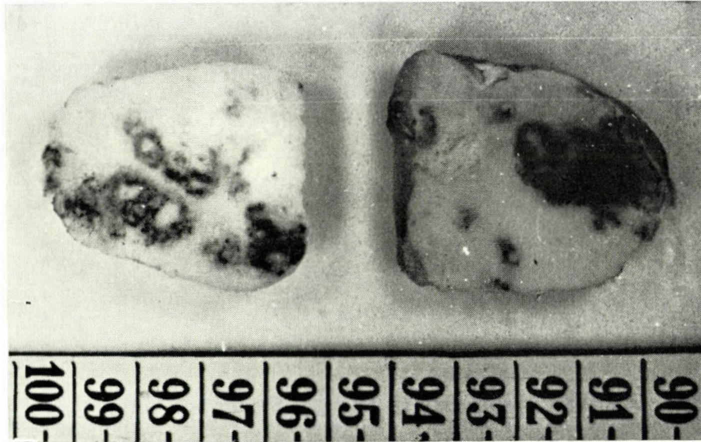

Fig. 1. Fresh infarctions in a supramammal lymph node (*Klebsiella*-mastitis)

The internal iliac lymph nodes and less often also other lymph nodes (praescapular, popliteal) ipsilateral to the affected (*s*) udder were swollen and exhibited reddening in 12 cases.

Infarctions of the regional lymph nodes were observed in *K. pneumonias*-mastitis and in one case of *Staph. aureus* mastitis (in three cases the internal iliac and in one case each the popliteal and praescapular lymph nodes were involved).

Spleen

Splenic lesions occurred in 51 cases; the most frequent change was a hyperaemic or hyperplastic swelling (Fig. 2) which was usually associated with processes due to *Staph. aureus*, *C. pyogenes* or *K. pneumoniae*.

Infarctions of the spleen occurred in 8 cases. Formerly these were observed exclusively in *K. pneumoniae* mastitis (Fig. 2), but in the present study two of the eight cases were associated with an actinomycosis-like chronic process due to *Ps. aeruginosa*.

Liver

Forty-one cows, a surprisingly high percentage of those diseased in mastitis had liver lesions: 16 showed fatty degeneration, 20 necrotic-inflammatory foci, 4 both kinds of change and one had a fresh, haemorrhagic infarction.

Fatty degeneration usually occurred in processes due to *C. pyogenes* (4 cases), *Staph. aureus* (2 cases) or *E. coli* (2 cases), but it was also observed

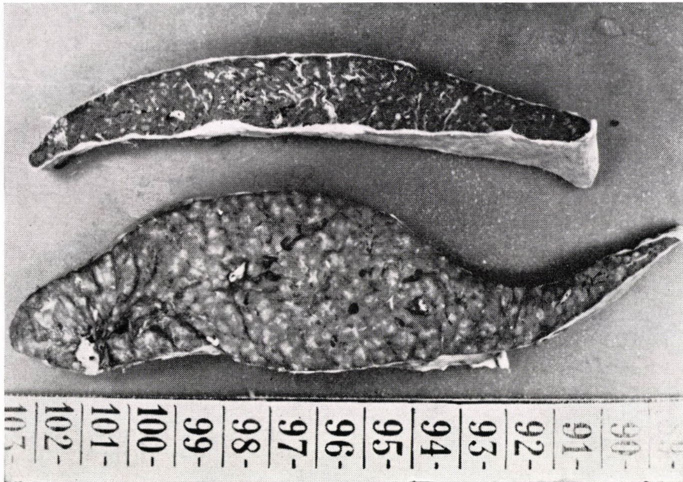

Fig. 2. Hyperplasial swelling of the spleen in *Klebsiella*-mastitis. At top; Control section from healthy animal

with *K. pneumoniae* (3 cases) and *Ps. aeruginosa* mastitis. Microscopically, the fatty degeneration was chiefly centrolobular. On the basis of case history bacteriological examination and post-mortem findings, the liver lesions were regarded as being aetiologically related to mastitis.

The necrotic-inflammatory foci were associated with *K. pneumoniae* and *Ps. aeruginosa* mastitis in all except two cases (one mixed infection with

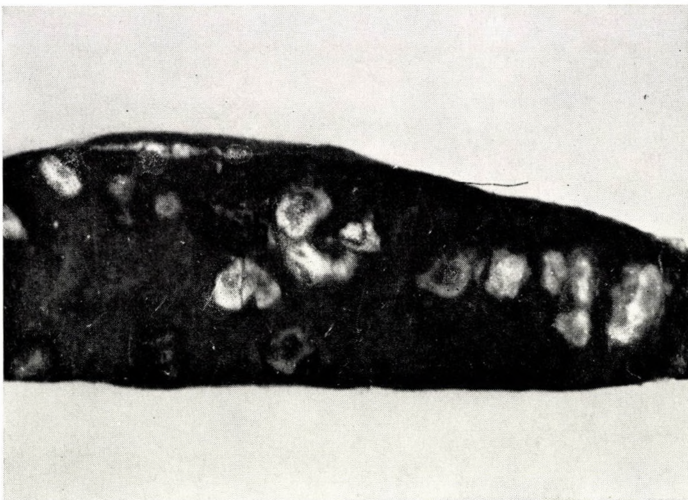

Fig. 3. Many infarctions in the spleen, part of them in the process of organization (*Klebsiella*-mastitis)

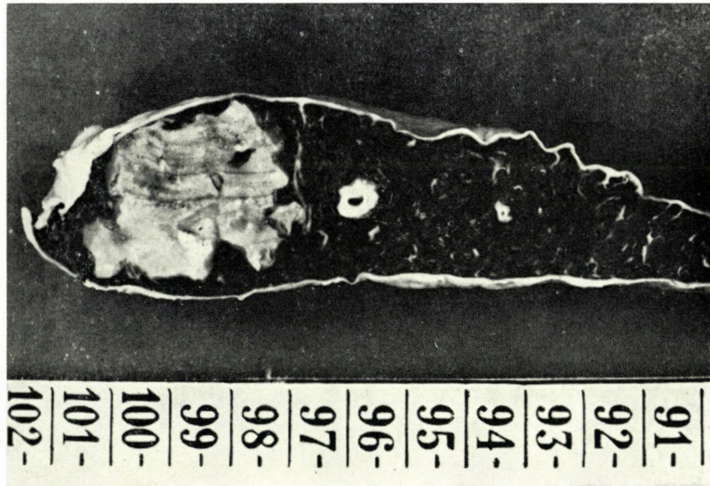

Fig. 4. Large anaemic infarction in the spleen of a cow diseased in chronic actinomycosis-like mastitis due to *Ps. aeruginosa*

E. coli + Staph. aureus and another with *Staph. aureus + C. pyogenes)* and were twice as frequent with *Klebsiella* mastitis.

The necrotic-inflammatory liver lesions caused by *K. pneumoniae* and *Ps. aeruginosa* differed slightly from one another in gross appearance. The foci occurring in *K. pneumoniae* mastitis ranged in size from that of a pin-head to a millet seed or even rice grain, and were numerous (Fig. 5), whereas those caused by *Ps. aeruginosa* were often hardly visible to the naked eye and usually fewer in number (Fig. 6).

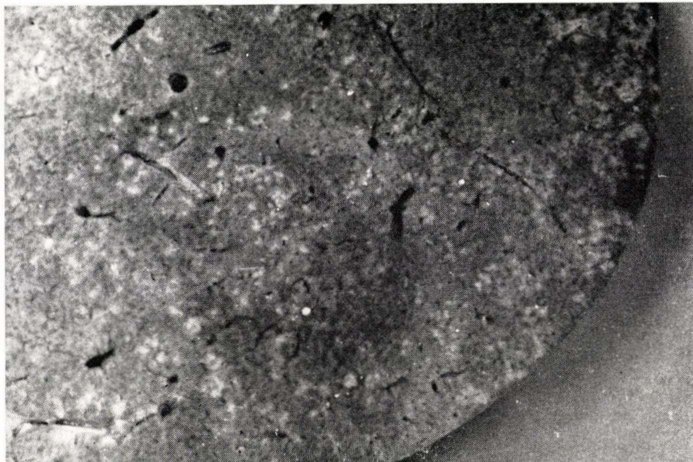

Fig. 5. Many inflammatory-necrotic foci and fatty degeneration in the liver (*Klebsiella*-mastitis)

Fig. 6. Inflammatory-necrotic foci occurring sporadically and in small groups in the liver of a cow diseased in chronic actinomycosis-like mastitis due to *Ps. aeruginosa*

In one case of actinomycosis-like, chronic *Ps. aeruginosa* mastitis, apart from an inflammatory cellular reaction in the periportal spaces, a fresh haemorrhagic infarction of the size of a walnut was found.

In eight cases, a double to treble enlargement and mural thickening of the gallbladder was observed along with the liver change. The contents of the involved cholecyst were thicker and darker than normally. Microscopically in six cases oedema and a very low degree of perivascular inflammation were found; in two cases there was a severe, acute cholecystitis.

In three cases the fatty degeneration and in one case the fatty degeneration + necrotic-inflammatory changes were accompanied by icterus (in two cases of *Ps. aeruginosa* and in one case each of *K. pneumoniae* and *C. pyogenes* mastitis).

Kidneys

Renal lesions are frequently associated to infectious bovine mastitis.

Although the kidneys of the diseased cows are often lighter in colour than normally, or even yellowish, a fatty degeneration of the kidney could be confirmed microscopically in only two cases. (In one case of *Staph. aureus* mastitis, fatty deposition was found in the tubular epithelium, in the other case, due to *Ps. aeruginosa*, the fatty degeneration was associated with a focal interstitial nephritis). In both cases, the agent was isolated, apart from the udder, also from the kidney, indicating a relationship of the fatty renal degeneration with mastitis.

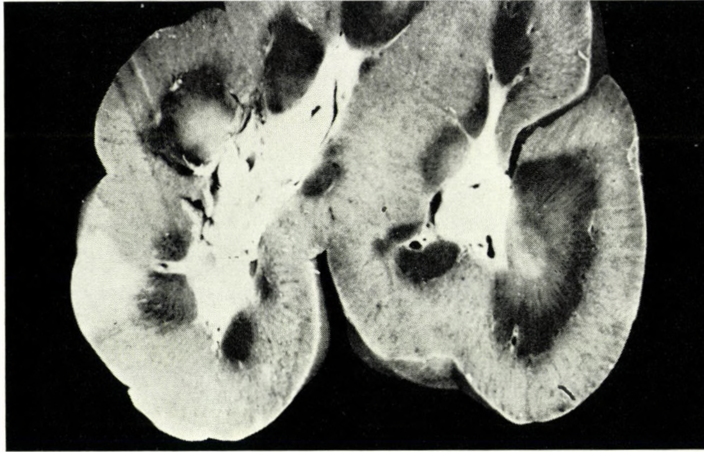

Fig. 7. Anaemic infarction in the kidney (*Klebsiella*-mastitis)

Metastatic purulent nephritis associated with microscopic abscess formation was rare, occurring only in one case each of *E. coli* and *K. pneumonias* mastitis.

Anaemic infarctions of the kidney were more frequent (8 cases); these were associated in five cases with *K. pneumoniae* mastitis (Fig. 7) and in one case each with *E. coli* and *Staph. aureus* mastitis. In the latter case, the necrotic change involved three complete renal lobules (Fig. 8).

Ps. aeruginosa mastitis was very often accompanied by focal interstitial nephritis (16 out of 33 cases). Latter occurred in only one case when

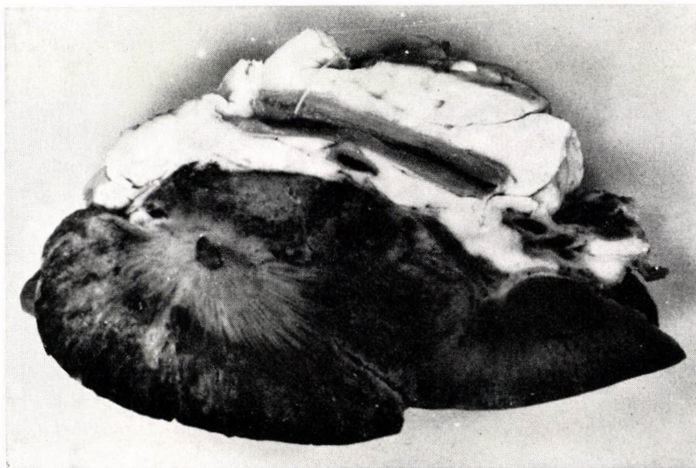

Fig. 8. Complete necrotized renal lobule in *Staph. aureus* mastitis

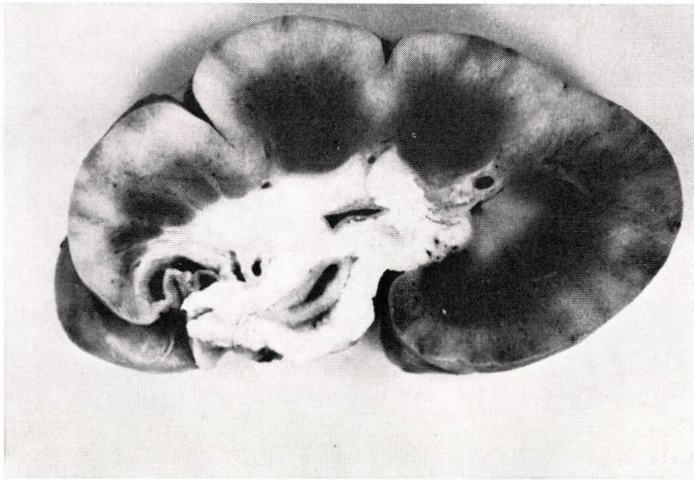

Fig. 9. Focal interstitial nephritis (actinomycosis-like chronic mastitis due to *Ps. aeruginosa)*

the pathogenic agent was different. In some cases the cortical lesions were hardly visible to the naked eye, in other cases they were severe and conspicuous (Fig. 9).

Lungs

The lungs were rarely involved, mainly in those cows whose general condition was very poor. A severe interstitial emphysema was always present with the lesions described below.

In one case each of *K. pneumoniae* and *C. pyogenes* mastitis a few sporadic, partly organizing septic infarctions were seen in the pulmonary parenchyma. One cow, diseased in *Staph. aureus* mastitis associated with septikaemia, had small metastatic abscesses in the bronchopneumonial lung areas, from which the causative agent of the udder process was isolated. Another cow, diseased in an actinomycosis-like, chronic *Ps. aeruginosa* mastitis, had a few metastatic abscesses of the actinomycosis type in the pulmonary lobes.

Joints

Arthritis occurred in 18 cases. Usually two or more joints become involved, chiefly the bilateral carpal, metacarpal and knee joints, less often the hip joint; involvement of a single joint was rare. A purulent arthritis was also rare: it was associated with one case each of *C. pyogenes* and *K. pneumoniae* mastitis.

The lesion was generally characterized by a serous-jellinaceus deposition in the capsule and periarthritic tissue, and accumulation of a medium quantity of serum in the articular cavity. Thinning of the cartilage was nearly always observed, along with usuration in an area of the size of a small coin. A fibrinous inflammation of the tendinaceous sheaths and synovial bursa was frequent. In a single case of *Ps. aeruginosa* mastitis, the causal agent was isolated from the synovial fluid.

Other organs

Other organs rarely showed lesions which could be regarded aetiologically related to mastitis. In one case of *E. coli* mastitis there was an extensive Zenker-type waxy degeneration of certain muscles, and in one case of *Staph. aureus* mastitis many muscular haemorrhages were found all over the body. A circumscribed fibrinous pleuritis was present in one case each of *E. coli* and *Ps. aeruginosa* mastitis and deficient bleeding was observed in a few other cases.

Changes unrelated to mastitis (perforation of the abomasum, metritis, etc.) were of course found at the post-mortem examination of several cows. These lesions were taken into consideration at evaluation.

Discussion

It follows from the present findings that in acute or chronic bovine mastitis caused by *Ps. aeruginosa*, *K. pneumoniae*, *E. coli*, *C. pyogenes*, *Staph. aureus* or their combinations, organs other than the udder (supramammary and other lymph nodes, spleen, liver, gallbladder, kidneys, lungs and joints) often become involved by the causal agent of the udder disease.

At the post-mortem examination of 100 cows slaughtered for mastitis, only 9 had no other but udder lesions.

Sixteen cows showed involvement of the supramammary lymph nodes and spleen, in the form of a slight or marked swelling.

Involvement of one or more organs apart from the udder was observed in 75 cases and a great variety of gross lesions was found. These were clearly septikaemic lesions due to the agent responsible for the mastitis. The spread of the causal agent *via* the blood stream was found to occur frequently in bovine mastitis; it had been demonstrated by one of us (SZÁZADOS, 1972) in 80, 61.1, 64.5, 59.4 and 50% of the cases due to *E. coli*, *Ps. aeruginosa*, *C. pyogenes*, *K. pneumoniae* and *Staph. aureus*, respectively. Roughly the same percentage occurrence was found in the present study.

The occurrence of organic lesions in association with bovine mastitis should always be considered for both clinical and post-mortem diagnosis.

The clinical consequences of organic lesions should briefly be discussed here. HEIDRICH and RENK (1963) as well as DIERNHOFER (1950) remarked that, apart from the regional changes of the udder and supramammary lymph nodes, acute grave bovine mastitis (acute parenchymatous mastitis) is characterized by a severe general condition. According to our own experience, the general symptoms are especially severe in the advenced stage of mastitis, when emergency slaughter becomes necessary. DIERNHOFER described the main symptoms as shivering, enteritis, functional disorders of the forestomachs, arthritis and nephritis; most of these can easily be explained by the organic lesions described in this report. The emergency-slaughter certificates presented with the cows examined by us often included data indicating pain in the forestomach region (perforation of abomasum) or diffuse abdominal pain (enteritis, metritis, nephritis) as the main trouble, and little mention, if any, was made of mastitis. Only post-mortem examination revealed that the case history was erroneous, mastitis being the basic disorder.

Consideration of the organic lesions is also useful for the post-mortem diagnosis; certain agents cause typical gross lesions which permit aetiological conclusions before the bacteriological examination is concluded.

The post-mortem findings are most characteristic with the *K. pneumoniae* mastitis (FORRAY et al., 1969). Apart from the grossly visible anaemic infarctions of the udder, such changes may be present in the supramammary and other lymph nodes, as well as in the spleen, kidneys and lungs. Although necrotic changes of the supramammary lymph nodes may sometimes occur in *E. coli* mastitis — a single case was encountered in this study — the simultaneous appearance of infarctions in several organs is of a diagnostic value for *K. pneumoniae* mastitis. Numerous, grossly easily visible necrotic-inflammatory foci in the liver are also indicative of the latter condition. *E. coli* mastitis, which can easily be misdiagnosed for *K. pneumoniae* mastitis if only the udder is examined, scarcely affects other organs. Swelling of the supramammary lymph nodes and spleen and less often a liver degeneration may occasionally be present.

The actinomycosis-like chronic mastitis due to *Ps. aeruginosa* is characterized by a focal interstitial nephritis, which seems to be exceptional in mastitides of other aetiology, and never was observed in mastitides of actinomycosis character caused by *Staph. aureus*. Other frequent changes occurring in association with the chronic *Ps. aeruginosa* mastitis are sporadic necrotic-inflammatory foci in the liver, which are as a rule hardly visible to the naked eye, and articular lesions (usuration of cartilage, swelling of capsule, *etc.)*.

The available observations suggest that the extramammary lesions are not conclusive of the aetiology of mastitis caused by *C. pyogenes* and *Staph. aureus*. Although purulent processes of the supramammary lymph nodes, kidneys and lungs were relatively often encountered in mastitides caused

by these agents, examinations on a larger group of animals are required to establish their diagnostic value.

*

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SUMMARY

One hundred cows with mastitis were examined after slaughter for gross and microscopic lesions as well as bacteriologically, with special regard to extramammary lesions. In both acute and chronic forms of mastitis caused by *Ps. aeruginosa*, *K. pneumoniae*, *E. coli*, *C. pyogenes*, *Staph. aureus* or their combinations, organic lesions indicative of the haematogenic spread of the causal agent (s) of mastitis were often encountered.

The percentage occurrences of the different kinds of lesions were as follows: swelling of the spleen, 43%; splenic infarctions, 8%; swelling of the supramammary lymph nodes, 50%; purulent inflammation of the supramammary lymph nodes, 12%; necrotic lesions of the supramammary lymph nodes, 7%; actinomycosis-type metastases in the supramammary lymph nodes, 3%; swelling of the regional lymph nodes, 12%; necrotic change of the regional lymph nodes, 5%; fatty degeneration of the liver, 16%; necrobiotic foci in the liver, 20%; fatty degeneration plus necrobiotic foci in the liver, 4%; cholecystitis, 2%; fresh haemorrhagic infarction of the liver, 1%; infarctions of the kidneys, 8%; purulent metastatic nephritis, 2%; fatty degeneration of the kidneys, 2%; focal interstitial nephritis, 17%; metastatic pulmonary processes, 4%; arthritis, 18%.

The pathological pictures in association with infectious mastitis, especially with those caused by *K. pneumoniae* and *Ps. aeruginosa*, are so characteristic that aetiological conclusions can be drawn prior to the completion of bacteriological examinations.

In mastitis due to *K. pneumoniae*, anaemic infarctions may be present apart from the udder in the supramammary and other lymph nodes and in the spleen, kidneys and lungs. Necrotic-inflammatory foci, ranging in size from pin-head to millet seed are as a rule simultaneously present in the liver.

In the actinomycosis-like chronic mastitis caused by *Ps. aeruginosa*, a focal interstitial nephritis would often occur in addition to actinomycosis-type foci in the udder. In this type of mastitis, the necrotic-inflammatory focal lesions of the liver are grossly scarcely visible, but the joints frequently become involved.

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A FURTHER TYPE OF CATTLE SERUM TRANSFERRIN

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Cattle serum transferrin has been shown to exist in several different genetically determined forms in various breeds of domesticated cattle *(Bos taurus* and *Bos indicus).* Apart from some abnormal cases (SARTORE and BERNOCO, 1966; SPOONER and BAXTER, 1969) so far twelve normal serum transferrin alleles — *i.e.* each exhibiting a set of four, five or six distinct protein bands depending upon the method applied — have been demonstrated by starch gel electrophoresis. These are in the order of decreasing electrophoretic mobility, using the nomenclature of JAMIESON (1966) in detail, as follows: Tf^{H}; $Tf^{A}1$; $Tf^{Pyhäjoki}$; $Tf^{A}2$; Tf^{B}; $Tf^{D}1$; $Tf^{D}2$; Tf^{F}; Tf^{N}; Tf^{E}; Tf^{G} and Tf^{X} (HICKMAN and SMITHIES, 1957; ASHTON, 1958, 1959; GAHNE, 1961; OSTERHOFF and VAN HEERDEN, 1964; ASHTON and LAMPKIN, 1965; KRISTJANSSON and HICKMAN, 1965; VASENIUS, 1965; SARTORE and BERNOCO, 1966; BRAEND and KHANNA, 1967; ABE et al., 1968). However, three of them, namely the first three, have not yet been compared directly. In his recent paper VASENIUS (1971) presents evidence for the new $Tf^{Pyhäjoki}$ variant on the basis of family material proving that the new variant was produced by mutation. The alleles $Tf^{A}2$; $Tf^{D}1$; $Tf^{D}2$ and Tf^{E} appear to be common to all breeds, but the others are present only in certain breeds and their gene frequencies are very low. Moreover, the gene frequencies of the above-mentioned four relatively frequent alleles show also wide differences among cattle breeds.

In this paper the occurrence of a new transferrin variant is reported, which was first found in two Hungarian Spotted (Fleckvieh) individuals during our studies on serum transferrins of different Hungarian cattle breeds. These two animals showed a set of slow-moving transferrin bands in heterozygous combination with the bands of allele $Tf^{D}2$. The new variant shows slower electrophoretic mobility than the bands of allele Tf^{E} (Fig. 1). The stability of these unusual patterns was confirmed by repeated blood collections from the same animals. In accordance with the suggestions of the FAO Nomenclature Committee (ASHTON et al., 1967) the new variant is called tentatively $Tf^{I}_{Hungary}$.

In order to disclose a possible identity with Tf^{X} (ABE et al., 1968), a comparison with a serum sample received from Dr. ABE, containing the allele Tf^{X}, was carried out. Starch gel electrophoresis was used following the technique of MAKARECHIAN and HOWELL (1966) with slight modifications. On the basis of this comparison the identity has been excluded (Fig. 1). Tf^{X} shows much slower electrophoretic mobility than the new variant. As regards the Tf^{G}, reported by OSTERHOFF and VAN HEERDEN (1964), we were unable to make any comparison, because reference samples containing this variant were not available (OSTERHOFF, 1971, personal communication).

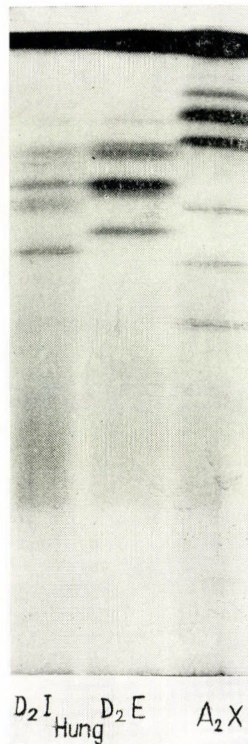

Fig. 1. Photograph of a gel demonstrating the transferrin phenotypes $D_2I_{Hungary}$, D_2E and A_2X

Further family studies were undertaken to find other individuals possessing this rare allele. Blood samples were collected from all the herds in which the occurrence of the new $Tf^{I}_{Hungary}$ was suspected. So the existence of this variant was proved in seven cases: one $Tf^{A}2/Tf^{I}_{Hungary}$ and six $Tf^{D}2/Tf^{I}_{Hungary}$ phenotypes were found. Such a distribution of phenotypes, however, is well explainable considering the gene frequency values in the breed (Soos et al., in preparation).

Further family investigations will be made to establish the manner of inheritance of the $Tf^{I}_{Hungary}$ variant.

SUMMARY

A new type of cattle serum transferrin, tentatively termed $Tf^{I}_{Hungary}$, was found in the Hungarian Spotted (Fleckvieh) breed. It has a slower electrophoretic migration rate than Tf^{E} in starch gel electrophoresis. Our family studies suggest that the new type is inherited as a co-dominant trait.

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STUDY OF *ACHOLEPLASMA* STRAINS ISOLATED FROM SWINE

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In the last years a great number of strains of *Mycoplasmatales* have been isolated from various organs of swine (MARTIN et al., 1968; DZU and SCHIMMEL, 1971; SCHIMMEL and PUSTOVAR, 1971). Most of the strains grew fast even on serum-free medium, and splitted glucose. In tube agglutination test, slide agglutination test read by phase-contrast microscope (SCHIMMEL and HUBRIG, 1968; MARTIN et al., 1968; SCHIMMEL and DZU, 1969) as well as in agar-gel precipitation test strains different in antigen structure have been distinguished.

Results of this investigation prompted us to examine one strain of each separated group biochemically and serologically comparing with internationally accepted reference strains.

Materials and Methods

Strains

The strains $D_2/1634$, $D_6/1010$, $D_7/10$ P, $D_8/2775$, $D_9/1816$, $D_{10}/1270$, $D_{11}/3097$ and $D_{12}/252$ were obtained from the collection of the Institut für bakterielle Tierseuchenforschung der Deutschen Akademie der Landwirtschaftwissenschaften zu Berlin, Jena-Zwätzen, DDR, the strains *A. laidlawii* A PG 8, *A. laidlawii* B F-8, *A. granularum* (Friend), *A. axanthum* (ATCC 25176) and *A. sp.* PG 49 (Squire) reference strains used for comparison from the FAO/WHO International Reference Centre for Animal Mycoplasmas (Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark).

The strains were in dried state in ampoules which were opened under separated conditions, they were inoculated into BE fluid medium, and cultivated for 3 days. One part of the cultures was dried again for storing, another part was filtered through Millipore (GSWP 02500, 25 ea GS 0.22 μm) filter, and the filtrates were inoculated on to BE agar plates. After 3 days of cultivation 2 colonies were picked up from each strain for further subcultivation and transferred into separated tubes containing BE fluid medium. Subcultures were filtered after 3 days and the whole procedure was repeated. All strains were cloned 3 times and the subcultures thus obtained were used for investigation.

Media

For examination of cultural properties of the strains the BE medium used in the WHO Centre was used. Biochemical, resistance and serological tests were performed in various modifications of BE medium.

Biochemical and resistance tests

The following properties of strains were examined: glucose- (under aerobic and anaerobic conditions), mannose-, D-galactose-, sucrose-, mannitol-, D-sorbit-, D-cellobiose- and xylose-decomposing, arbutin and aesculin hydrolysation, 2,3,5-triphenyltetrazoliumchloride-, potassium tellurite-, and methylene blue-reduction (under aerobic and anaerobic conditions), cholesterol requirement, serum digestion, phosphatase production, "film and spot" formation, sensitivity to sodium chloride, methylene blue, Tween 80, sodium azide, sodium taurocholate, sodium polyanetholsulphonate (KUNZE, 1971), digitonin, pH : 5.5 and pH: 9.5, Erythromycin, Kanamycin and Polymyxin B.

Serological tests

The strains were examined in growth-inhibition (CLYDE, 1964) test on various agar media and at different temperatures, in indirect haemagglutination test (KROGSGAARD, 1972), and in immunofluorescence test (ROSENDAL and BLACK, 1971).

Results

All strains grew well in BE medium, all except for D_2/1634 grew both under aerobic and anaerobic conditions. The D_2/1634 strain produced visible "fried egg" colonies after 4—5 days, while others like the *A. laidlawii* reference strains, after 1—2 days. Examining the properties characteristic of acholeplasmas, it was found that all strains required cholesterol, grew on serumfree medium and at temperature 22 °C, they were resistant to digitonin and to sodium polyanethol sulphonate.

All strains decomposed glucose under aerobic and anaerobic conditions, but did not split mannose, sucrose, D-galactose, D-sorbit, mannitol and xylose, did not hydrolyse arbutin and did not form "film and spot", did not produce phosphatase, did not digest serum and did not reduce potassium tellurite. They reduced 2,3,5-triphenyltetrazoliumchloride under both aerobic and anaerobic conditions, and reduced methylene blue under anaerobic conditions.

Table I

Results of biochemical assays of *Acholeplasma* strains isolated from swine

Strains	Biochemical tests				
	Aesculin	Arbutin	Galactose	Cellobiose	Xylose
D_6/1010	+	—	—	+	—
D_{10}/1270	+	—	—	+	—
D_7/10 P	—	—	—	+	—
D_8/2775	—	—	—	+	—
D_9/1816	—	—	—	—	—
D_{11}/3097	—	—	—	—	—
D_{12}/252	—	—	—	—	—
D_2/1634	—	—	—	—	—

Different results were obtained in splitting of cellobiose and in aesculin-hydrolysing tests (Table I). Aesculin was hydrolysed by strain D_6/1010 and D_{10}/1270, cellobiose by D_6/1010, D_{10}/1270, D_8/2775 and by D_7/10 P. The other strains were negative.

All strains were sensitive to sodium taurocholate and to methylene blue and resistant to sodium azide. The strains showed different properties in other sensitivity tests too (Table II). The D_2/1634 and D_8/2775 strains were sensitive to sodium chloride, the D_2/1634 strain was sensitive to Tween 80, pH: 5.5 and 9.5, while D_6/1010 and D_8/2775 failed to grow at pH: 5.5, other strains grew only in the first passage and were not transferable on to agar

Table II

Behaviour of *Acholeplasma* strains isolated from swine in resistance tests

Strains	Tests			
	Sodium chloride	Tween 80	pH: 5.5	pH: 9.5
D_6/1010	R	R	S	R
D_{10}/1270	R	R	R(1)	R
D_7/10 P	R	R	R(1)	R
D_8/2775	S	R	S	R
D_9/1816	R	R	R(1)	R
D_{11}/3097	R	R	R(1)	R
D_{12}/252	R	R	R(1)	R
D_2/1634	S	S	S	S

Symbols used: S, sensitive; R, resistant

Table III

Serological tests of *Acholeplasma*

Strains	Reference sera: *A. axanthum* ATCC 25176			*A. laidlawii* A PG 8		
	test: GI	IF	IHA	GI	IF	IHA
$D_6/1010$	—	—	—	4.0	80	—
$D_{10}/1270$	—	—	—	P 1.5	80	4096
$D_7/10$ P	—	—	—	P 2.5	80	80
$D_8/2775$	—	—	—	P 1.5	10	2048
$D_9/1816$	—	—	—	—	10	1024
$D_{11}/3097$	—	—	—	—	10	4096
$D_{12}/252$	—	—	—	—	20	1024
$D_2/1634$	—	—	—	—	—	—
Homologous antigen	3.0	80	1280	P 3.0	80	4096

Symbols used: GI, growth inhibition test; IF, immunofluorescence test; IHA, in-

medium of pH: 5.5. Behaviour of the strains against antibiotics was alike, namely they were sensitive to Erythromycin, their sensitivity to Kanamycin was weak and they were resistant to Polymyxin B.

Serological investigation

The strains reacted mostly with the *A. laidlawii* reference sera. In the growth inhibition test the *A. laidlawii* A PG 8 reference serum gave positive reaction with strains $D_6/1010$, $D_{10}/1270$, $D_7/10$ P and $D_8/2775$, but not with the aesculin and cellobiose-negative strains $D_9/1816$, $D_{11}/3097$ and $D_{12}/252$. In contrast to these results, the *A. laidlawii* B F-8 reference serum inhibited all the above-mentioned strains. Other *Acholeplasma* sera did not inhibit the growth of the strains.

In the immunofluorescence test the *A. laidlawii* A PG 8 reference serum reacted up to its end titre with strains $D_6/1010$, $D_{10}/1270$ and $D_7/10$ P but only in a very low (1 : 10) dilution with $D_8/2775$ and with the aesculin- and cellobiose-negative strains. At the same time the *A. laidlawii* B F-8 serum was bound to colonies of all strains except for $D_8/2775$ up to its end titre. There was no reaction with other sera used.

In the indirect haemagglutination test the *A. laidlawii* A PG 8 and *A. laidlawii* B F-8 sera agglutinated most of the strains approximately up to their end titres. The *A. granularum* (Friend) serum reacted only in a very

strains isolated from swine

Reference sera								
A. laidlawii B F-8			*A. granularum* (Friend)			*A. sp.* PG 49 (Squire)		
GI	IF	IHA	GI	IF	IHA	GI	IF	IHA
5.0	640	320	—	—	—	—	—	—
P 1.5	640	640	—	—	160	—	—	—
P 2.5	640	4096	—	—	—	—	—	—
P 3.0	10	4096	—	—	40	—	—	—
P 2.0	640	1024	—	—	20	—	—	—
P 1.0	640	4096	—	—	—	—	—	—
P 1.5	640	4096	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—
4.5	640	8192	3.0	20	4096	4.0	80	1280

direct haemagglutination test

low dilution with D_{10}/1270, D_8/2775 and D_9/1816; the *A. axanthum* (ATCC 25176) and *A. sp.* PG 49 (Squire) sera were negative.

There was no positive serological reaction between the D_2/1634 strain and *Acholeplasma* sera.

Conclusion

The first species of sterol-nonrequiring *Acholeplasma*, called *A. laidlawii* (Edward and Freundt, 1956), was isolated by Laidlaw and Elford (1936). Subsequently the belonging to this species were generally regarded as saprophytes. Since, however, such were very often isolated from pathological materials of human, bovine and swine origin, a possible pathogenic role of the species *A. laidlawii* was raised. Up to now however there have been no data concerning differentiation of the saprophytic and pathogenic *A. laidlawii* strains. Originally 3 types of *A. laidlawii* were described: types A, B and C (Laidlaw and Elford, 1936; Pirie, 1937). For the time being only types A and B are accepted. Their intensive biological and serological examination has not revealed any differences (Tully and Razin, 1968). There were some divergences in the electrophoretic patterns of membrane proteins of the strains (Rottem and Razin, 1967).

Recently *A. laidlawii* strains serologically identical with, but biochemically different from, the reference strains have been isolated, *viz.* glucose-

negative strains (HOARE and HAIG, 1964; LEACH, 1967), glucose and mannose-positive strains (LEMCKE, 1964; HAYFLICK and STANBRIDGE, 1967), and aesculin-negative (WILLIAMS and WITTLER, 1971) strains.

In an earlier work SCHIMMEL and PUSTOVAR (1971) made distinction between *A. laidlawii* strains of different serological groups by slide agglutination, tube agglutination and agargel-precipitation tests. In the present work we have differentiated (1) aesculin- and cellobiose-positive, (2) aesculin-negative and cellobiose-positive, and (3) aesculin- and cellobiose-negative strains. The reference strains, on the other hand, were aesculin-positive and cellobiose-negative. So our strains differed from the reference strains. $D_6/1010$ and $D_{10}/1270$ belong to group (1), $D_6/1010$ strain differed from the reference strains in agglutination with the *A. laidlawii* A PG-8 serum, which was negative, and in sensitivity to pH 5.5. Group (2) includes $D_7/10P$ and $D_8/2775$. $D_8/2775$ was different from the reference strains in being sensitive to pH 5.5 and to sodium chloride and reacting with reference *A. laidlawii* sera in the immunofluorescence test in a low dilution. Group (3) was represented by strains $D_9/1816$, $D_{11}/3097$ and $D_{12}/252$, which were not inhibited in the growth-inhibition test by the *A. laidlawii* A PG-8 reference serum and reacted with it only in a low dilution in the immunofluorescence test.

There was no correlation between the biochemical properties and the results of resistance and serological tests, except for the aesculin- and cellobiose-negative group, which was not inhibited in the growth-inhibition test by the *A. laidlawii* A PG-8 reference serum and reacted with it in the immunofluorescence test in a low dilution.

It is therefore evident that biochemically different variants exist in the *A. laidlawii* species, but it is necessary to reveal more detailed properties of the strains.

The differences between our strains and the reference strains can explain the existence of pathogenic strains in this species. According to earlier studies among the strains examined by us. $D_8/2775$, $D_{10}/1270$ and $D_{12}/252$ proved to be pathogenic (MARTIN et al., 1968; DZU et al., 1971; ROMVÁRY et al., 1970).

One of the examined strains was diffferent from all the used *Acholeplasma* species in the biochemical, resistance and serological tests, suggesting that further, still unknown *Acholeplasma* species may exist.

*

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SUMMARY

Eight *Acholeplasma* strains isolated from the respiratory tract of swine were studied biochemically, serologically and in resistance tests. One of the strains was different from the *A. laidlawii* A PG 8, *A. laidlawii* B F-8, *A. granularum* (Friend), *A. axanthum* (ATCC 25176) and *A. sp.* PG 49 (Squire) reference strains. Others are regarded as *A. laidlawii*, but some of them were different from the *A. laidlawii* A PG 8, and *A. laidlawii* B F 8 reference strains in aesculin-hydrolysing and in cellobiose-decomposing ability, some — in absence of aesculin-hydrolysing but at the same time in presence of cellobiose-decomposing ability, and some in absence of aesculin and cellobiose-splitting effect.

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IDENTIFICATION OF BOVINE *MYCOPLASMA* STRAIN ISOLATED FROM CATTLE

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Intensive mycoplasma research was begun in the late forties and resulted in isolation of mycoplasmas from different organs of cattle; respiratory tract (HAMDY, 1968) genital tract (BAKOS et al., 1962; BŐGEL et al., 1962; AFSHAR et al., 1966), udder (HALE et al., 1962), blood, lymph nodes, spleen, liver (JASPER, 1967), joints (HUGHES et al., 1966), eye (JAIN et al., 1969) and surface of the skin (HAUKE et al., 1967). Detailed studies of the isolates have shown that they usually differ from the previously isolated mycoplasma species known as *M. mycoides* var. *mycoides* (EDWARD and FREUNDT, 1956), responsible for bovine contagious pneumonia (NOCARD and ROUX, 1898) and often also from one another. As a result of comparative studies of bovine mycoplasma strains and types, LEACH established in 1967 eight independent species, or groups. In the meantime the new species *M. arginini* (BARILE et al., 1968), *M. dispar* (GOURLAY and LEACH, 1970), *M. oculi* and some new types (AL-AUBAIDI, 1969) have been added to the range of known bovine mycoplasmas.

The abruptly increasing number of isolations during the recent years has hampered strain typing and the frequent exchange of strains resulted in the contamination of the reference strains with other mycoplasmas. Certain authors (AL-AUBAIDI and FABRICANT, 1971) have therefore questioned the validity of the existing classification of bovine mycoplasma strains. Some groups (types) of the classification scheme are in fact not satisfactorily characterized, *e.g.* Leach's group 6, which comprises relatively few strains. The bovine origin of the first two strains of this group, isolated by LANGER and CARMICHAEL in the USA (1963), has been disputed (LEACH, 1967) because they were isolated by alternate serial passages in tissue culture. Evidence of bovine origin has recently been presented by the isolation of a similar strain in Hungary (BOKORI et al., 1971), for this grew out on artificial medium inoculated directly with thoracal puncture fluid from diseased animals.

Due to the recent controversies on bovine mycoplasma type and species determination, the mycoplasma research groups working under the auspices of the FAO and WHO have initiated detailed biochemical and serological investigations into a home bovine mycoplasma isolate, using the reference strain *A. sp.* PG 49 (Squire). Apart from presenting experimental proof that our isolate belongs to the group Squire (Leach's group 6), the further aim of our studies was to determine the main properties of this group.

Materials and Methods

The home strain No. 292, isolated by BOKORI et al. (1971) from bulls and the *A. sp.* PG 49 (Squire) strain, from the collection of the FAO/WHO International Reference Centre for Animal Mycoplasmas (Institute of Medical

Microbiology, University of Aarhus, Aarhus, Denmark) were used throughout. The strains were grown in liquid medium, the cultures were passed through 0.22 μm Millipore filter, the filtrates were streaked on solid agar plates and individual colonies grown out on the streak plates were used for further cultivation. This procedure was repeated three times and the subcultures thus obtained were used for further study.

Medium:

The following medium was regularly used for culturing:

Heart Infusion Broth (Difco) (according to the producer's instructions)	90	ml
Inactivated horse serum	20	ml
Fresh yeast extract	10	ml
0.2% DNA-extract (SIGMA)	1.2	ml
10% thallium acetate (BDH Chemicals, England)	1.0	ml
0.25 ml (200,000 U/ml) penicillin	0.25	ml
1% triphenyltetrazoliumchloride (TTC) solution (MERCK)	1.0	ml

For certain biochemical examinations, this medium was modified in different ways.

Biochemical examinations

The following tests were performed with the strains: carbohydrate decomposition (glucose under aerobic and anaerobic conditions; mannose, galactose, sucrose, xylose, sorbitol, mannitol, cellobiose), arginine decarboxylation, hydrolysis of urea, aesculin and arbutin, phosphatase activity, reduction of 1—5% TTC—0.0002% methylene blue (under aerobic and anaerobic conditions), resistance to 3% NaCl, 0.02% methylene blue, 0.2% Tween 80, 0.5—1.0% sodium taurocholate, 5% sodium polyanetholsulphonate, 0.01% digitonin, pH 5.5 and pH 9.5 Growth at 22 °C and film and spot formation of the strains were also tested. The examinations were conducted according to the standard prescriptions of the FAO/WHO International Reference Centre for Animal Mycoplasmas.

Serological examinations

The strains were studied on four kinds of growth-inhibitory media (Clyde, 1964) at 37 and 27 °C and by metabolic inhibition (Taylor-Robinson et al., 1966), indirect haemagglutination (Rosendal and Black, 1972) and

immunofluorescence (KROGSGAARD, 1971) tests. The following hyperimmune sera were used for serological testing: antisera to *M. pneumoniae* FH, *M. hominis* I. (PG 21), *M. fermentans* PG 18, *M. orale* I CH, *M. orale* II CH20247, *M. orale* III, *M. salivarium* PG 20, *M. mycoides* var. *mycoides* PG 1, *M. bovigenitalium* PG 11, *A. laidlawii* A PG 8, *M. bovirhinis* PG 43, *M. agalactiae* var. *bovis* (Donetta), *A. sp.* PG 49 (Squire), *M. sp.* PG 50 (N29), *M. sp.* PG 51 (D 12), *M. arginini* G230, *M. bovigenitalium* (Kalle), *M. hyorhinis* PG 29, *A. granularum* (Friend), *M. gallisepticum* X95, *M. gallinarum* PG 16, *M. meleagridis* 17529, *M. anatis* 1340, *M. iners* PG 30, *M. spumans* PG 13, *M. canis* PG 14, *M. maculosum* PG 15, *M. felis*, *M. gateae*, *M. pulmonis* (Ash), *M. arthritidis* PG 6, *M. neurolyticum* Sabin A, *M. leonis* LL, *M. lipophilum* (MABY), *M. agalactiae* PG 2, *M. mycoides* var. *capri* PG 3, *A. axanthum* ATCC 25176, *A. laidlawii* B (F8), Strain No. 292. All antisera but the last named serum were prepared in the Reference Centre (Aarhus).

Polyacrylamide gel electrophoresis was performed by the method of RAZIN and ROTTEM (1967).

Results

In growth inhibition, immunofluorescence, metabolic inhibition and indirect haemagglutination tests, the antiserum to strain No. 292 reacted to a similar degree as with the homologous antigen only with the reference strain *A.sp.* PG 49 (Squire) of all acholeplasmas tested (Table I). The reactions of the same serum in the metabolic inhibition and indirect haemagglutination tests were nevertheless weaker with the reference strain than with the homologous antigen.

The anti-*A.sp.* PG 49 (Squire) reference serum did not react with antigens other than the homologous strain. Strain No. 292, however, reacted with the above reference serum in all tests, giving even more intensive reactions than the homologous antigen.

Strain No. 292 reacted with *Acholeplasma* reference sera other than anti-*A. sp.* PG 49 (Squire) only in the indirect haemagglutination test at low dilutions (1 : 8, 1 : 32), similarly to the *A.sp.* PG 49 reference strain (Table II).

Strain No. 292 gave no positive reaction with the mycoplasma reference sera in the growth-inhibition test, but reacted with several of them in the indirect haemagglutination test and, above all, in the metabolic inhibition test. These reactions occurred only at very low serum dilutions compared to the controls (Table III).

Photometrically evaluated curves for the polyacrylamide-gel-electrophoretograms of strains No. 292 and *A.sp.* PG 49 (Squire) are shown in Figs 1 and 2. The number, shape and size of the peaks representing the indi-

Table I

Serological testing of strain No. 292 and *Acholeplasma* reference strain

Antigen	Antiserum N . 292				
	IF		GI	MI	IHA
	1 : 10	*1 : 160*			
A. laidlawii A PG 8	—	—	—	—	—
A. laidlawii B F 8	—	—	—	—	—
A. granularum (Friend)	—	—	—	—	—
A. axanthum ATCC 25176	—	—	—	—	—
A. sp. PG 49 (Squire)	+++	+++	5.0	1 : 640	1 : 1280
Strain No. 292	+++	+++	6.0	1 : 2560	1 : 2560

Symbols used: IF, immunofluorescence; GI, growth inhibition test; MI, metabolic

vidual fractions on the two curves seemed to correspond roughly already on visual inspection. Measurements of colour changes of each fraction have shown that all peaks (fractions) of the curve for the strain No. 292 correspond also in absolute value to the appropriate peaks of the curve for the reference strain. Although the peaks designated as *b* appear uniform, the fraction is broader on the reference-strain curve. The absolute values for peaks *c* were uniform, but these peaks consisted of at least 3 parts, of which peaks c^1 were uniform, where as peaks c^2 and c^3 were variable. The two strains behaved identically in the biochemical tests. Glucose decomposition by strain No. 292 became positive 1—2 days later than in the case of the reference strain. Aerobic

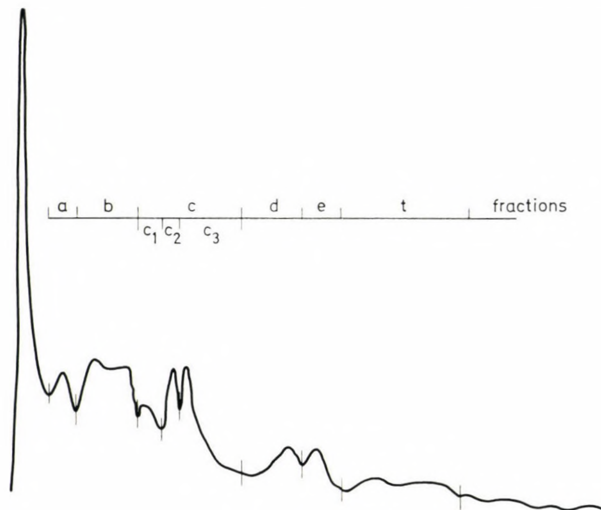

Fig. 1. Polyacrylamide gel electrophoretic patterns of cell proteins of the strain 292/3a

with hyperimmune sera to strains No. 292 and *A. sp.* PG 49 (Squire)

Antigen	Antiserum *A. sp.* PG 49 (Squire)				
	IF		GI	MI	IHA
	1 : 10	*1 : 320*			
A. laidlawii A PG 8	—	—	—	—	—
A. laidlawii B F 8	—	—	—	—	—
A. granularum (Friend)	—	—	—	—	—
A. axanthum ATCC 25176	—	—	—	—	—
A. sp. PG 49 (Squire)	+++	+++	3.0	1 : 320	1 : 640
Strain No. 292	+++	+++	5.0	1 : 640	1 : 2560

inhibition test; IHA, indirect haemagglutination test

reduction of TTC occurred only in rabbit serum containing broth, but even then it was of low degree. Both strains reduced TTC and methylene blue under anaerobic conditions. The strains grew well on a 20% horse serum containing aesculin medium, but did not decompose aesculin and there was little growth, if any, on aesculin medium prepared with PPLO serum fraction (Table IV).

The two strains behaved similarly also in the resistance tests (Table V): they resisted pH 9.5, sodium polyanetholsulphonate and digitonin. Both

Table II

Testing of strains No. 292 and *A. sp.* PG 49 (Squire) with *Acholeplasma* reference sera

Antigen	Test	Reference sera							
		A. laidlawii A PG 8		*A. laidlawii* B F-8		*A. granularum* Friend		*A. axanthum* ATCC 25176	
Strain No. 292	IF	*1*	*2*	—	—	—	—	—	—
	GI	—		—		—		—	
	MI	—		—		1 : 10		1 : 10	
	IHA	1 : 8		1 : 32		1 : 16		1 : 8	
Strain *A. sp.* PG 49 (Squire)	IF	—	—	—	—	—	—	—	—
	GI	—		—		—		—	
	MI	—		—		1 : 10		1 : 10	
	IHA	1 : 8		1 : 8		1 : 8		—	

Remarks for symbols used see Table I;
1, result obtained with 1 : 10 diluted serum;
2, result obtained from dilution of end titre of the homologous serum

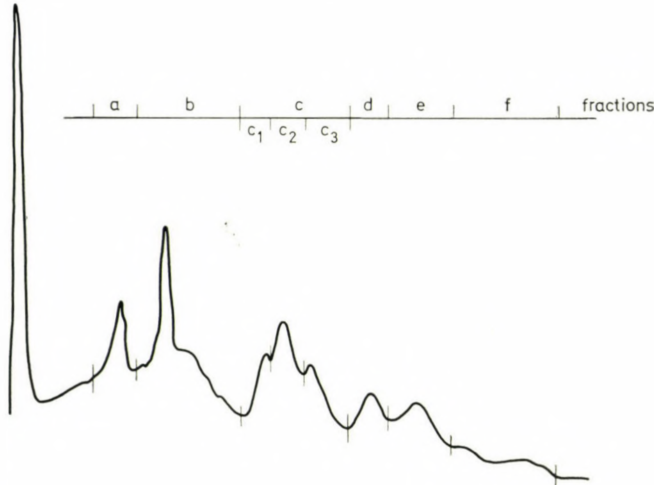

Fig. 2. Polyacrylamide gel electrophoretic patterns of cell proteins of the *A. sp.* PG 49 (Squire) strain

Table III

Inhibition of strain No. 292 by mycoplasma reference sera

Sera		Metabolic inhibition test	Indirect haemagglutination test	Growth inhibition test
Mycoplasma mycoides var. *mycoides*	PG 1	1 : 10	—	—
M. bovirhinis	PG 43	—	1 : 10	—
M. agalactiae var. *bovis* (Donetta)		1 : 20	—	—
M. sp. D 12	PG 51	1 : 20	—	—
M. arginini	G 230	1 : 20	1 : 10	—
M. hyorhinis	PG 29	—	1 : 10	—
M. anatis	1340	1 : 10	—	—
M. maculosum	PG 15	—	1 : 10	—
M. felis	CO	1 : 20	—	—
M. gateae	CS	1 : 20	—	—
M. leonis	LL	1 : 20	—	—
M. agalactiae	PG 2	1 : 10	—	—
M. mycoides var. *capri*	PG 3	1 : 10	—	—

Negative results in all tests with sera:

M. bovigenitalium	PG 11	*M. neurolyticum*	Sabin A
M. bovigenitalium	Kalle	*M. lipophilum*	MABY
M. sp. N29	PG50	*M. orale* III.	DC-333
M. gallisepticum	X95	*M. pneumoniae*	MAC (LI)
M. meleagridis	17529	*M. hominis* I	PG21
M. iners	PG30	*M. fermentans*	PG18
M. spumana	PG13	*M. salivarium*	PG20
M. canis	PG14	*M. orale* I.	CH 19299
M. pulmonis	Ash	*M. orale* II.	CH 20247
M. arthritidis	PG6	*M. gallinarum*	PG16

Table IV

Results of biochemical assays of strains No. 292 and *A. sp.* PG 49 (Squire)

Tests	Results	
	Strain No. 292	Reference strain *A. sp.* PG 49 (Squire)
Decomposition of glucose aerobic	+	+
anaerobic	+	+
Decomposition of mannose	—	—
Decomposition of sucrose	—	—
Decomposition of galactose	—	—
Decomposition of mannitol	—	—
Decomposition of sorbitol	—	—
Decomposition cellobiose	—	—
Decomposition of xylose	—	—
Decarboxylation of arginine	—	—
Hydrolysis of urea	—	—
Reduction of TTC aerobic	(+)	+
anaerobic	+	+
Reduction of methylene blue aerobic	—	—
anaerobic	+	+
Aesculin	—	—
Arbutin	—	—
Phosphatase	—	—
"Film and spot" formation	—	—
Cholesterol requirement	—	—
Serum digestion	—	—

Table V

Behaviour of strain No. 292 and reference strain *A. sp.* PG 49 (Squire) in resistence tests

Tests	Strain No. 292	Reference strain *A. sp.* PG 49 (Squire)
Sodium polyanetholsulphonate	R	R
Digitonin	R	R
NaCl	S	S
pH: 5.5	S	S
pH: 9.5	R	R
Methylene blue	S	S
Tween 80	S	S
Bile salt 0.5%	S	S
1.0%	S	S
Growth at 22 °C	R	R

Symbols used: S, sensitive; R, resistant

strains grew at 22 °C, but at a much lower rate than the controls kept at 37°C.

Neither strain displayed a phosphatase activity or formed "film and spot". They grew well in cholesterol-less medium.

Discussion

In biochemical and resistance tests strain No. 292 and reference strain *A. sp.* PG 49 (Squire) were identical; in all serological tests, strain No. 292 reacted with the aforementioned reference serum and antiserum to strain No. 292 reacted with the reference strain. The intensity of the reactions differed only slightly. In contrast, strain No. 292 failed to react with heterologous acholeplasma or mycoplasma antisera in the growth-inhibition test and reacted only at very low dilutions in the indirect haemagglutination and metabolic inhibition tests. These findings have affirmed that strain No. 292 is a group-6 bovine *Mycoplasma* strain according to LEACH's classification. The results of polyacrylamide-gel-electrophoresis served further evidence, because apart from two subfractions of fraction *c*, all electrophoretic fractions of strain No. 292 were found to be identical with the fractions of the reference strain. The minor difference between the two *c*-subfractions can account for the minimal dissimilarities of serological behaviour, but it cannot prove the serological non-identity of the two strains. This is in support of the earlier observations of BOKORI et al. (1971).

Results of the determination of cholesterol requirement and resistance to sodium polyanetholsulphonate and digitonin, as well as growth ability at 22 °C suggest, in conformity with EDWARD's (1971) view, that strains of the group *A. sp.* PG 49 (Squire) represent an *Acholeplasma*.

Apart from the observations made in biochemical and resistance tests, the following properties seem to be characteristic of the group *Acholeplasma:* distinct glucose decomposing ability under both aerobic and anaerobic conditions, absence of mannose-, galactose-, sucrose-, mannitol-, sorbitol-, cellobiose- and xylose-decomposing activity, no arginine-decarboxylating and urea-hydrolysing activity, aerobic decomposition of TTC exclusively in the presence of rabbit serum, but vigorous TTC and methylene blue-reducing activity under anaerobic conditions. Neither phosphatase activity, nor "film and spot" formation could be demonstrated. The strains studied grew well in a medium adjusted to pH 9.5, but did not grow at pH 5.5, and they were sensitive to the applied concentrations of NaCl, Tween 80 and methylene blue.

Detailed serological studies of strain No. 292 have shown that this group of *Acholeplasma* clearly differs from the other known *Acholeplasma* species *(Acholeplasma laidlawii* A and B, *A. axanthum*, *A. granularum)*. Certain

Acholeplasma and *Mycoplasma* antisera have nevertheless cross-reacted weakly with the strain in the indirect haemagglutination test. The behaviour of the strain in the metabolic inhibition test was in good accordance with the observation of AL-AUBAIDI (1969) that strains belonging to this *Acholeplasma* group may be inhibited, although to a minor degree, by antisera to certain mycoplasmas. No such phenomenon occurred in the growth inhibition test.

*

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SUMMARY

Comparative biochemical and serological examinations of a group-6 (according to LEACH) bovine *Mycoplasma* strain isolated in Hungary were carried out using the reference strain *A. sp.* PG 49 (Squire). Apart from presenting evidence for the identity of the two strains, the main properties of this *Acholeplasma* group were determined.

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ULTRASTRUCTURAL CHANGES IN THE LIVER OF EQUINE FETUSES ABORTED DURING RHINOPNEUMONITIS

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Earlier light-microscopic studies on the pathogenesis of equine rhinopneumonitis showed that the fetuses of the diseased mares exhibit chiefly liver changes including focal necroses and acute or subacute hepatitis (KAPP et al., 1969). Suckling syrian hamsters infected experimentally with the equine rhinopneumonitis virus developed a lethal hepatitis in which the causative role of the virus was demonstrated by light and electron microscopy (KAPP, 1972). The liver lesions of the aborted equine fetuses and experimentally infected hamsters resembled morphologically the changes encountered in the viral hepatitis of man and animals.

This paper reports electron-microscopic examinations of the livers of equine fetuses aborted in the course of a recent severe outbreak of rhinopneumonitis in a horse stock.

Materials and Methods

Livers from two equine fetuses, aborted after 7 weeks and 10 months of intrauterine life, respectively, were examined electron-microscopically.

The 7-week-old fetus had been aborted by a mare which was emergency-slaughtered for paralytic symptoms in the terminal stage of rhinopneumonitis. The fetal liver was dark brownish-red, swollen, and granular over the surface; the parenchyma was slightly rigid and friable. A severe, subacute hepatitis was observed grossly and by light-microscopic examination. There was gastroenteritis, but no other organic change.

The other fetus, which survived abortion in the 10th month by two hours, also had liver lesions. The external and cut surfaces of the enlarged liver were greyish-white and spotted with greyish-red areas. Inside the latter a few sporadic greyish-white foci of the size of a pin-head were seen. The parenchyma was more compact than normally and resisted tearing. According to the post-mortem examination, the foal died of chronic hepatitis.

Three samples, each from a different part of the liver, were taken from either fetus, from the 7-week-old one immediately after the emergency slaughter of the mare and from the other immediately after its death. The specimens were fixed in OsO_4, buffered according to Pallade, dehydrated in step-graded ethanol and embedded in Durcupan. Ultra-thin sections were cut with a

Reichert ultra-microtome and electron micrographs were prepared with a Tesla BS 613 type electron microscope. The semi-thin sections were stained with toluidine blue.

Electron microscopic examinations

Semi-thin sections from the liver of the 7-week-old fetus showed that the greater part of the parenchymal cells were severely damaged in the affected lobules. Cowdry's type-A nuclear inclusions were seen in many cells, often in those close by the sinusoids (Fig. 1). In other areas, the parenchymal cells showed hyperchromatosis, karyorrhexis or karyolysis. Multinucleated giant cells formed from liver cells occurred sporadically in liver lobules. Besides the nuclear changes, the cytoplasma of single cells or in groups of cells stained homogeneously (acidophilic necrosis) and round, homogeneous bodies were formed from necrotic cells.

Electron-microscopically the cells containing Cowdry's type-A nuclear inclusions had enlarged nuclei with an undulating nuclear membrane. Part of the nuclear chromatin aggregated along the membrane. Granular and filamentous chromatin residue usually unsingled with virus particles accumulated in the centre of the enlarged nuclei (Figs 2 and 3). The liver cells showing signs of virus synthesis usually had recognizable cytoplasmic organelles. The coarse endoplasmic reticulum increased in most cells and its membranes were arranged in parallel rows (Fig. 4); free ribosomes were seen between the membranes in many places. Cyst-like dilatations of the endoplasmic reticulum, containing many virus particles, were also seen (Figs 4 and 5). In most cells, cyst-like dilatations appeared in the Golgi apparatus as well. The mitochondria enlarged and rounded off their external membrane became lysed here and there and the cristae often appeared fragmented. The damaged mitochondria gave rise to autophagous vacuoles and there was frequently a myeline-like degeneration of the mitochondrial membranes. The changed parenchymal cells usually contained very few glycogen granules and a lysis of the cytoplasmic membrane was seen in many places. The intercellular space was occasionally dilated and a finely granular electron-dense substance and virus particles appeared in it (Fig. 5). Some sinusoids and Disse's gaps also contained virus particles.

Apart from the marked parenchymal cell lesions, inflammatory phenomena were seen in other parts of the liver. The sinusoids were distended and the swollen cells of the endothelial lining often became desquamated into the sinusoidal lumen, admixing therein with leucocytes and residues of deteriorated liver cells. The Disse gaps were also dilated in most places, containing a mass of a granular electron-dense substance and deteriorated cell organelles; the swollen microvilli of the liver cells extended into the gaps. The interlobular septa were infiltrated by lymphocytes in many sites.

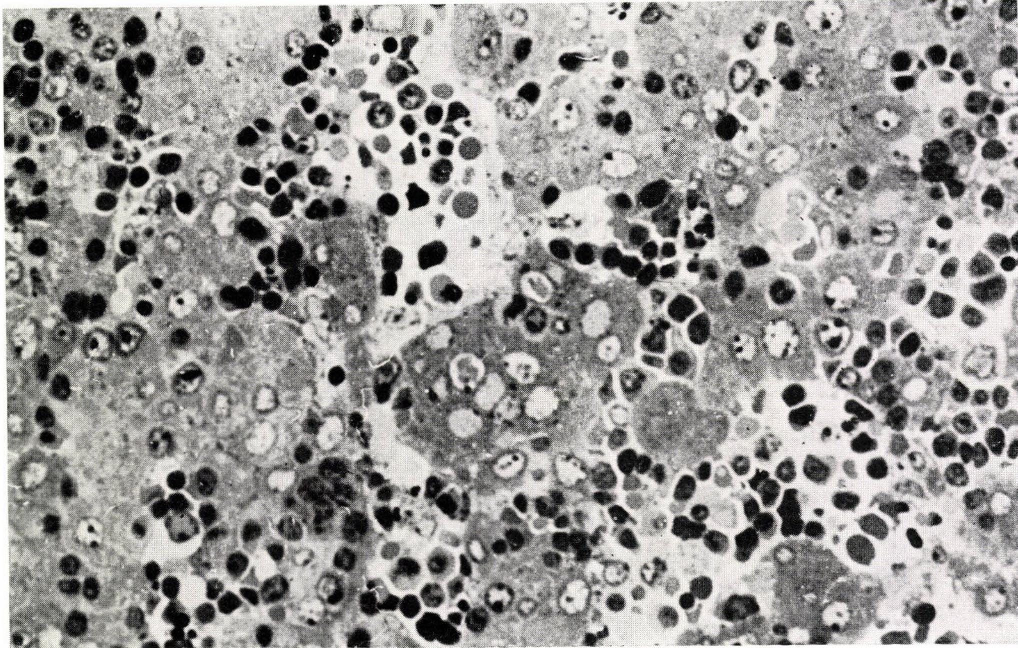

Fig. 1. Liver of 7-week-old equine fetus. Cowdry type A intranuclear inclusions (centre), hyperchromatosis, karyorrhexis and karyolysis in liver cells. The lumen of the distended sinusoids contains swollen, desquamated endothelial cells and leucocytes. Toluidine blue staining ×1000

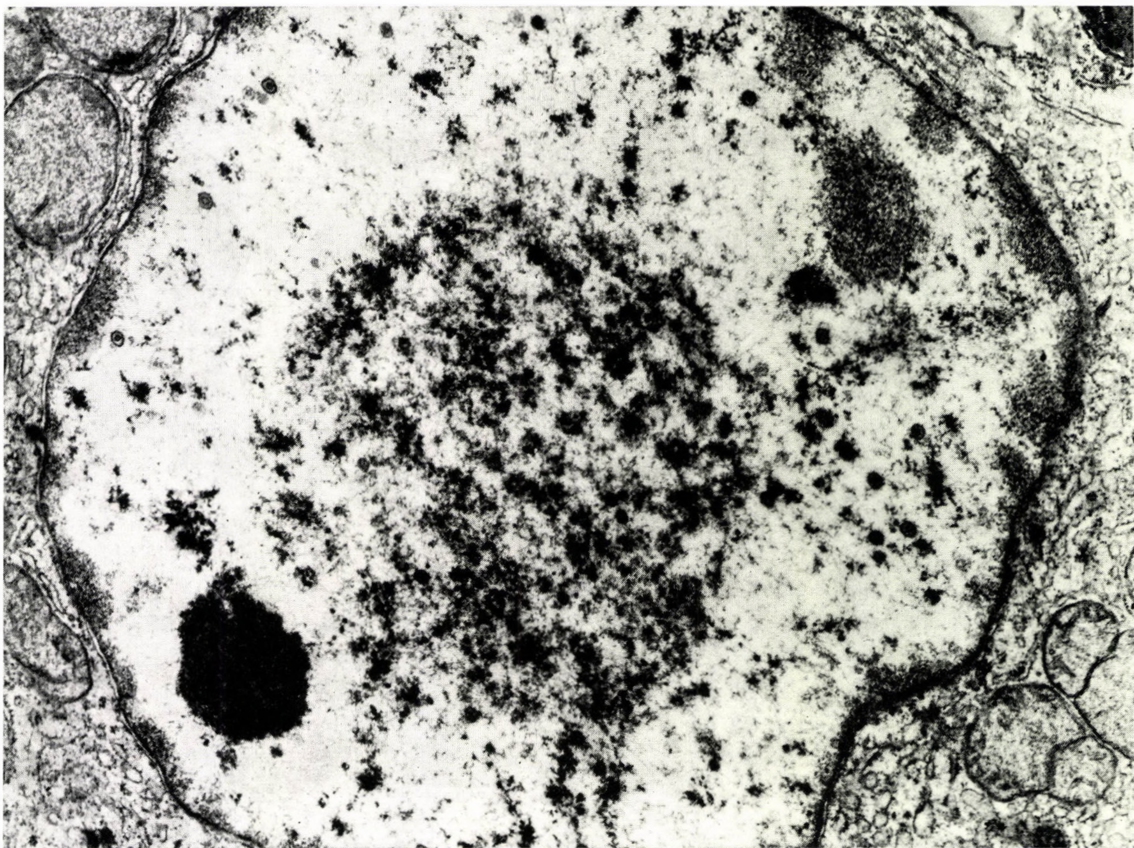

Fig. 2. Liver of 7-week-old equine fetus. Centrally placed granular-filamentous chromatin residue and scattered virus particles in the enlarged liver cell nucleus. ×19,800

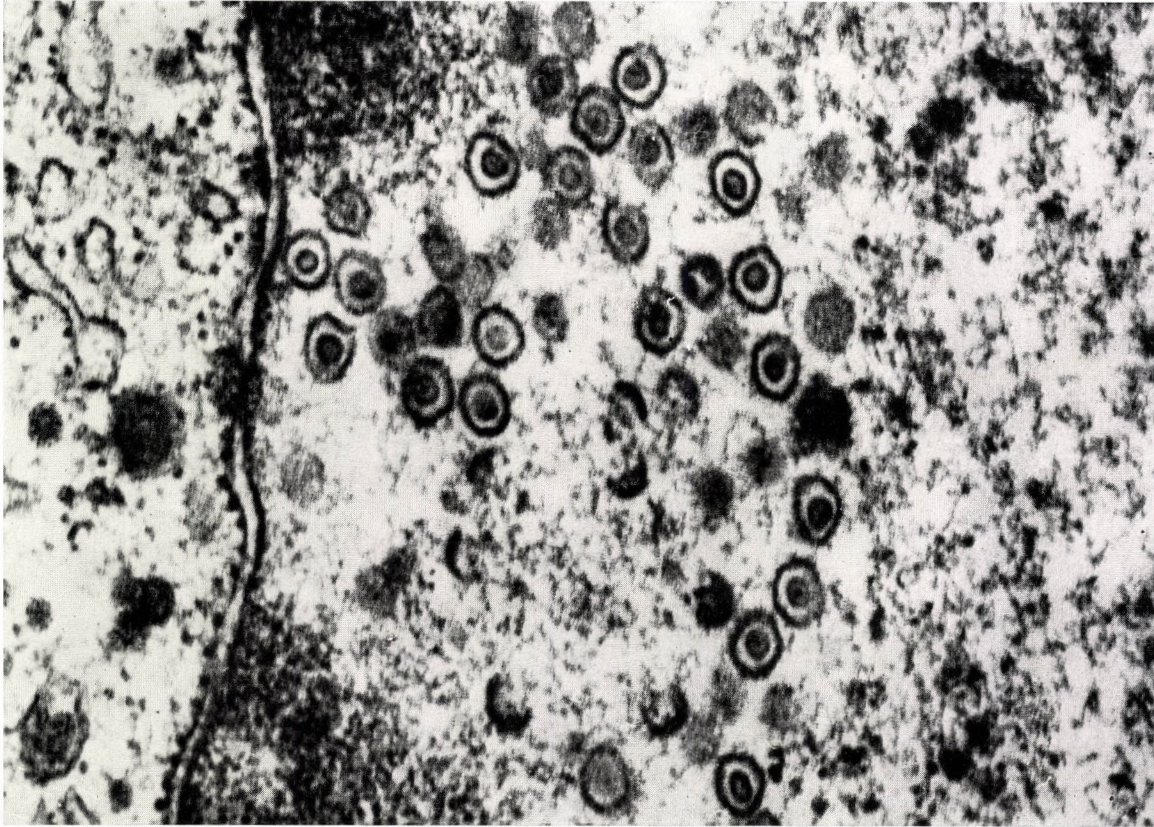

Fig. 3. Liver of 7-week-old equine fetus. Virus particles in the liver cell nucleus. ×84,000

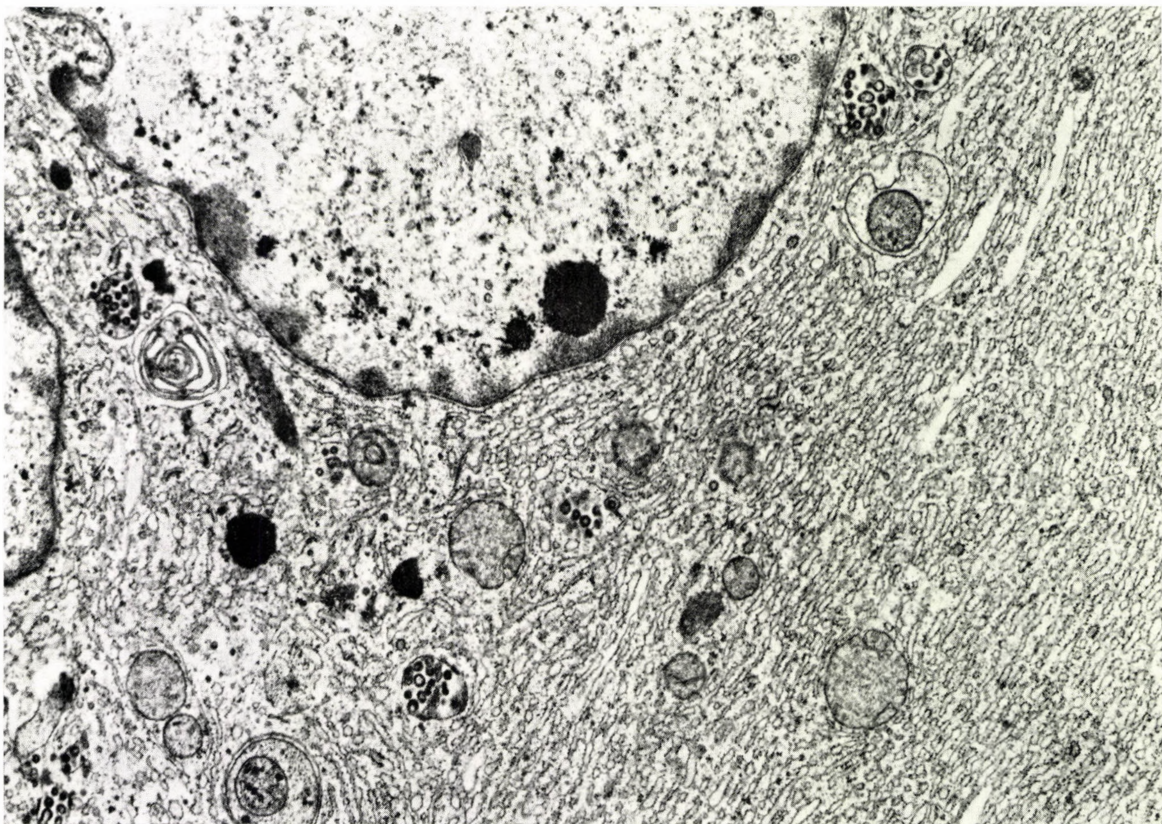

Fig. 4. Liver of 7-week-old equine fetus. The coarse endoplasmic reticulum hypertrophied and its distended cyst-like parts contain virus particles. Autophagous vacuoles are also present in the cytoplasm. ×29,700

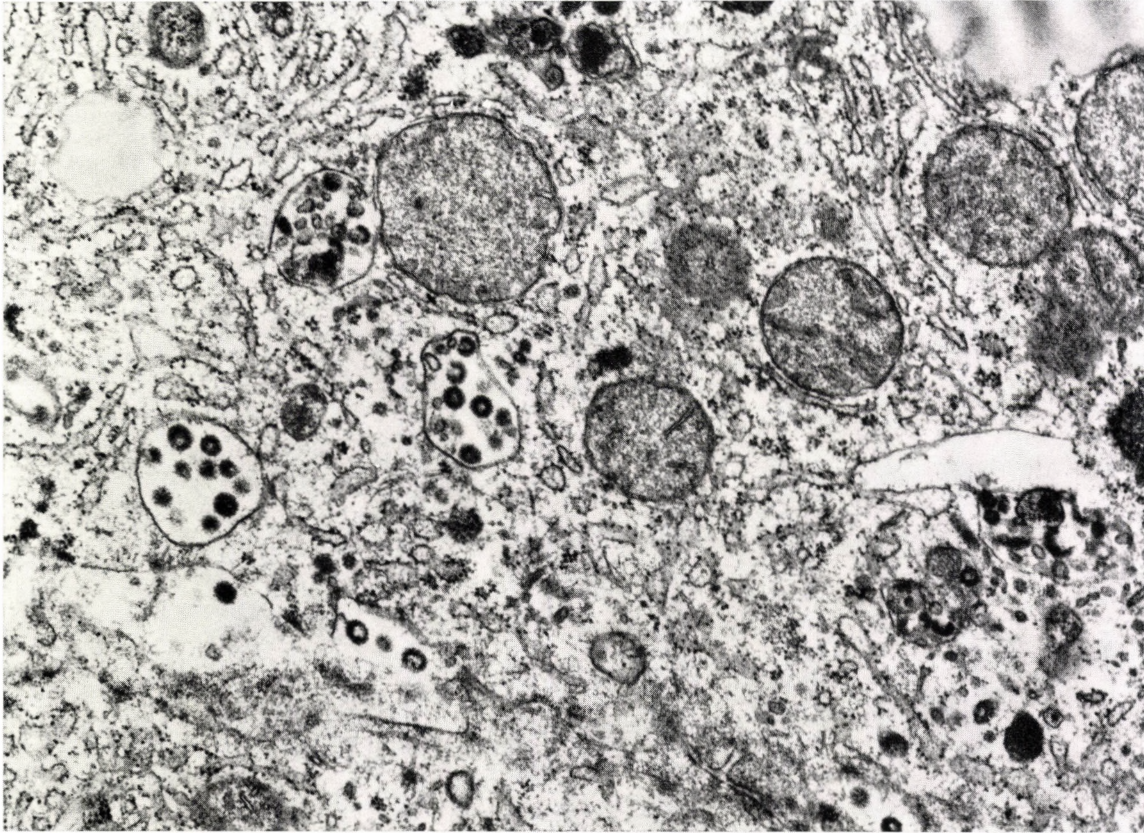

Fig. 5. Liver of 7-week-old equine fetus. Virus particles in the cytoplasmic cyst-like structures and in the intercellular space. ×42,000

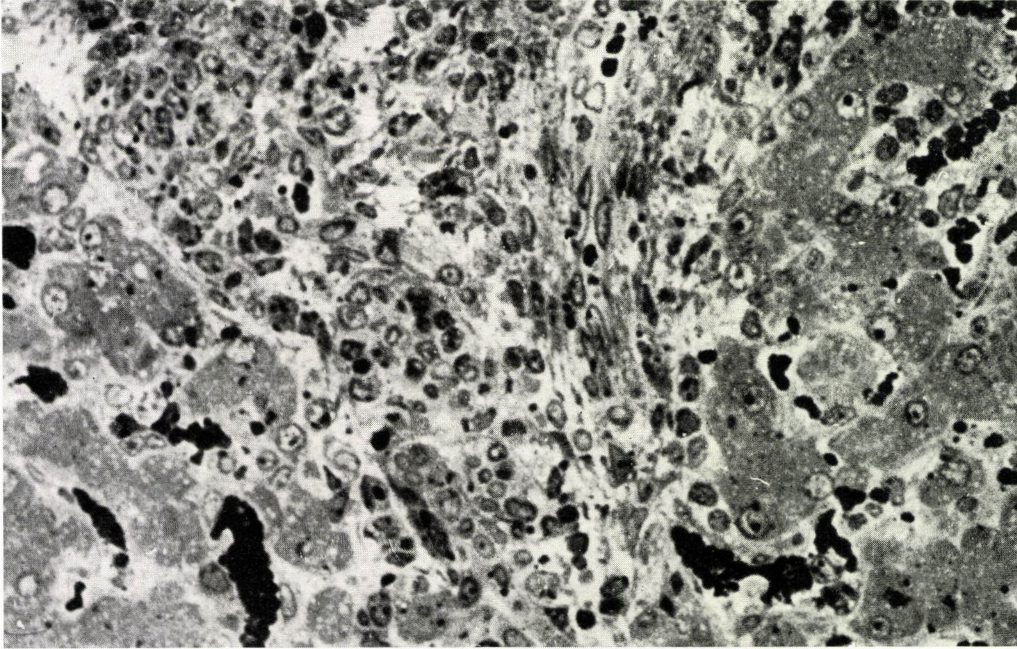

Fig. 6. Liver of equine fetus miscarried in the 10th month. Infiltrating lymphocytes and histiocytes in the thickened interlobular septum. Toluidine blue staining. ×600

The liver lesions of the fetus aborted in the 10th month were chiefly chronic and were limited to the mesenchymal parts of the liver. The thickened interlobular septa showed a marked histio-lymphocytic infiltration and protruded into the lobular parenchyma (Fig. 6). Fibroblasts also appeared among the inflammatory cells and the formation of collagen fibrils was seen in many cells and their immediate surroundings. The biliary ducts in the interlobular septa were dilated and highly electron-dense cytoplasmic granules accumulated in the biliary duct epithelial cells. In some of these cells the endoplasmic reticulum showed cyst-like dilatations (Fig. 7). The microvilli of the liver cells were swollen and the Disse gaps were markedly dilated in many places and a loose granular substance accumulated inside the gaps. A highly electron-dense continuous basement membrane was seen in the immediate neighbourhood of the sinusoidal lining endothelial cells (Fig. 8). The latter were usually enlarged and contained rounded mitochondria, electron-dense granules, and several autophagous vacuoles. In many places the RHS cells increased in number forming small foci. Occasionally Cowdry's type-A nuclear inclusions occurred in RHS cells. Some liver cells adjacent to the sinusoids showed lysis of the cytoplasmic membrane and a vesicle-like dilatation of the smooth and coarse endoplasmic reticulum. The mitochondrial membranes in the liver cell were also damaged. In places the sinusoids contained necrotic cell organelles and hyaline bodies formed from necrotic liver cells (Councilman bodies) (Fig. 9).

In certain areas of the lobules, the biliary canalicules dilated, the microvilli of the liver cells deteriorated and highly electron-dense microbodies appeared in the peri-canalicular perenhymal cells (Fig. 10). The coarse endoplasmic reticulum distended forming vesicles and ribosomes tended to detach from its membrane. The tubules of the Golgi apparatus also showed a vesicle-like distension. The inner structure of the mitochondria became indistinct and their outer membrane lysed. In other places the lumen of the biliary canalicules was filled by elongated, irregularly-shaped microvilli of the liver cells. At the marginal parts of the latter, close by the microvilli, a granular electron-dense substance had accumulated and filaments appeared in the hyaloplasm among the distended, vesicle-like endoplasmic reticulum (Fig. 11).

No virus particles were seen in the electronmicrographs prepared from three different parts of the liver of the fetus aborted in the 10th month. Light-microscopic sections of the surroundings of the grossly visible greyish-white foci showed, however, Cowdry's type-A nuclear inclusions in the parenchymal cells (Fig. 12).

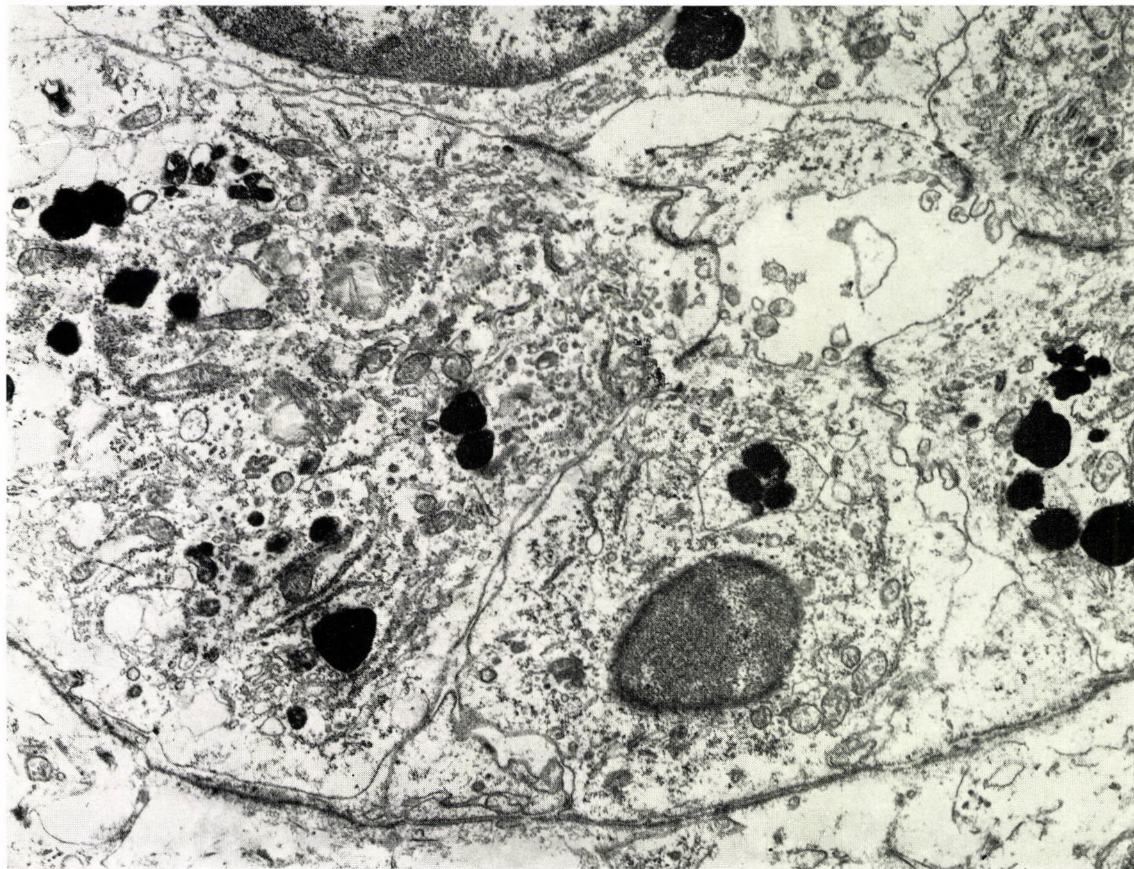

Fig. 7. Liver of equine fetus miscarried in the 10th month. Irregularly shaped, dilated biliary duct lumen. The epithelial cells contain many highly electrondense granules and the endoplasmic reticulum distended in a cyst-like manner in several places. ×9260

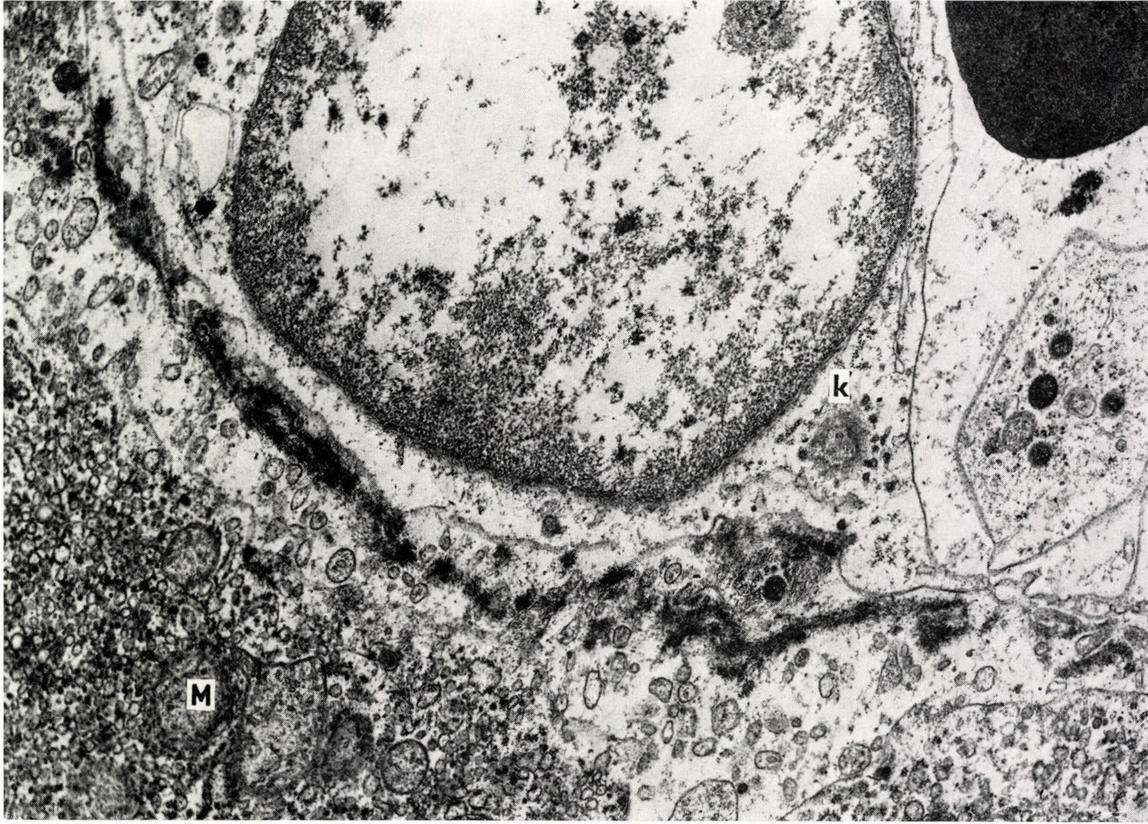

Fig. 8. Liver of equine fetus miscarried in the 10th month. Continuous basement membrane formation in the distended Disse's gaps and swollen microvilli on the surfaces of parenchymal cells. K, Kupffer cell; M, detail of liver cell. ×13,890

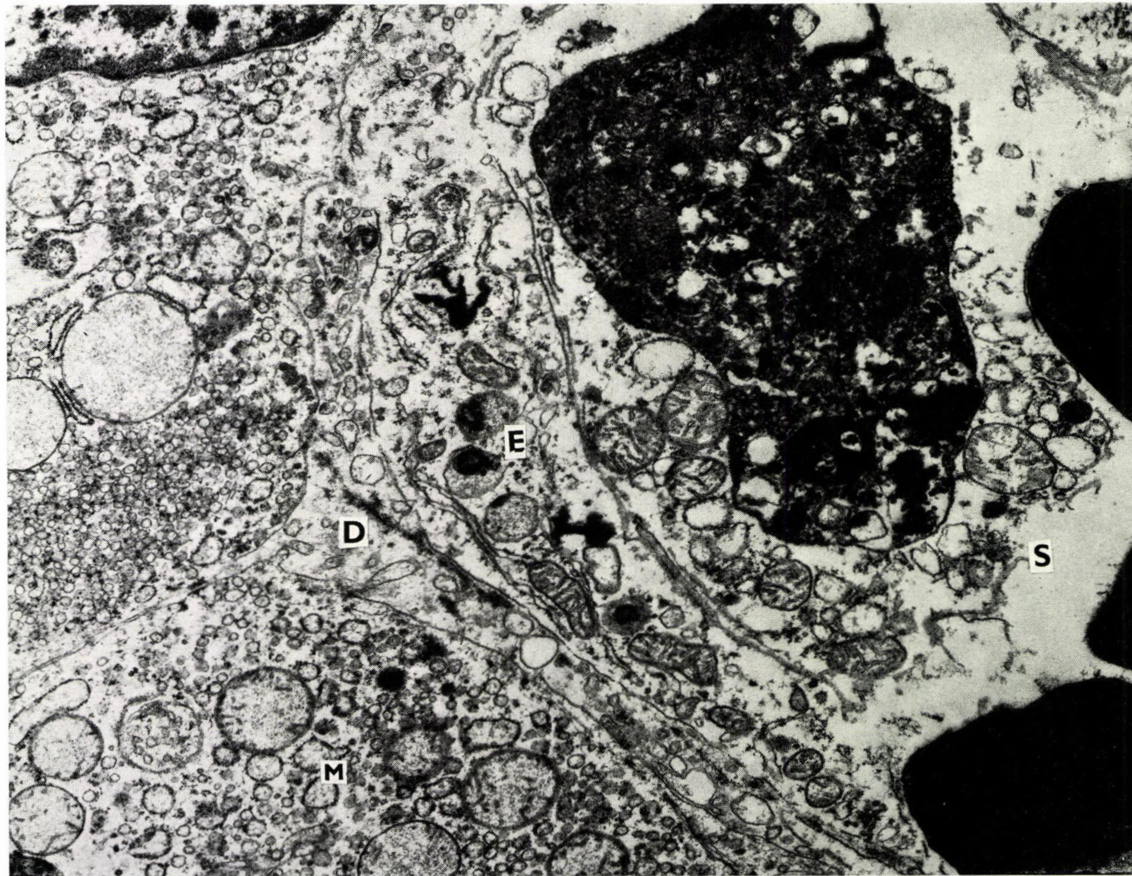

Fig. 9. Liver of equine fetus miscarried in the 10th month. Hyaline body (Councilman body) in the sinusoid. S, sinusoid; E, endothelial cell; D, Disse's gap; M, liver cell. $\times 9260$

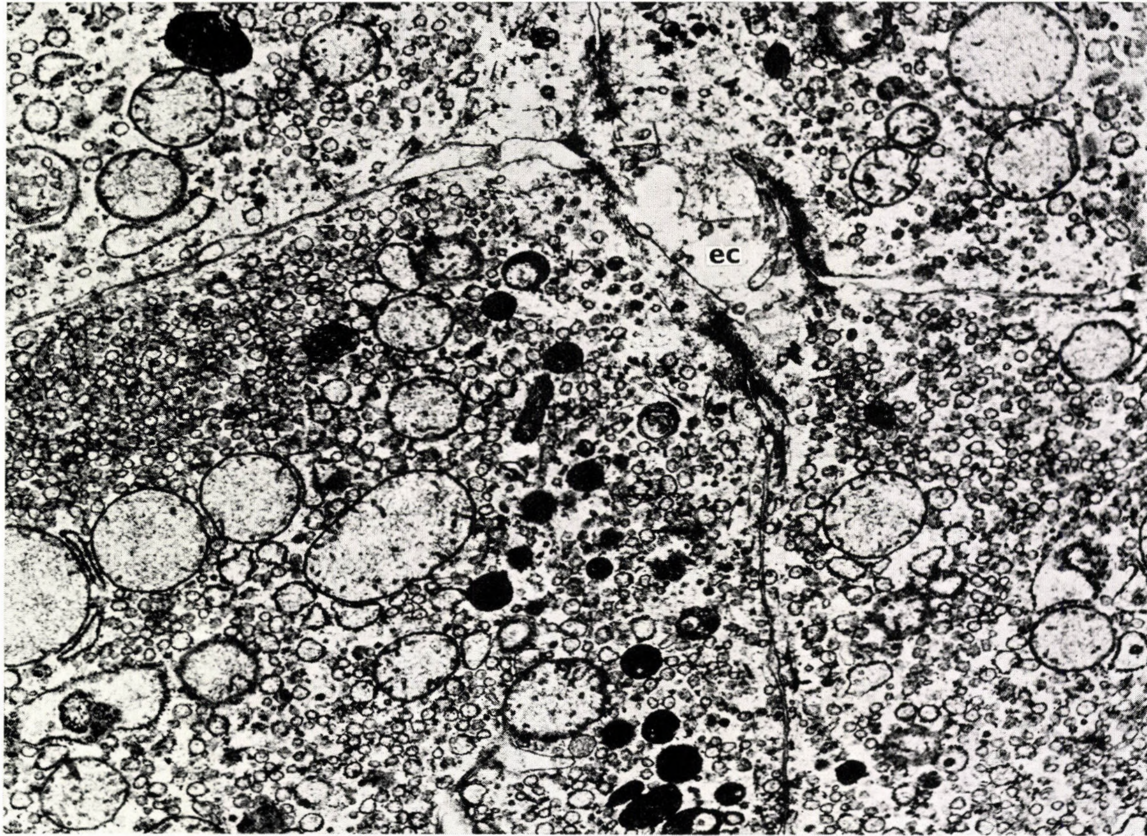

Fig. 10. Liver of equine fetus miscarried in the 10th month. Irregularly-shaped biliary canalicules scarcely containing microvilli. Vesicle-like dilatation of the endoplasmic reticulum. ×13,890

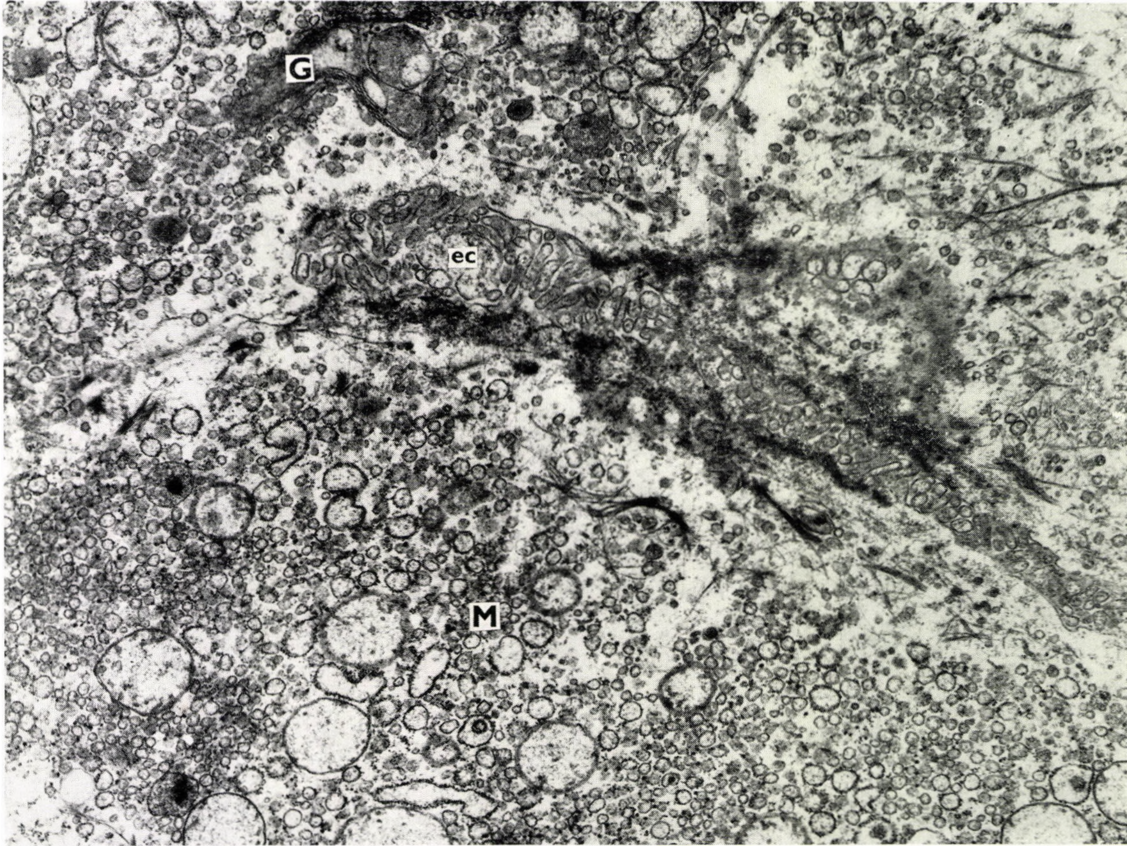

Fig. 11. Liver of equine fetus miscarried in the 10th month. Irregularly-shaped, elongated biliary canalicule (EC), containing irregularly-arranged microvilli. The liver cells contain filaments and the endoplasmic reticulum distended in a cyst-like manner. M, detail of liver cell; G, Golgi-apparatus. ×12,000

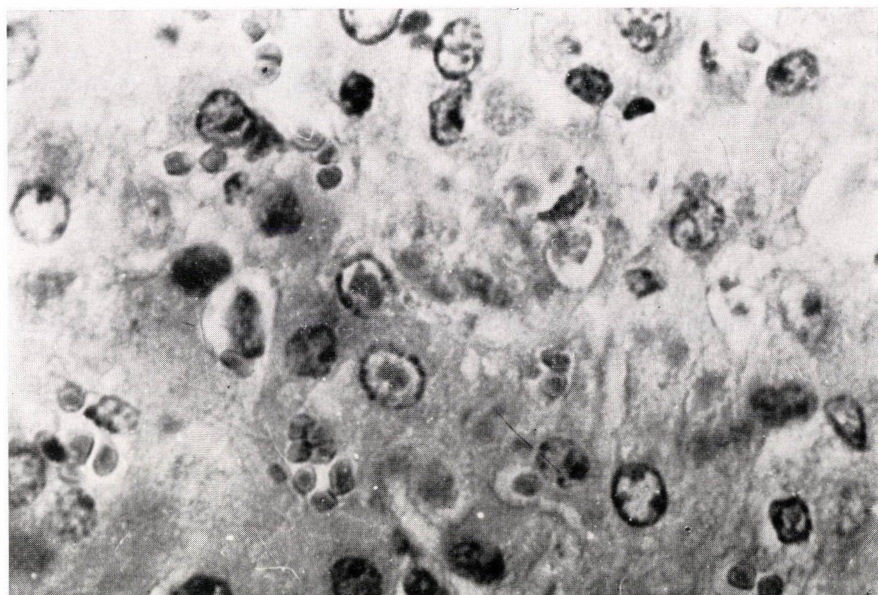

Fig. 12. Liver of equine fetus miscarried in the 10th month. Liver cell adjoining the necrotic areas contain Cowdry's type-A intranuclear inclusions. Haematoxylin and eosin stain. ×1600

Discussion

A 7-week-old and a 10-month-old fetus, aborted in the course of a severe outbreak of rhinopneumonitis among adult horses, chiefly pregnant mares, were examined. Gross and light-microscopic examinations showed that the fetal disease caused by equine rhinopneumonitis virus chiefly involved the liver, and both fetuses died of hepatitis. Detailed examinations of the organs of the dead fetuses weighed in favour of our earlier hypothesis that the main target organ of rhinopneumonitis virus infection is the liver.

In the younger fetus, the main electronmicroscopic change was the lesions of single and groups of liver cells. Acidophilic necrosis of cells, Cowdry's type-A nuclear inclusions and other nuclear damages were often seen. There was also a sporadic giant cell formation. The glycogen contents of the damaged cells decreased, the coarse endoplasmic reticulum usually became hypertrophic and many mitochondria as well as the Golgi apparatus were damaged to different degrees. In addition to parenchymal cell changes, exudation and inflammatory cells filled the lumen of the sinusoids and Disse's gaps. Particles showing the characteristics of herpesvirus were seen in large numbers in the damaged cells and their nuclei, especially in those containing nuclear inclusions as well as in the intercellular space, Disse's gaps and the sinusoids. It is

thus obvious that the acute hepatitis, involving extensive parenchymal cell lesions, was caused directly by the rhinopneumonitis virus. It should be mentioned that the light-microscopic and ultrastructural changes seen in the liver of the 7-week-old fetus were in every respect similar to those previously observed in the livers of suckling Syrian hamsters which had died a few days after experimental infection with rhinopneumonitis virus.

The older fetus, aborted in the 10th month, showed chiefly mesenchymal lesions in the liver. The interlobular septa were infiltrated by lymphocytes and histiocytes, many fibroblasts appeared in them and the collagen fibres increased in number. The RHS cells showed a focal and diffuse proliferation and a continuous basement membrane formation took place in Disse's gaps. Apart from changes in the mesenchymal parts, there was a sporadic necrosis of liver cells resulting sometimes in formation of Councilman bodies. Although no virus particles could be detected in the liver of the 10-month-old fetus, Cowdry's type-A intranuclear inclusions, suggesting the effect of a DNA virus, were present in parenchymal cells as well as in the proliferating RHS cells.

The present morphological observations confirm the causal relationship of both the acute and chronic fetal liver changes with equine rhinopneumonitis virus. This is in good accordance with our earlier hypothesis that also the chronic liver changes of the equine fetus are elicited and maintained by the agent of rhinopneumonitis.

The organic change primarily responsible for fetal death in rhinopneumonitis has been much disputed. JELEFF (1957, 1964) although having observed hepatitis in most cases, postulated that the change of the central nervous system also plays a role in the fatal disease. JACKSON and KENDRICK (1971) and PETZOLDT et al. (1972) failed to substantiate this assumption on the basis of virological examinations; they did not exclude that the agent might enter the central nervous system during the stage of viraemia, but pointed out that virus particles are detected in the liver and lungs most presently. SÁLYI (1941) failed to detect any notable lesions in the central nervous system of a large group of equine fetuses aborted in the course of rhinopneumonitis outbreaks.

The severity, extension and nature of the liver lesions found in the present study suggest that these changes play the leading part in the fatal outcome of the fetal rhinopneumonitis virus disease. It is remarkable that a greater part of the liver lesions associated with the acute and chronic hepatitis due to rhinopneumonitis virus are of similar nature to those encountered in the various virus hepatitides of man and animals (intranuclear inclusions, single cell acidophilic necrosis, Councilman bodies, focal and diffuse proliferation of RHS cells, etc.).

According to present knowledge, equine rhinopneumonitis is caused by

an agent of the Herpesvirus group. It has long been known from experimental infections (GOODPASTURE and TEAGUE, 1923; ANDERSON, 1940) that the most characteristic member of this group, the herpes simplex virus, affects the meso- and entodermal structures to the same degree as the ectodermal tissues. It is of interest from the viewpoint of comparative pathology that the herpes simplex virus causes a severe disease in the visceral organs of newborn infants: hepatitis and generalized herpes chiefly affect prematurel babies. In the former case a lethal hepatitis develops, accompanied by the formation of necrotic foci, giant cells and intranuclear inclusions. In the generalized form, necrotic-inflammatory foci also arise in the spleen, adrenals and other organs of the newborn. In animal experiments (ANDERSON, 1940), the course of the herpes simplex virus infection was found to depend greatly on the age of fetus: younger fetuses usually developed the generalized form, whereas older ones developed hepatitis. Similar observations were made on the human infant (ZUELCER and STULBERG, 1952). The lethal form of equine rhinopneumonitis also occurs in the fetal or newborn age. It is known that herpes simplex virus may persist for a long time in the organs of infants. Such a persistence may account for the occasional chronic liver lesions in equine fetuses during rhinopneumonitis outbreaks.

Thus, the herpes simplex virus infection of human infants and the fetal equine hepatitis caused by rhinopneumonitis virus have several common features in respect of morphological picture and pathogenesis. This conclusion and the results of the pathological examinations of equine fetuses diseased during rhinopneumonitis outbreaks serve as an additional proof of CORREA and NILSSON's (1966) earlier hypothesis that rhinopneumonitis is the viral hepatitis of the horse.

*

Acknowledgements. The author is indebted to Mrs. GY. PETHES for preparation of the ultra-thin sections and electron micrographs and to Mrs. J. NAMÉNYI for the photocopies.

SUMMARY

Liver lesions of two equine fetuses, aborted in the course of a rhinopneumonitis outbreak after 7 weeks and 10 months of intrauterine life, respectively, were examined by electron microscopy. The younger fetus had an acute hepatitis with extensive parenchymal cell damage (Fig. 1). Liver cell necrosis and Cowdry's type-A intranuclear inclusions were frequent and a sporadic giant cell formation was seen. The coarse endoplasmic reticulum was often hypertrophic and also the other cytoplasmic organelles were severely damaged (Figs 4 and 5). Inflammatory phenomena were seen in the sinusoids and Disse's gaps. Many virus particles, showing the characteristics of herpes virus, appeared in the damaged liver cells and their nuclei as well as in the intercellular space (Figs 2—5).

The older fetus, which died shortly after having been aborted, showed chronic liver lesions. The interlobular septa were infiltrated by histiocytes and lymphocytes (Fig. 6), with an increased number of fibroblasts and collagen fibres, there was a focal and diffuse proliferation of RHS cells, a continuous basement membrane formation took place in Disse's

gaps (Fig. 8), the biliary ducts were damaged (Figs 7, 10 and 11), single cell lesions and (Fig. 12) formation of Councilman bodies (Fig. 9) occurred sporadically in the liver lobules. Intranuclear inclusions indicative of the action of a DNA virus were present in liver cells and RHS cells, but no virus particle was found (Fig. 12).

These findings and previous observations of the author unequivocally suggest that during outbreaks of rhinopneumonitis, affected equine fetuses chiefly develop a lethal hepatitis of an acute or chronic course. Comparative examinations have disclosed a great morphological similarity of the related lesions to those associated with other forms of viral hepatitis. On a morphological basis, equine rhinopneumonitis can well be regarded as viral hepatitis of the horse.

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ULTRASTRUCTURAL DETAILS OF THE CORONARY BAND OF SWINE FOOT

By

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The frequent occurrence of foot diseases in swine indicates that in certain cases the poor quality, reduced strength or elasticity of the horny part of the foot may predispose for such conditions. The prevention of foot diseases would be of immediate interest in large-scale swine farms, but this requires a thorough knowledge of the development of foot horn and of the factors influencing it.

As far as we are informed, of the horny structures of swine only the skin has been studied by electron microscopy (JENKINSON, 1965; KARASEK and OEHLERT, 1968), but no data are available on the development of foot horn and ultrastructure of the coronary band. It seemed, therefore, worthwhile to investigate the histological structure of the coronary band of swine foot and the mechanism of keratinization.

This paper reports ultrastructural studies of the epidermis, with regard to a possible relation between fine-structural details and age.

Materials and Methods

Healthy white bacon pigs maintained on a commercial diet were used. Two animals each were killed at 2, 10, 20, 40 and 80 days of age and blocks of one cu. mm volume were cut from the coronary band of the anterior left foot; keratinization proceeds most vigorously in this part. The specimens were fixed in a 2% osmium tetroxide solution buffered according to Pallade, dehydrated in step-graded ethanol and embedded in Durcupan. The ultra-thin sections were cut with a Reichert Ultramicrotome and electron micrographs were prepared in a TESLA type Bs 613 electron microscope. Semi-thin sections were stained with toluidine blue.

Results

The ultra-thin sections showed that the germinative layer of the epidermis is separated from the corium by an undulating basement membrane,

passing parallel to the margin of the germinative cell layer. The basement membrane is finely granular, about 500 Å wide, located about 300—400 Å away from the basal cells of the epidermis (Figs 1—3). A delicate net of loosely arranged reticular filaments is seen between the basement membrane and the collagen fibrils of the corium (Fig. 2).

The cells of the germinative layer have a granular hyaloplasm of medium electron density. The nuclei are round or oval, with several invaginations and a conspicuous nucleolus (Figs 1 and 2). The basal surface of the cells is slightly undulating at 10 and 20 days of age (Figs 1 and 2), but deeply invaginated at 80 days (Fig. 4). The cells are connected with the basement membrane by many semidesmosomes; the opposite side of the membrane is slightly thicker and much more electron dense (Figs 2, 3 and 13/a). Most cytoplasmic organelles are of juxtanuclear position.

The mitochondria are roundish, with well-visible cristae and some of them contain many osmiophilic granules. The Golgi apparatus is well-developed. The smooth and coarse endoplasmic reticulum have a less well defined structure and appear dilated in places. Many free ribosomes are present in the hyaloplasm. The cytoplasm contains bundles of tonofibrils (epitheliofibrils) in a roughly radial arrangement. At 80 days of age, the tonofibrils are more numerous and form thicker bundles than in younger age. The neighbouring cells are linked by dense interdigitations inside which regular desmosome structures are seen. The intercellular space is dilated in places. A few melanocytes occur among the basal cells.

The stratum spinosum consists of two to several rows of cells. Neighbouring cells are linked with one another by digit-like structures. Between the cells there are many 1800—4000 Å long desmosomes, whose lateral, intermediary electron dense part can easily be differentiated from their medial, intercellular contact layer (Fig. 13b). The cells of the stratum spinosum have centrally placed nuclei; these are round or oval and in the cells immediately above the germinative layer often lobular. Towards the stratum granulosum, the longitudinal nuclear axis is parallel with the surface of the epidermis. The nuclei of most cells are surrounded by an organelle-free zone (Fig. 6). The tonofibrils are ordered concentrically around the nucleus and are more numerous than in the stratum germinativum cells. Greater numbers and larger bundles of tonofibrils were found in specimens from 40-day and 80-day old pigs compared to younger animals (Figs 5 and 6). The constituent filaments of most tonofibrils are well-defined. Condensation of tonofibrils and the presence between them of roundish, electron dense cytoplasmic bodies, described by ZELICKSON (1967) as "membrane-coating granules" (MCG), was often seen in cells adjacent to the stratum granulosum (Fig. 7). The dense bodies were 700—2500 Å in diameter. The stratum spinosum cells contained fewer mitochondria and organelles than the stratum germinativum cells.

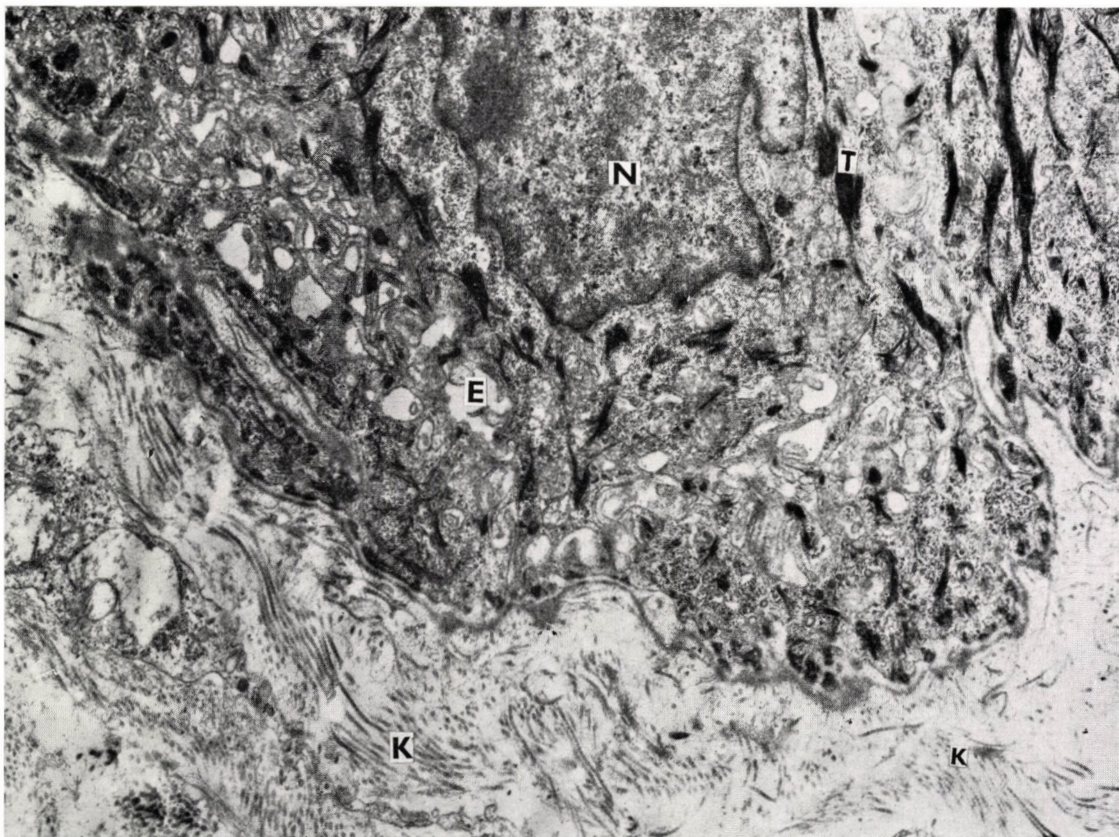

Fig. 1. Transition between epithelium and corium. Cells of the stratum basale contain tonofibrils (T), the corium contains collagen fibrils (K). The endoplasmic reticulum (Er) is dilated 10-day-old piglet. ×12,040. N, nucleus

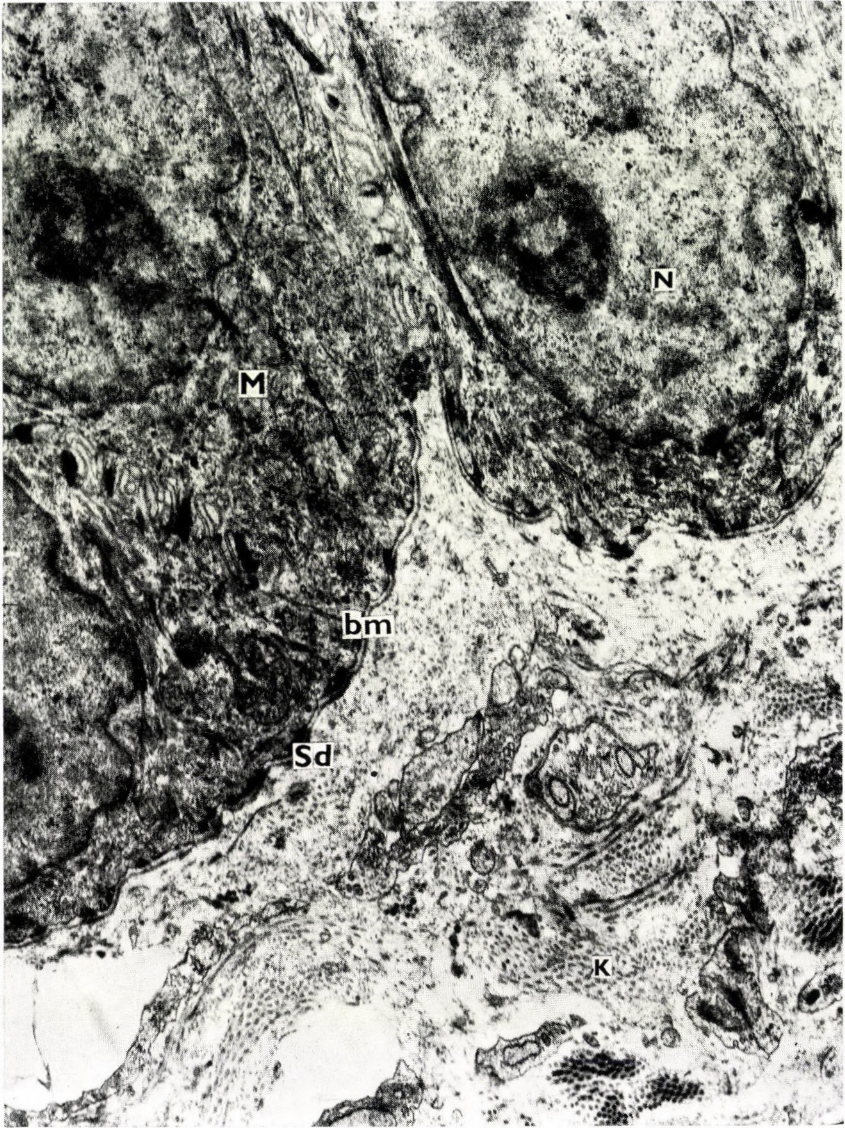

Fig. 2. Borderline between corium and epidermis; cells of the stratum germinativum. 10-day-old pig. N, nucleus; M, mitochondrion; K, collagen fibres in the corium; bm, basement membrane; Sd, semidesmosomes ×12,040.

The stratum granulosum consists of one or two cell rows. The cells have flat, oval nuclei, arranging parallel to the surface of the epidermis. The cytoplasmic tonofibrils also run parallel to the surface. The cells contain highly electron dense keratohyalin granules which are roundish and quite small at

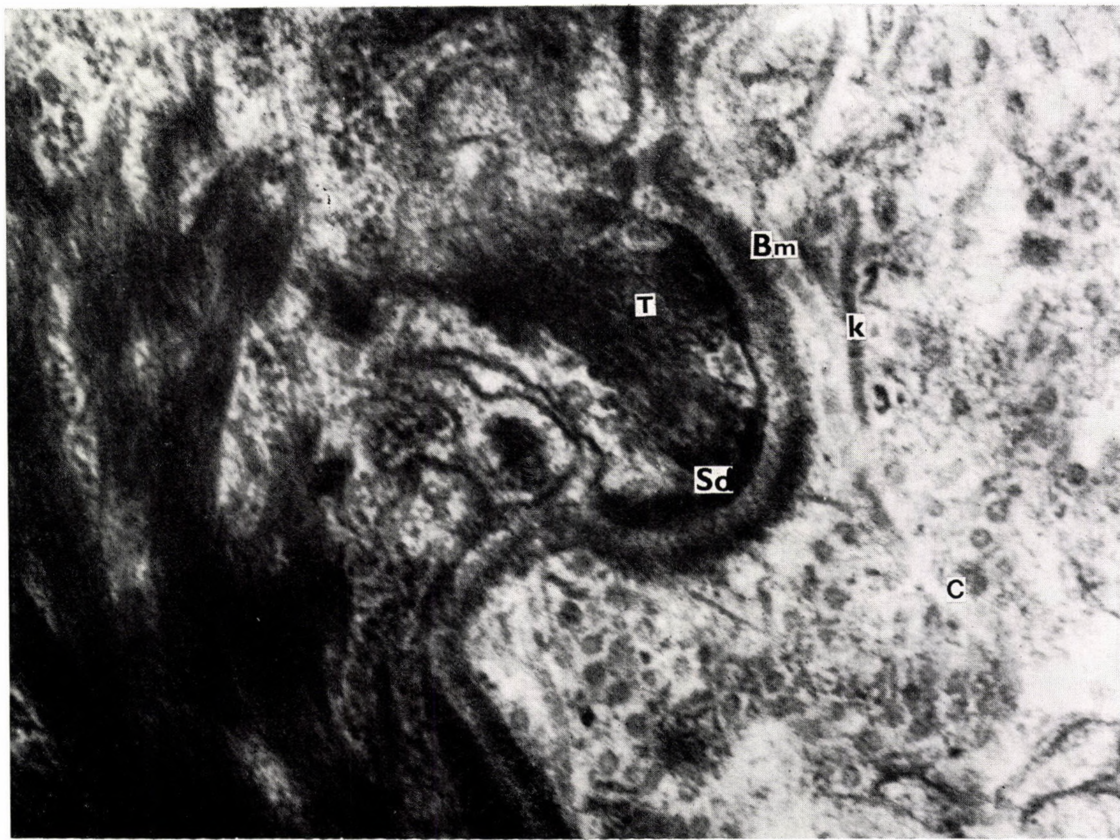

Fig. 3. Transition between epithelium and corium. Detail of a stratum germinativum cell. Note association between semidesmosome and tonofibril, widenings of the basement membrane and increase of electron density. 20-day-old pig. ×72,800. T, tonofibril; Sd, semidesmosome; C, corium; K, collagen fibres; Bm, basement membrane

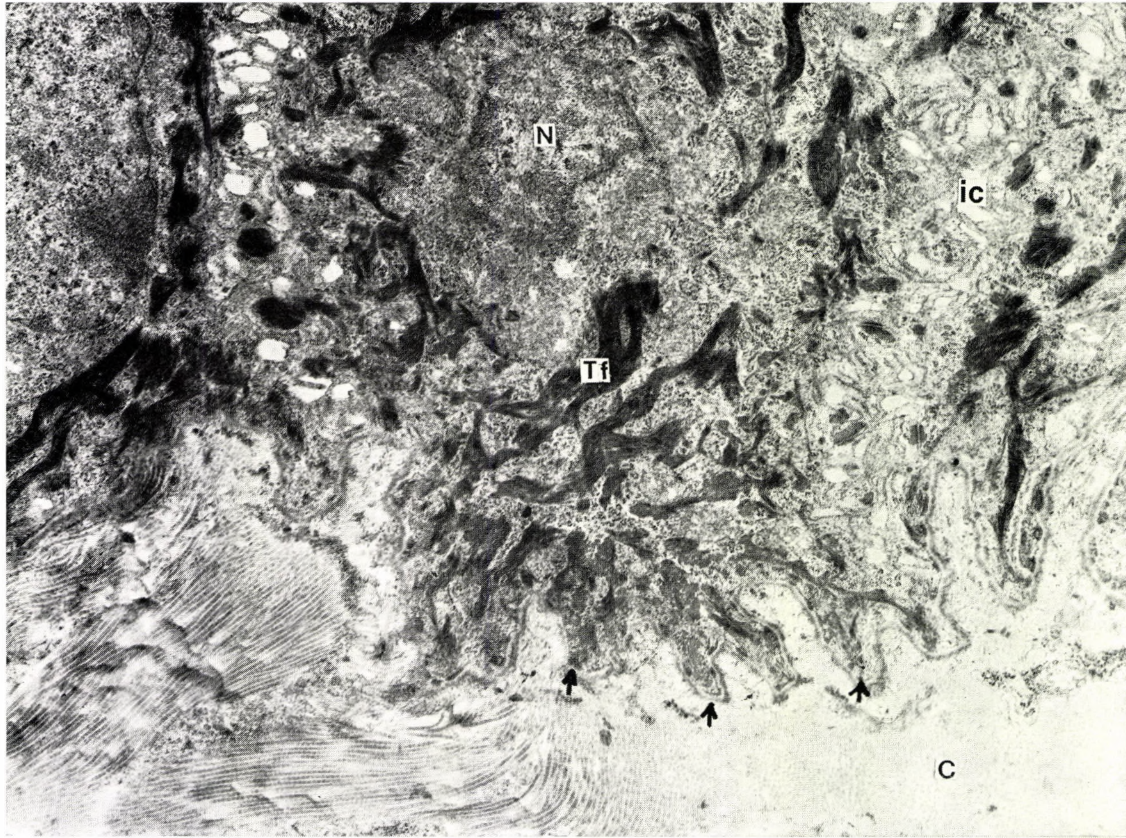

Fig. 4. Transition between epithelium and corium. The processes of stratum germinativum cells (→) are well-visible. 80-day-old pig. ×12,960. N, nucleus; Tf, tonofibril; C, corium; ic, intercellular space

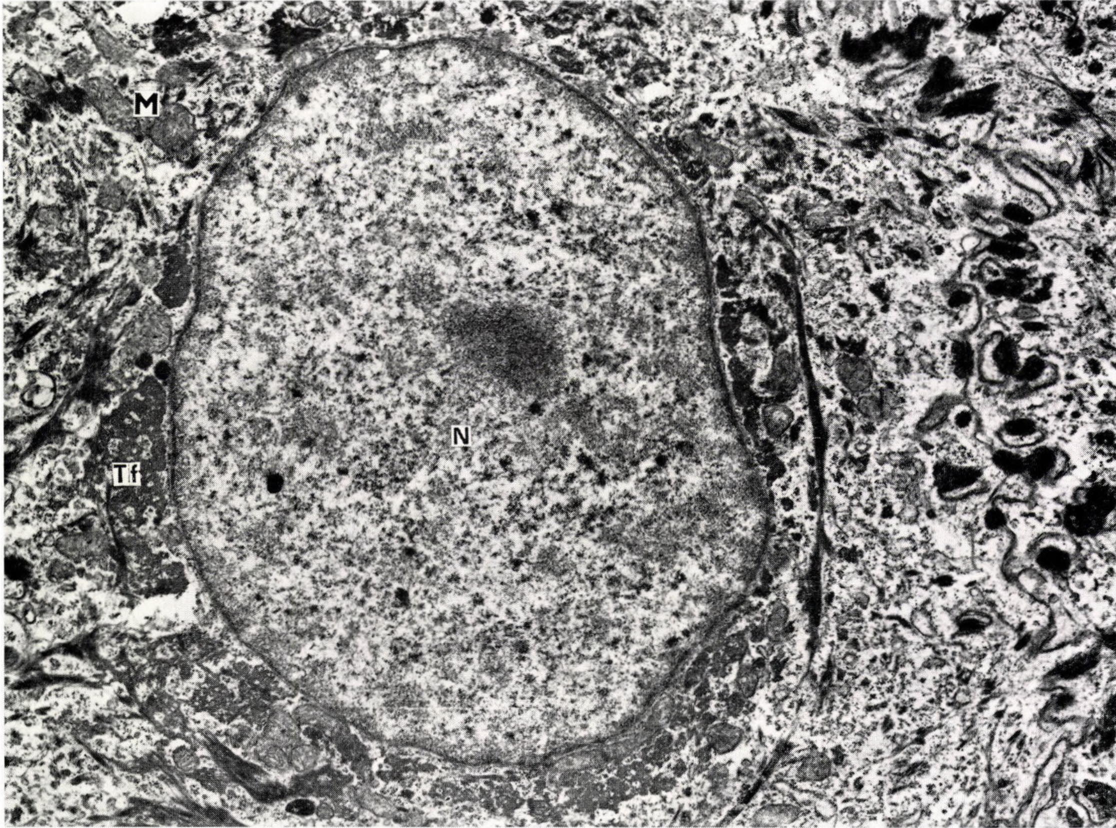

Fig. 5. Stratum spinosum cell from 2-day-old piglet. Many desmosomes link the cells and longitudinal and cross-sections of tonofibrils are seen in the cytoplasm. ×12,500. N, nucleus; M, mitochondrion; Tf, tonofibril

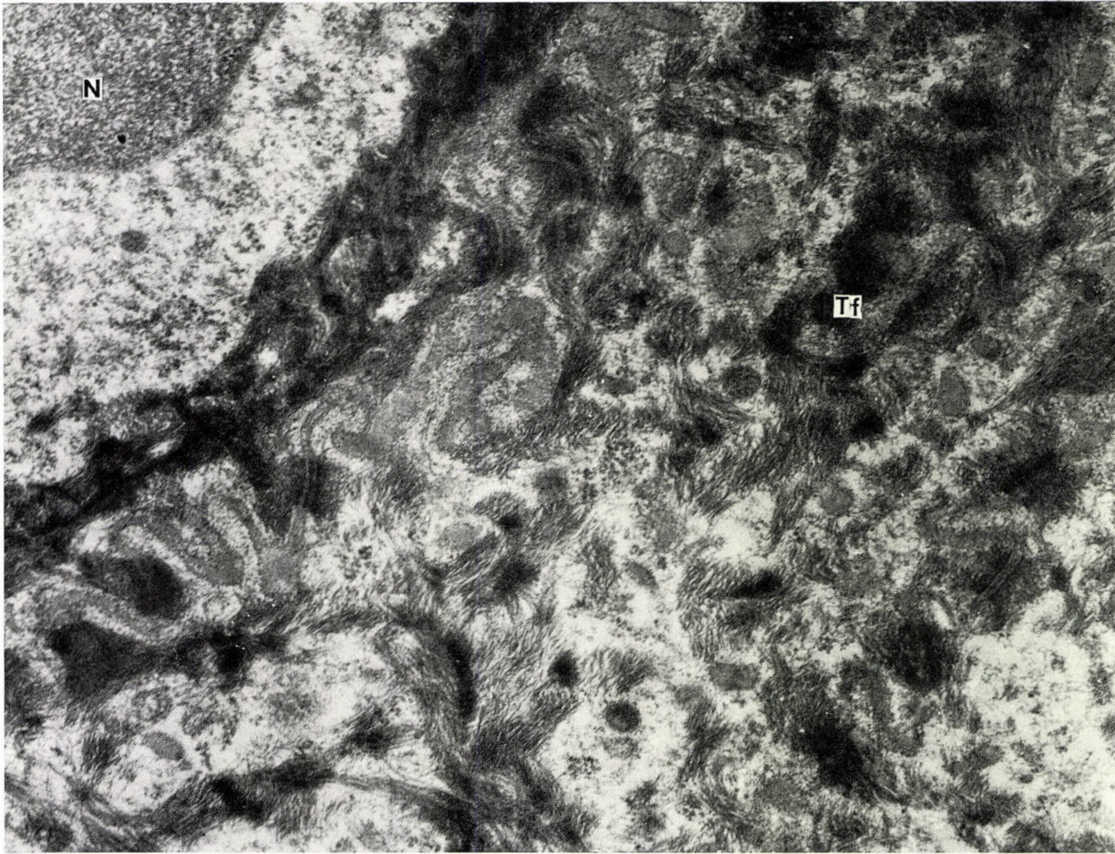

Fig. 6. Detail of stratum spinosum cell from the coronary band of the nail of a 40-day-old pig. The nucleus (N) is surrounded by an organelle-free zone, but the cytoplasm is packed with dense bundles of tonofibrils (Tf). The microfibrils are well-visible. ×25, 740

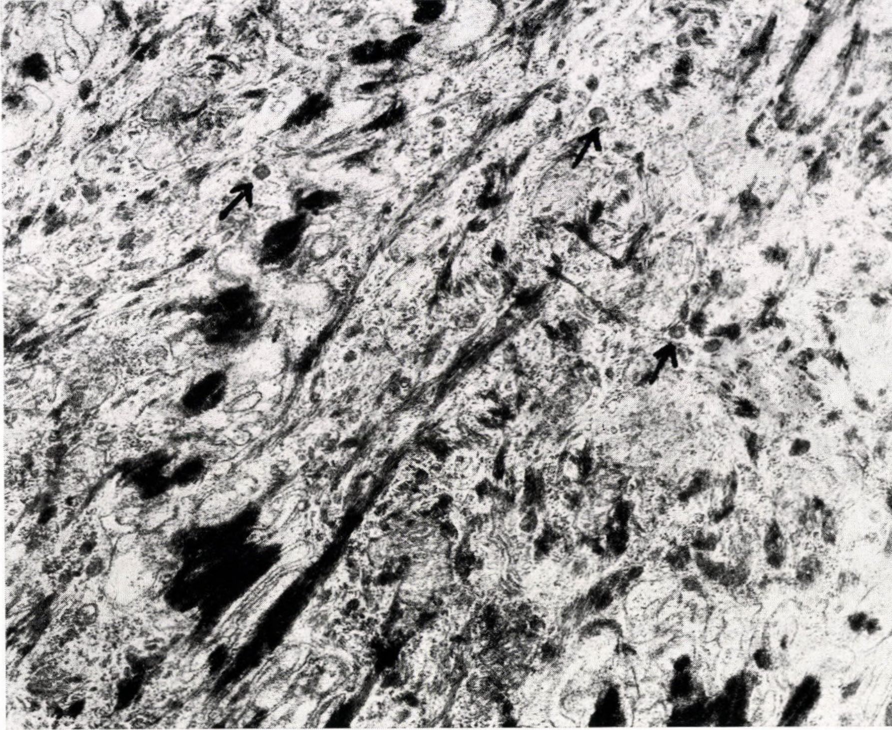

Fig. 7. Membrane-coating granules (→) in stratum spinosum cells. 10-day-old pig. ×18,500.

2 and 10 days of age, but irregular in shape and larger later (80 days) (Figs 8 and 9). In most cases, small keratohyalin granules also in the nucleus, followed by a decrease in density and loss of definition of the nuclear structure. As long as only few keratohyalin granules are present in the cell, certain organelles can still be recognized in the hyaloplasm (Fig. 9). When the granules grow larger, the cytoplasm becomes homogeneous and the organelles entirely loose definition (Fig. 8). MCG are present in a greater number than in the stratum spinosum cells.

The cell membrane is undulating, slightly thicker than usually and highly electron dense. The structure of the desmosomes corresponds essentially to that observed in the stratum spinosum (Fig. 13c).

Flat transitory cells, containing masses of keratohyalin, occasionally even keratin-like structures, are seen in many places immediately below the stratum corneum (Figs 10 and 11). The nucleus of such cells is scarcely visible, if at all. In other places fully keratinized cells are seen immediately above the stratum granulosum.

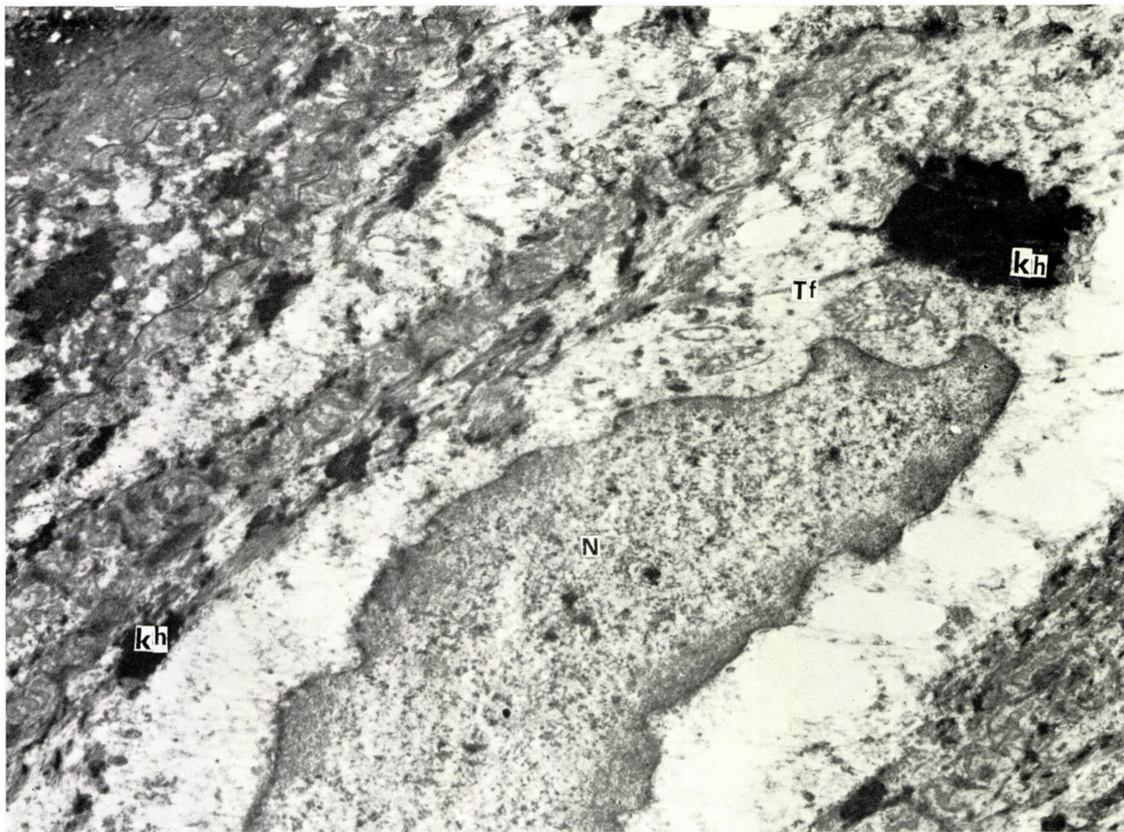

Fig. 8. Stratum granulosum cell. Note the irregularly shaped keratohyalin granules (Kh) in the cytoplasm and their association with tonofibrils (Tf). 80-day-old pig. ×12,960. N, nucleus

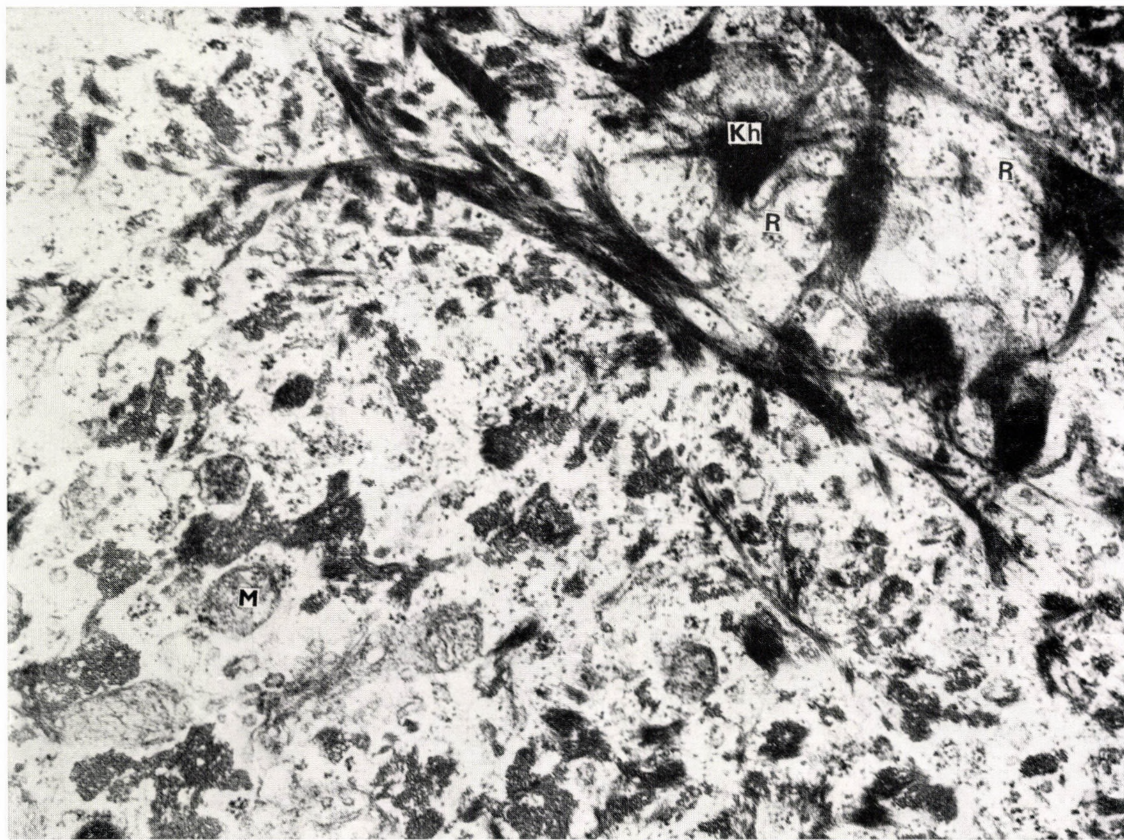

Fig. 9. Detail of stratum granulosum cell. The tonofibrils are closely associated with keratohyalin granules (Kh), and free ribosomes (R) are seen in their surroundings. Mitochondria (M) or their remnants localize nearby the nucleus. 2-day-old piglet. ×37,800

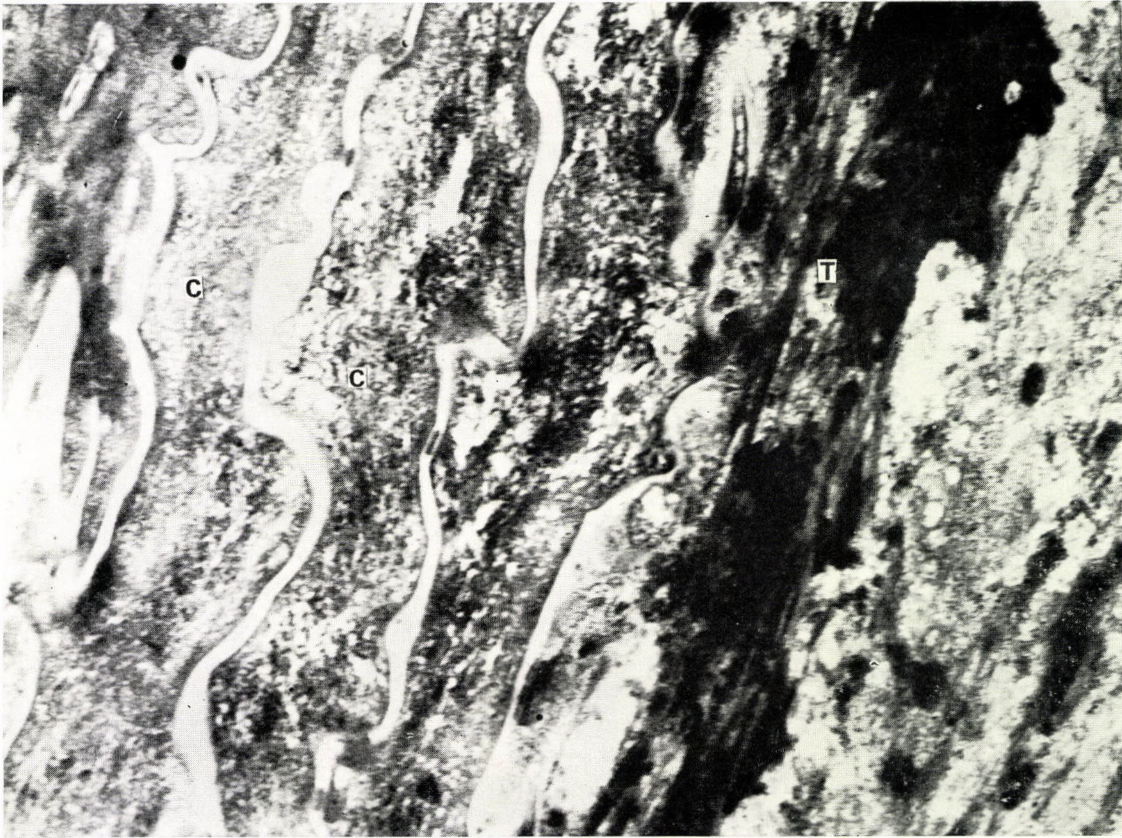

Fig. 10. Transition between stratum corneum and stratum granulosum. Note the highly electron dense keratohyalin granules in the transitory-type cells (T). Three cells of the stratum corneum (C) show a foam structure. 10-day-old pig. $\times$36,400

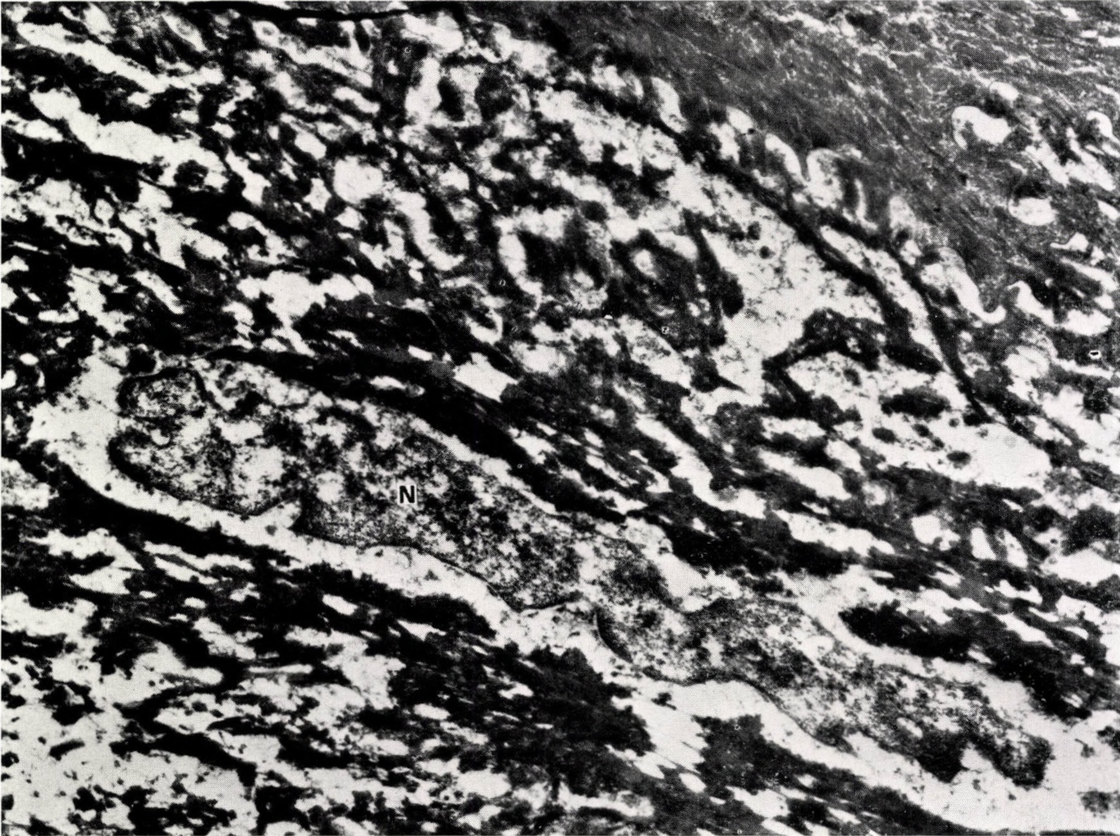

Fig. 11. Transitory-type cell rich in keratohyalin granules, even in the flattened nucleus (N). 80-day-old pig. ×12,960

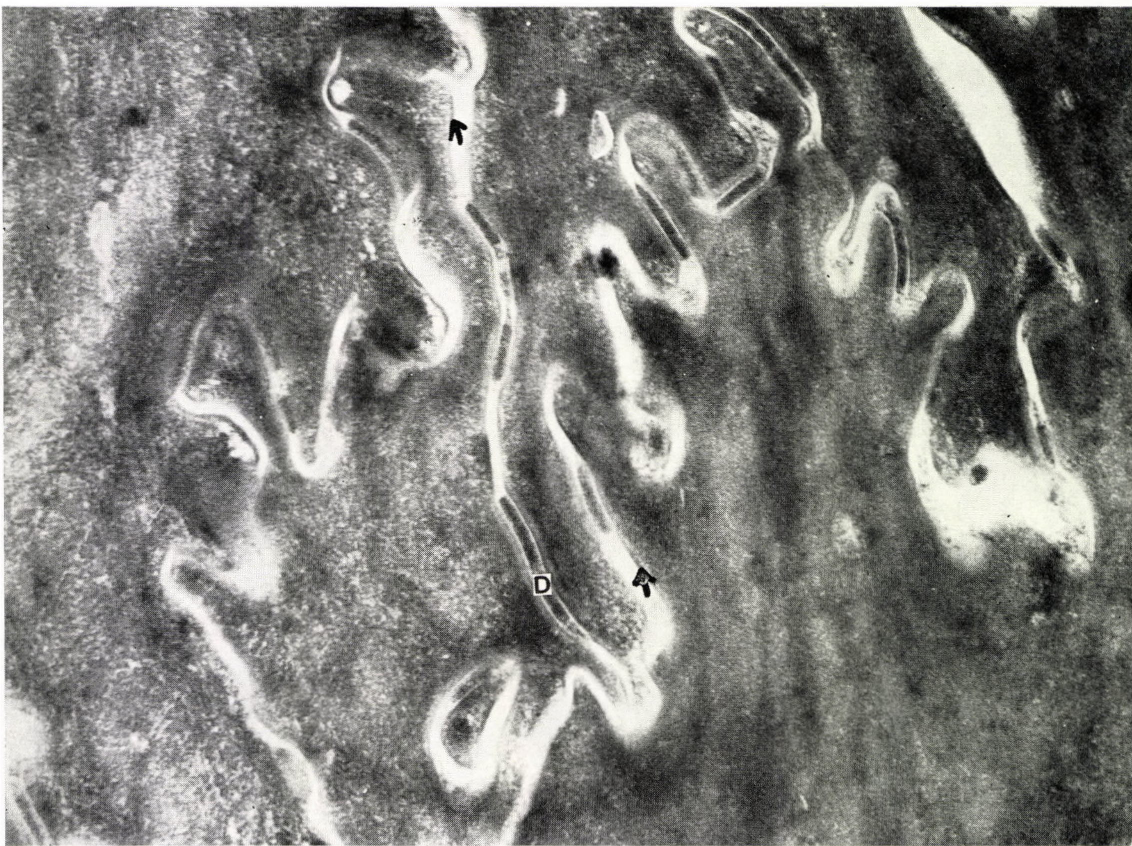

Fig. 12. Stratum corneum cells of a 20-day-old pig. The thickened cell membrane forms an electron dense layer (↑). Note the desmosomes (D) and the granular substance in the intercellular space and the keratin structure in the cytoplasm. ×12,000

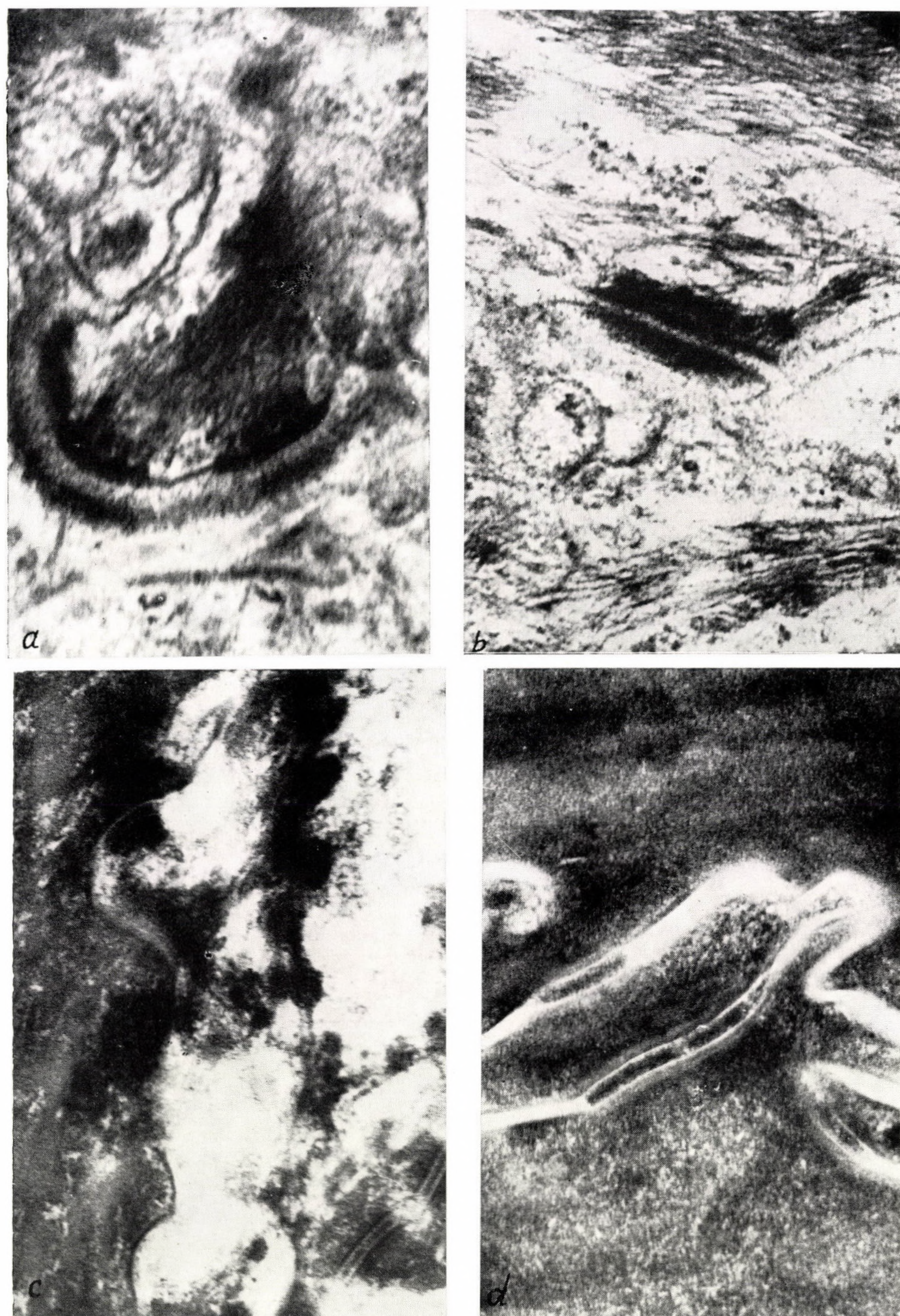

Fig. 13 a–d. Ultrastructure of semidesmosome and desmosome. *a*, in stratum germinativum (×70,000); *b*, between stratum spinosum cells (×70,000); *c*, in stratum granulosum (×54,600); *d*, between stratum corneum cells (×70,000)

The cells of the stratum corneum are quite flat, with no indication of nucleus or organelles. Deep invaginations alternate with processes on the surface of such cells (Fig. 12). The intercellular space contains a finely granular, in places vacuolar, substance and many desmosomes. The latter differ ultrastructurally from the desmosomes of the spinosum and granulosum cells; they have a highly electron dense central core which is connected with the cell membranes on both sides by a thin, less dense layer (Fig. 13d). The cell membranes appear slightly thickened and highly electron dense, merging with the similarly dense cytoplasmic keratin. The structure of the keratin can be recognized in some cells, but the cytoplasm is in places loose and spongious; other cells appear to consist of an entirely homogeneous, highly electron-dense substance in which keratin appears as a structureless mass.

Discussion

Electron micrographs of the coronary band of the foot horn of young (2—80 days old), healthy pigs show that the epithelium and corium are separated by a basement membrane about 500 Å in thickness. This follows the contours of the epithelial cells and is accordingly more or less undulating. Many semidesmosomes link the basement membrane with the epithelial cells.

The processes linking the stratum germinativum cells with the corium are more developed in older animals than in very young ones.

The epithelial cells of certain layers of the coronary epidermis differentiate for a special function. The stratum germinativum cells are of medium electron density and characterized by the presence of mitochondria and ribosomes, an active Golgi apparatus and dilated endoplasmic reticulum. They also contain tonofilaments and tonofibrils, which increase in number with the age. The variety of cell organelles indicate a high activity and functional capacity of the basal cells. The stratum germinativum of the skin of miniature pigs was found to contain cells of different density and structure (Karasek and Oehlert, 1968). No such phenomenon was observed in the present study of swine coronary band; ultrastructural dissimilarities of the same type of cells may probably result from the different methods of processing.

The increase of tonofilaments and their aggregation to tonofibrils, during which the cell organelles tend to vanish, begins in the stratum spinosum cells. Apart from the free perinuclear zone, these cells contain many bundles of tonofibrils, especially in older (80-day) animals. This indicates that the mechanical strain of the horny epithelium of the foot tends to increase with age and the structural development of the epithelial cells takes place accordingly. Like in the skin of miniature pigs (Karasek and Oehlert, 1968) the

constituent filaments of tonofibrils were generally well-visible in the stratum spinosum of the coronary band.

It should be mentioned that a condensation of the cytoplasmic tonofibrils had already taken place in certain areas of the upper stratum spinosum layers.

Apart from the numerical increase in tonofibrils, cytoplasmic "membrane coating granules" (MCG) appeared in the spinosum and granulosum cells in the further phase of keratinization. The granules increased in number in the area of the stratum granulosum and aggregated along the cytoplasmic membrane. This phenomenon clearly indicates that the granules pass into the intercellular space during the further process of keratinization. The manner of their escape from the cell is still a matter of dispute. Several investigators (Matoltsy, 1966; Rupec and Braun-Falco, 1965; Zelickson, 1967) hold the view that the cytoplasmic granules fuse with the cell membrane, causing its transformation and thickening, and therafter pass into the intercellular space to form the protective or cementing coat of the horny layer. Others (Frei and Sheldon, 1961) are of the opinion that the cell membrane invaginates to help the escape of the granules.

The most characteristic event of the ultrastructural course of keratinization was the appearance of keratohyalin granules in the granulosum cells of the epidermis. At a young age (2—20 days) the keratohyalin bodies were small and roundish or oval, subsequently (80 days) larger, and irregular in shape. Regardless of age, the keratohyalin was highly electron dense and different amounts of it were present in the cells, while cell organelles or their residues were still seen. The close association of the keratohyalin granules with tonofibrils and ribosomes was clearly seen in most cells. It is known that the keratohyalin granules of the stratum granulosum play an important role in keratinization. It has been suggested that the keratohyalin bodies are incorporated in the tonofibrils to form the interfilamentary substance of keratin. According to an alternative hypothesis keratohyalin is the direct precursor of keratin (Sugár, 1966).

According to Horstmann (1961), the stratum granulosum is absent over the coronary band and keratinization takes place in this area without keratohyalin formation. We saw both the granulosum layer and keratohyalin-containing cells in this study.

The final issue of the specialized transformation of epithelial cells can be seen in the stratum corneum. Transition of the granulosum layer into the keratinized layer was seen to occur in places by the development of so-called transitory cell types; in other places the transition was direct, without transitory forms. The epithelial cells of the hornified layer had become quite flat, with undulating margins, and a regular pattern of keratin structure filled the cytoplasm. Although some of the stratum corneum cells had a

spongious structure with an indistinct pattern of keratin, no layers of different cell types could be differentiated in contrast to the observation of others (BRODY, 1959; KARASEK and OEHLERT, 1968; NIX et al., 1964) in different material.

Parallel to the differentiation of the layers of the epidermis, the desmosomes (Fig. 13) developed as described in detail in the foregoing.

The keratinization processes demonstrated by electron microscopy in the epidermis of the coronary band of the swine foot correspond essentially to those observed in the epidermis of humans and different animals, except that the mechanical strain increasing with age induces characteristic structural changes, including an extraordinarily powerful development of the epitheliofibrils within the epidermis cells.

It appears that the mechanical factors related to active movement have a decisive role in the appropriate progress of keratinization and through it on the normal production of horny matter.

*

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SUMMARY

Specimens of the coronary band of foot horn of pigs aged 2—80 days were examined electron-microscopically. The relations between epidermis and corium (Figs 1—3) and the cell constituents of the germinative (Figs 1—4), spinosum (Figs 5—7), granulosum (Figs 8—11) and corneum (Fig. 12) layers of the epidermis are described. The ultrastructure and specialized transformation of the cells are analyzed and the process of keratinization is followed up electron microscopically with regard to age. Observations on the transformation of desmosomes, which link the cells with one another in certain layers of the epidermis, are also reported (Fig. 13/a, b, c).

The ultrastructural details were found to resemble those described by others for the epidermis of man and different animals, except that the swine coronary band undergoes a specialized structural transformation under the influence of the increase of mechanical strain with age. The main characteristic of this structural change is an increased formation of epithelio-fibrils (tonofibrils). It is concluded that the normal progress of keratinization and normal production of horny matter are greatly influenced by the mechanical factors related to active movement.

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ISOLATION OF *ACHOLEPLASMA AXANTHUM* FROM SWINE

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The following members of *Mycoplasmatales* have been isolated so far from swine: *Mycoplasma hyorhinis* (SWITZER, 1955; DINTER et al., 1965), *M. granularum* (SWITZER, 1964), *M. hyopneumoniae (suipoeumoniae)* (MARE and SWITZER, 1965; GOODWIN et al., 1967) and *M. hyosynoviae* (ROSS and DUNCAN, 1970; FRIIS, 1970); there are only few publications concerning the occurrence of *Acholeplasma* species in swine (DINTER et al., 1965). In contrast to this in our countries *Acholeplasma* could be isolated very often from swine (SCHIMMEL and PUSTOVAR, 1971; ROMVÁRY et al., 1971).

So far three sterol-nonrequiring *Acholeplasma* species (EDWARD and FREUNDT, 1970) are known; *A. laidlawii*, *A. granularum* and *A. axanthum* (TULLY and RAZIN, 1968, 1969). In contrast to the members of the first two species occurring very often in natural circumstances, only two strains of *A. axanthum* have been isolated up to now, both (S-410 and S-743) from mouse leukaemia cell lines (FRIEND et al., 1966a, b) and there is no information about the occurrence of this species in animals.

In earlier studies several sterol-nonrequiring *Acholeplasma* strains were isolated. Their serological examination including tube agglutination, slide agglutination read by phase-contrast microscopy and agar-gel precipitation showed strains of different antigenic structure (MARTIN et al., 1968; SCHIMMEL and PUSTOVAR, 1971).

During more detailed examination of the strains and their comparison with international reference *Acholeplasma* strains we found two *A. axanthum* strains. Results of this examination are presented in this paper.

Materials and Methods

Strains. Strains $D_1/58$, a strain isolated from the joint of a pig, and $D_3/1796$, isolated from the nasal cavity of a pig, were obtained from the collection of one of us (D. SCH.).

After opening the ampoules the strains were rehydrated and inoculated on to BE agar medium and into BE fluid medium. The 5-day-old fluid cultures were filtrated through Millipore filter (GSWP 02500,25 ea GS 0.22), filtrates were spread on agar medium, plates were cultivated in candle jar for 5 days. Then 2 subcultures were made from two single colonies. The subcultures were cloned 3 times including filtration of fluid cultures through Millipore filter each time. Subcultures obtained in this way were used for further examination.

For comparison of the strains studied the following reference strains were used: *A. laidlawii* A PG 8, *A. laidlawii* B F-8, *A. granularum* (Friend), *A. axanthum* ATCC 25176, and *A. sp.* PG 49 (Squire). The reference strains were obtained from the FAO/WHO International Reference Centre for Animal Mycoplasmas (Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark).

Media

The cultural properties of the strains were studied on the BE medium used in the WHO Centre: 90 ml of Heart Infusion Broth (DIFCO), or Heart Infusion Agar (DIFCO) prepared according to the instruction, 20 ml of inactivated horse serum, 10 ml yeast extract (Hayflick-type), 1.0 ml 10% thallium acetate (FISHER Scientific Company, USA), 1.0 ml 1% 2,3,5-triphenyl tetrazolium chloride (MERCK, BRD), 1.2 ml 0.2% deoxyribonucleic acid from calf thymus (SIGMA, USA), 0.25 ml 200,000 U/ml penicillin.

Antigens for indirect haemagglutination and immunization and agar cultures for immunofluorescence were prepared in BE medium without serum. Various modifications were used for the growth inhibition, metabolic inhibition and for the different biochemical and resistance tests.

Examination of biochemical properties and resistance

The following properties were examined: glucose (aerobic and anaerobic conditions), mannose, galactose, sucrose, cellobiose, mannitol, xylose splitting; hydrolysis of aesculin and arbutin; tetrazolium chloride, methylene blue, potassium tellurite reduction, "film and spot"-formation, phosphatase production, serum digestion, cholesterol requirement. The resistance tests included examination of sensitivity to 3% sodium chloride, 0.02% methylene blue, 0.2% Tween 80, 0.5% sodium taurocholate, 0.01% sodium azide, 5% sodium polyanethol sulphonate, digitonin, erythromycin, kanamycin, polymyxin B, pH 5.5 and pH 9.5.

Serological tests

The strains were examined in growth inhibition, metabolic inhibition (Taylor-Robinson et al., 1964), immunofluorescence (Rosendal and Black, 1972) and indirect haemagglutination (Krogsgaard, 1972), using *A. laidlawii* A PG 8, *A. laidlawii* B F 8, *A. granularum* (Friend), *A. axanthum* (ATCC 25176) and *A. sp.* PG 49 (Squire) reference sera obtained from the WHO Centre (Aarhus, Denmark).

Results

Cultural properties

Both D_1/58 and D_3/1796 strains produced well-visible colonies within 5 days on BE agar plates. On the same medium *A. laidlawii* A and *A. laidlawii* B strains colonies appeared within 1—2 days, *A. granularum* (Friend) and *A. axanthum* (ATCC 25176) and *A. sp.* PG 49 (Squire) within 2—3 days. The strains grew better under O_2 deficient conditions in candle jar, in the presence of CO_2, than in O_2-rich conditions. They produced "fried-egg" colonies on BE plates with 20% serum. On the plates with PPLO Serum Fraction or without it the peripheral zones of the colonies were very narrow, or only the central part of the colonies were seen. Similar properties were observed only with the *A. axanthum* (ATCC 25176) reference strain. Maximal CFU reached in BE fluid medium was 1×10^6 in 1.0 ml, at the same time control reference strains produced 1×10^8 and 1×10^9 CFU/ml.

To exclude L-form of bacteria D_1/58 and D_3/1796 strains were cultivated in the same medium without penicillin and thallium acetate over 10 passages. Neither bacterium-like colonies on agar plates, nor bacterium-like forms in Gramstaining were observed at the end of the passages.

Biochemical properties

According to their biochemical behaviour the strains studied reminded of *A. axanthum* (Table I). They decomposed glucose under both aerobic and anaerobic conditions, hydrolysed aesculin. They were mannose-, sucrose-, galactose-, mannitol-, xylose-, arginin- and urea-negative. Both strains reduced tetrazolium chloride under aerobic and anaerobic conditions, but potassium tellurite, they reduced methylene blue only under aerobic conditions. They did not digest serum and produced neither "film and spot", nor phosphatase. They grew at 22 °C and on agar plates without serum, but the maximal CFU/ml on such plates was by 1—2 log lower than in serum-containing medium. The strains did not require cholesterol.

D_1/58 strain differed from reference *A. axanthum* (ATCC 25176) strain in the absence of arbutin-hydrolysing ability. The D_3/1796 strain hydrolysed arbutin but it did not split cellobiose and xylose.

Results of resistance tests

Both strains were resistant to Tween 80, sodium azide, digitonin and sodium polyanethol sulphonate, but they proved to be sensitive to sodium chloride, sodium taurocholate, methylene blue and pH 9.5. The strains were

Table I

Results of biochemical assays

Tests		$D_1/58$ strain	$D_3/1796$ strain	*A. axanthum* (ATCC 25176) reference strain
Decomposition of glucose	aerobic	+	+	+
	anaerobic	+	+	+
Decomposition of mannose		—	—	—
Decomposition of sucrose		—	—	—
Decomposition of galactose		+	+	+
Decomposition of mannitol		—	—	—
Decomposition of cellobiose		+	—	+
Decomposition of xylose		—	+	—
Decomposition of sorbitol		—	—	—
Aesculin		+	+	+
Arbutin		—	+	+
Decarboxylation of arginine		—	—	—
Hydrolysis of urea		—	—	—
"Film and spot" formation		—	—	—
Growth at 22°C		+	+	+
Serum digestion		—	—	—
Phosphatase		—	—	—
Reduction of tetrazolium	aerobic	+	+	+
	anaerobic	+	+	+
Reduction of methylene blue	aerobic	—	—	—
	anaerobic	+	+	+
Reduction of potassium tellurite	aerobic	—	—	—
	anaerobic	—	—	—
Cholesterol requirement		—	—	—

Table II

Resistance tests

Tests	$D_1/58$ strain	$D_3/1796$ strain	*A. axanthum* (ATCC 25176) reference strain
NaCl	S	S	S
Sodium azide	R	R	R
Sodium taurocholate	S	S	S
Methylene blue	S	S	S
Tween 80	R	R	R
Sodium Polyanethol Sulphonate	R	R	R
Digitonin	R	R	R
Erythromycin	22 mm	24 mm	17 mm
Kanamycin	8.0 mm	9.0 mm	7.0 mm
Polymyxin B	—	—	—
pH: 5.5	S	R	S
pH: 9.5	S	S	S

Symbols used: S, sensitive; R, resistant

very sensitive to erythromycin, but resistant to polymyxin B. Their sensitivity to kanamycin was weak. D_3/1796 strain differed from reference *A. axanthum* (ATCC 25176) strain in its resistance to pH 5.5 (Table II).

Results of serological examination

Both strains were inhibited only by *A. axanthum* (ATCC 25176) reference serum in the growth inhibition test (Table III). The reaction was weak but it could be regarded as positive because it was detected with all the three dilution and on all the four media.

Table III

Growth inhibition tests

Strains	Reference sera				
	A. axanth. ATCC 25176	*A. laidl.* A PG 8	*A. laidl.* B F-8	*A. gran.* (Friend)	*A. sp.* PG 49 (Squire)
D_1/58	1.5 mm	—	—	—	—
D_3/1796	1.5 mm	—	—	—	—
A. axanthum (ATCC 2 5176)	4.0 mm	—	—	—	—
A. laidlawii A PG 8	—	3.0 (part)	2.0	0.5	—
A. laidlawii B F 8	—	2.5 (part)	4.5	0.5	—
A. granularum (Friend)	—	—	—	3.0	—
A. sp. PG 49 (Squire)	—	—	—	—	4.0

The strains reacted only with the *A. axanthum* (ATCC 25176) reference serum in the immunofluorescence test, too (Table IV). In this test the reaction was also weaker than with the homologous antigen. Antigens prepared from the strains were agglutinated in the indirect haemagglutination test by *A. axanthum* (ATCC 25176) reference serum. At the same time other *Achole-*

Table IV

Immunofluorescence tests

Strains	Reference sera				
	A. axanth. (ATCC 25176)	*A. laidl.* A PG 8	*A. laidl.* B F-8	*A. gran.* (Friend)	*A. sp.* PG 49 (Squire)
D_1/58	1 : 40+++	—	—	—	—
D_3/1796	1 : 40++	—	—	—	—
A. axanthum (ATCC 25176)	1 : 80+++	—	—	—	—
A. laidlawii A PG 8	—	1 : 80+++	1 : 320+++	—	—
A. laidlawii B F 8	—	1 : 80++	1 : 640+++	—	—
A. granularum (Friend)	—	—	—	1 : 20++	—
A. sp. PG 49 (Squire)	—	—	—	—	1 : 80+++

Table V

Indirect haemagglutination

Antigen	Reference sera				
	A. axanth. ATCC 25176	*A. laidl.* A PG 8	*A. laidl.* B F-8	*A. gran.* (Friend)	*A. sp.* PG 49 (Squire)
$D_1/58$	1 : 40	—	—	—	—
$D_3/1796$	1 : 80	—	—	—	—
A. axanthum (ATCC 25176)	1 : 1280	—	—	—	—
A. laidlawii A PG 8	—	1 : 4096	1 : 4096	1 : 20	—
A. laidlawii B F 8	—	1 : 4096	1 : 8192	1 : 20	—
A. granularum (Friend)	—	1 : 256	1 : 256	1 : 4096	—
A. sp PG 49 (Squira)	—	—	—	—	1 : 1280

plasma reference sera did not react with them (Table V). Again, the titres were weaker than the titres with the homologous antigen.

In the metabolic inhibition test the strains studied were inhibited only by the *A. axanthum* (ATCC 25176) reference serum (Table VI).

Table VI

Metabolic inhibition tests

Antigen	Reference sera				
	A. axanthum (ATCC 25176)	*A. laidl.* A PG 8	*A. laidl.* B F-8	*A. gran.* (Friend)	*A. sp.* PG 49 (Squire)
$D_1/58$	1 : 320	—	—	—	—
$D_3/1796$	1 : 640	—	—	—	—
A. axanthum (ATCC 25176)	1 : 1280	—	—	1 : 10	—
A. laidlawii A PG 8	—	1 : 1280	1 : 1280	—	—
A. laidlawii B F 8	—	1 : 1280	1 : 1280	—	—
A. granularum (Friend)	—	—	—	1 : 5120	—
A. sp. PG 49 (Squire)	1 : 10	1 : 10	1 : 20	1 : 10	1 : 640

To prove the results of typing, hyperimmune serum was prepared to the $D_3/1796$ strain and tested against the *A. axanthum* (ATCC 25176) reference strain and the $D_1/58$ strain. The inhibition zone produced by the $D_3/1796$ antiserum was 6.0 mm with the homologous strain, while 2.0 mm with $D_1/58$ strain and 3.0 mm with *A. axanthum* (ATCC 25176) reference strain. Positive reaction was obtained in other tests, too.

Conclusions

Investigation of cholesterol requirement of the tested strains and their resistance to sodium polyanethol sulphonate and digitonin and their ability to grow at 22 °C proved that they were Acholeplasmas. Their cultural and

morphological properties were similar to that of the *A. axanthum* (ATCC 25176) reference strain. In the serological tests the D_1/58 and D_3/1796 strains reacted only with the *A. axanthum* (ATCC 25176) reference serum and not at all with other *Acholeplasma* sera. The reactions were consistent though, weaker than with the homologous antigen. So our strains should be regarded as *A. axanthum.* This conclusion is supported by biochemical similarity between our strains and the reference strains. Divergences in some tests in comparison with the reference strain: as absence of arbutin-hydrolysing ability of D_1/58 strain or presence of xylose-splitting ability, by absence of cellobiose decomposing effect and sensitivity to pH 5.5 of D_3/1796 strain can explain the weaker serological reactions. The fact that our strains slightly differed from each other in the biochemical and resistance tests allow to suppose an existence of several variants in this species as it is known in *A. laidlawii* species.

Our results have confirmed WILLIAMS and WITTLER's (1971) data concerning the aesculin test as a characteristic property of *A. axanthum.* Both our strains hydrolysed aesculin. New results were obtained with the arbutin test. This test was negative with most of the strains of bovine origin, but it was positive with the *A. axanthum* (ATCC 25176) reference strain and strain D_3/1796.

Until recently there was no information about the occurrence of *A. axanthum* in animals (TULLY and RAZIN, 1969). This study served the first evidence of *A. axanthum* species in swine. Since attempts to infect swine with the D_1/58 strain intranasally have failed (MARTIN et al., 1968), the pathogenic role of this species has remained uncertain.

*

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SUMMARY

On the basis of cultural, morphological, biochemical (glucose-, mannose-, mannitol-, cellobiose-, suçrose-, xylose-, aesculin-, arbutin-, arginine-, and urea-splitting, reduction of tetrazolium, methylene blue and potassium tellurite, phosphatase production, "film and spot" formation, cholesterol requirement, resistance to sodium chloride, methylene blue, Tween 80, sodium azid, sodium taurocholate, sodium polyanethol sulphonate, pH 5.5 and 9.5 and digitonin) and serological (growth inhibition, metabolic inhibition, indirect haemagglutination, immunofluorescence) properties two *Acholeplasma* strains isolated earlier from swine were indentified as *A. axanthum.*

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HISTOGENESIS OF THE ACUTE (FORM OF) MAREK'S DISEASE

By

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(Received April 2, 1973)

There are only few papers among the numerous English, German and Japanese publications (Bayer and Urbaneck, 1972; Biggs and Payne, 1967; Fletcher et al., 1971; Fujimoto et al., 1971; Gratzl and Köhler, 1968; Maas et al., 1968; Malik, 1969; Payne and Biggs, 1967; Solisch, 1972; Witter et al., 1970) dealing with the histological lesions of the acute Marek's disease (MD) which give account of the histological lesions in certain organs of chicks artificially infected with MD virus.

In 1970 and 1971 we studied the histological lesions and the time of their appearance in largescale poultry farms where the disease was constantly prevalent for years. An account was given of the results last year (Rátz et al., 1972).

Since in our earlier examinations histological lesions suggesting MD have been found in certain organs at a surprisingly young age (2- to 3-day-old animals). This prompted us to study their origin and specific character experimentally.

Materials and Methods

Eggs and day-old-chicks from acute MD-free stocks were used. After disinfection the eggs were hatched and kept in an isolator tent. The day-old-chicks were divided into 3 groups.

I. Eight chicks were inoculated intraperitoneally with 0.2 ml of MD virus marked 7/11, 55,300 PFU and one animal was killed on each of days 1, 2, 3, 4, 6, 8, 11 and 13 post infection.

II. Eight chicks were exposed to natural infection and were killed on the same days as those in group I.

III. As negative control 26 day-old chicks from a similarly MD-free livestock were used whose extraneural organs and central nervous system (CNS) were prepared in the same way. Control chicks were killed on the same days as the infected ones and, besides, on days 12, 14, 16, 18 and 20.

Material was taken in each case from the heart, both lobules of the liver, both kidneys, the glandular stomach, the spleen, the red marrow of a thigh bone and from the skin always from the same area. Besides, sagittal sections from the total brain and frontal sections from the cervical and lumbosacral intumescences in the spinal cord were examined from each animal.

The organ specimens were immediately fixed in 10% formalin solution and embedded in paraffin. Sections from all organs were stained with haemalaun-eosin. In addition, some sections were stained with methylgreen-pyronine to detect RNA.

Results

I. Initial lesions in the liver and heart of chickens artificially infected with MD virus occurred as early as on the 1st, more pronouncedly on the 2nd day after inoculation. They appeared in the wall of certain blood vessels as initial focal proliferation of round, indistinct cells (reticular cells) having vesicular nuclei. Besides reticular cell proliferation, large lymphoblasts with dark nuclei rich in chromatin were noticed in the liver (Figs 1 and 2) and especially, in the myocardium of the chicken killed 2 days after inoculation (Figs 3 and 4). From the 3rd day on the latter lesions appeared very marked,

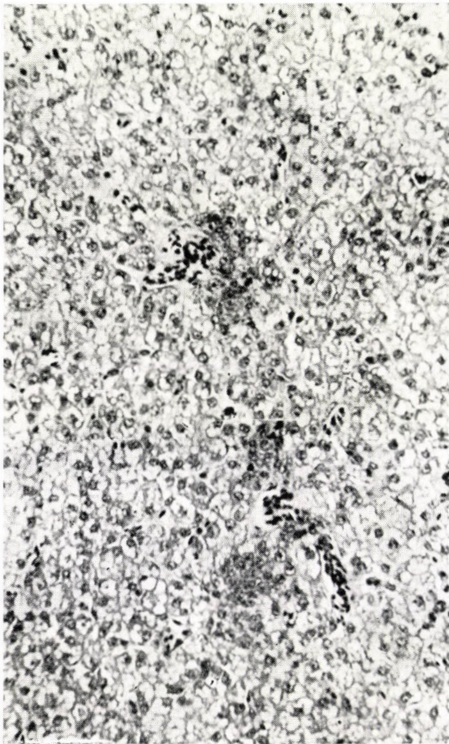

Fig. 1. Focal reticular proliferations around blood vessels in the liver on the second day after inoculation. Haemalaun-eosin. ×240

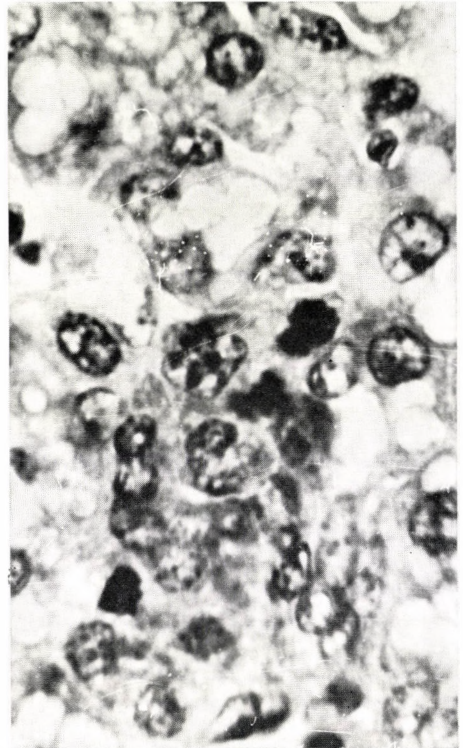

Fig. 2. Detail of Fig. 1. ×1400

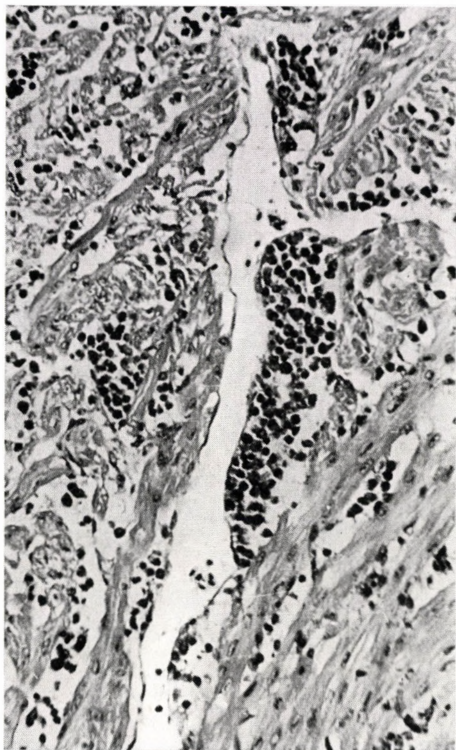

Fig. 3. Focal reticular cell and lymphoblast proliferations in the myocardium 2 days after inoculation. Haemalaun-eosin. ×240

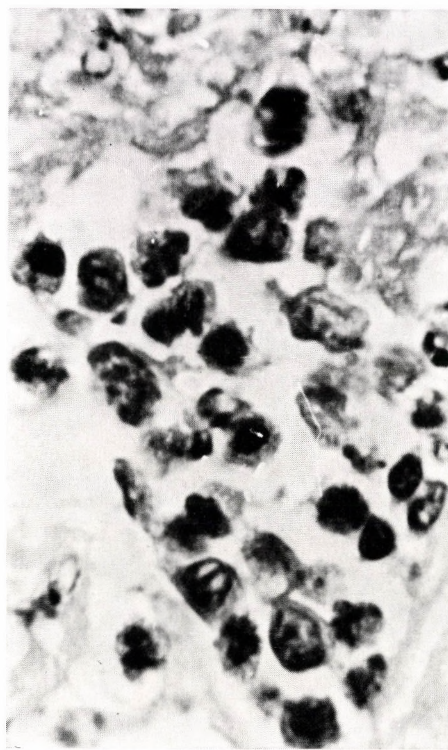

Fig. 4. Detail of Fig. 3. ×1400. Mitosis in several cell nuclei

especially in the liver (Figs 5 and 6) and in the heart (Figs 7 and 8). Mitosis was often observed in proliferating reticular cells and lymphoblasts. The cytoplasm showed pronounced pyroninophilia suggesting a high RNA content. Besides lymphoblasts, foci consisting of small, mature lymphocytes appeared in the mentioned organs along the wall of certain blood vessels of the chicken killed after 4 days (Figs 9 and 10). In the glandular stomach among the ducts of the deep propria gland of this animal initial, focal lymphoid-cell infiltrations were observed. The glandular cells nearby showed lesions of different phase (Figs 11 and 12).

Six days after inoculation numerous focal, sometimes neoplastic, tissue proliferations mainly consisting of lymphoid cells were found in the liver (Fig. 13) and in the renal parenchyma along several blood vessels (Fig. 14). Similar lesions were observed — to a lesser extent — in the myocardium and around certain blood vessels of the corium (Fig. 15). The described lesions seemed to be even more pronounced in the extraneural organs of the chickens

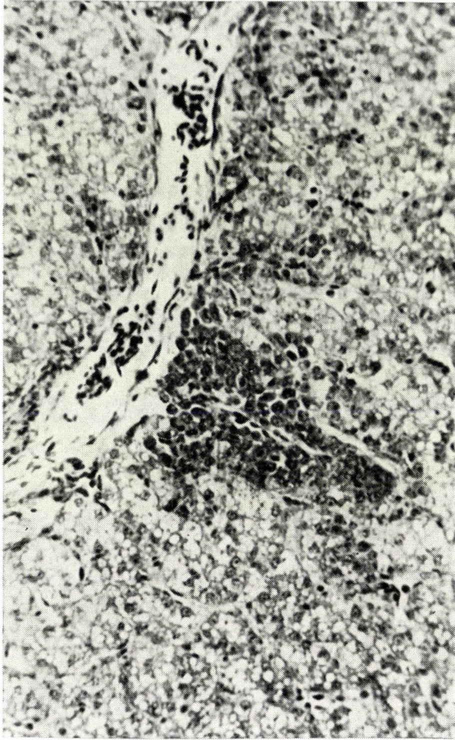

Fig. 5. Stronger focal proliferations mainly consisting of lymphoblasts in the liver 3 days after inoculation. Haemalaun-eosin. ×240

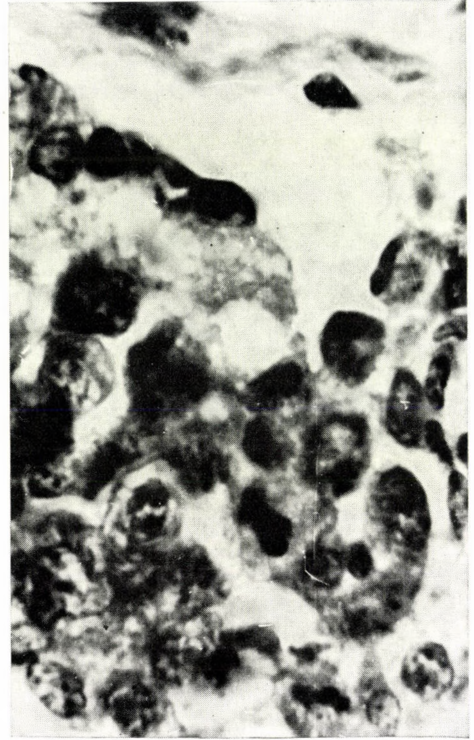

Fig. 6. Detail of Fig. 5. ×1400

killed on days 8, 11 and 13 and similar lesion were found in the kidney, bone marrow as well as in the deep propria glands of the glandular stomach.

Slight lesions — becoming more and more pronounced — were first found in the brain and in the intumescences of the spinal cord i.e., in the CNS. These were equally found in the cerebellum, in the lobus opticus, in the cerebrum and in the choroid plexus. The lesions consisted of lymphoid cell infiltrations around blood vessels which first manifested themselves in the form of foci, later in the formation of one- or two-line cuffings. In the wall of the affected small blood vessels the nuclei of endothelial cells became sometimes enlarged, swollen, in other cases they were shrunken. Most frequently they occurred in the medullary nucleus (Figs 16 and 17) and around blood vessels in the granular (Purkynje) and molecular layer of the gray substance, sometimes in the corpus medullare of the cerebellum. Lesions occurred in the blood vessels of choroid plexus in the interstitial substance as cuffings and as foci consisting of smaller or larger lymphoid cells. Cell

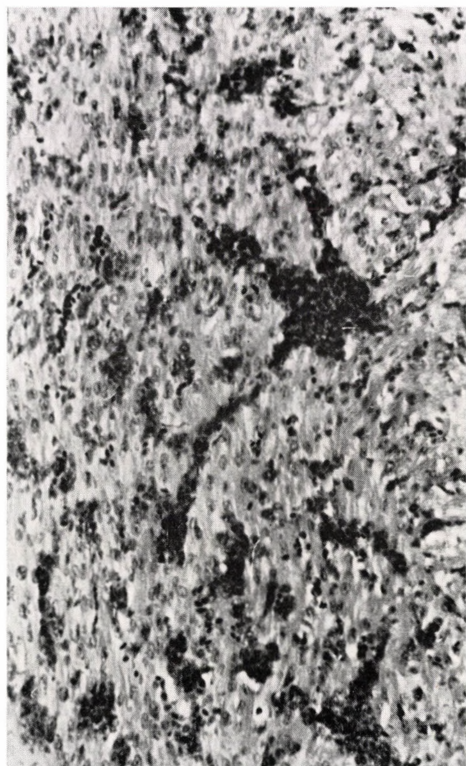

Fig. 7. Several foci of reticular and lymphoblast cell proliferation in the myocardium 3 days after inoculation. Haemalaun-eosin. ×240

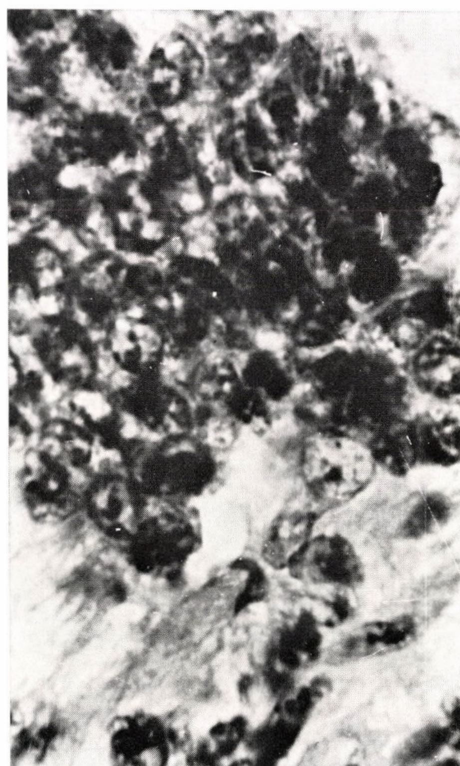

Fig. 8. Detail of Fig. 7. ×1400

infiltration or smaller foci consisting of lymphoid cells were observed around blood vessels in all layers of the optic lobe, in the optic zone, in the median zone and in the deep medullary zone (Fig. 18). Infiltrations were also found in the striatum of the cerebral hemisphere and around blood vessels in the meninges covering the cerebral hemispheres.

The lesions in the intumenscences of the spinal cord of the chicken killed 8 days after inoculation appeared mainly in the white substance or in the wall of blood vessels in the areas of the grey substance adjacent to the white substance (Fig. 19) as well as among the myelinated neurofibrils of afferent and efferent nerves. Identical lesions were found around some blood vessels in the lumbosacral organ of the lumbosacral intumescences. Besides the described lesions, lymphoid cell infiltrations occurred among the ganglion cells of the spinal ganglions in the intumescences of the spinal cord of the chicken killed 11 days after inoculation. By that time the wall of some blood vessels of the spinal leptomeninx also showed similar lesions.

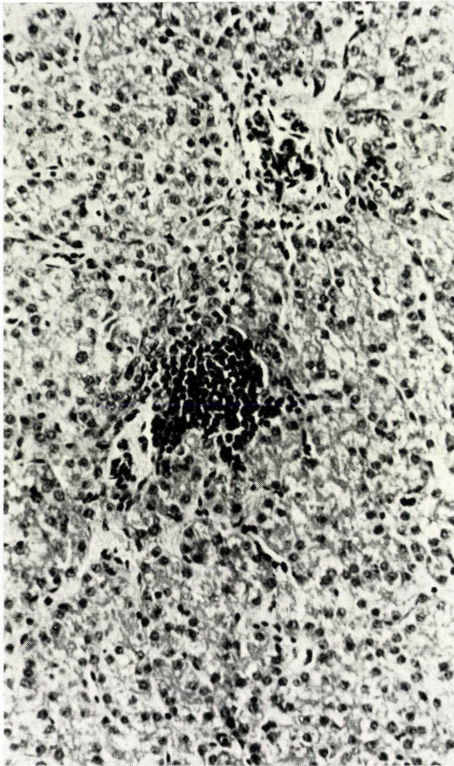

Fig. 9. Focus mainly consisting of perivascular lymphocytes in the liver 4 days after inoculation. Haemalaun-eosin. ×240

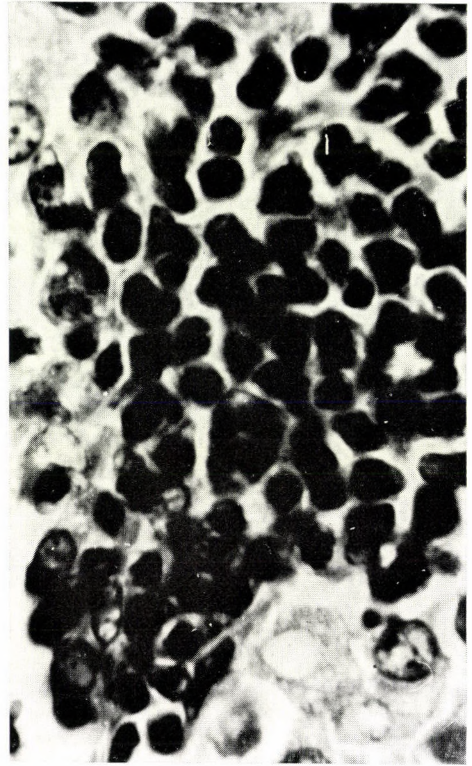

Fig. 10. Detail of Fig. 9. ×1400. The infiltration mainly consists of mature lymphocytes

II. In the same extraneural organs of naturally infected chickens lymphoreticular cell proliferation occurred similarly, but 3—4 days later than in the chickens inoculated. However, no lesion had been found in the cerebrum and in the spinal cord of these chickens up to the 13th day.

III. No lesions were found in the liver (Figs 20 and 21), heart and CNS of the control chickens.

Discussion

In the wall of the blood vessels of the liver and the heart in chickens infected strong proliferation of the active mesenchyma cells (reticular cells) was noticed as early as on the first, and even more pronouncedly on the 2nd, day after intraperitoneal inoculation. On the 3rd day numerous lymphoblasts, on the 4th day foci consisting of mature lymphoid cells appeared among the cells. The cytoplasm of the latter cells was poor, whereas in that of the

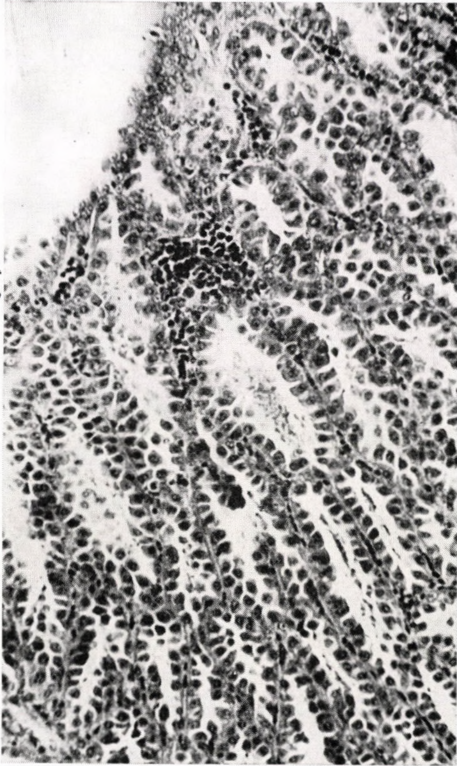

Fig. 11. Lymphoreticular cell proliferations among the ducts of the deep propria-gland of the glandular stomach 4 days after inoculation. Haemalaun-eosin. ×240

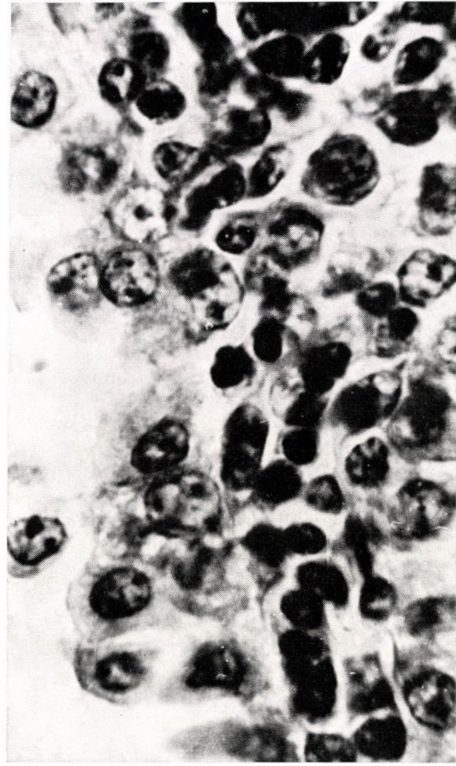

Fig. 12. Detail of Fig. 11. ×1400. Dissociated, degenerated glandular epithelial cells beside proliferating cells

former it was very rich, in RNA. This phenomenon and the numerous mitoses suggest a high degree of vitality. The lymphoreticular tissue starts an ever increasing, neoplastic proliferation from the 6th day on especially in the liver, later in other extraneural organs, too (e.g., in the heart, glandular stomach, skin, kidney, etc.). This time the neoplastic forms of an ever increasing number of lymphoid cells or reticulohistiocytes are to be found in the foci.

In the same organs of the animals exposed to natural infection similar tissue lesions were found 3—4 days later than in those of the inoculated ones. Since these were not found in the organs of the controls of the same age, it was concluded that the lesions occurring in the extraneural organs of both inoculated and naturally infected animals were caused by the MD virus. The lesions found in the extraneural organs of artificially and naturally infected chickens are regarded as a pathological process which is the partly hyper-

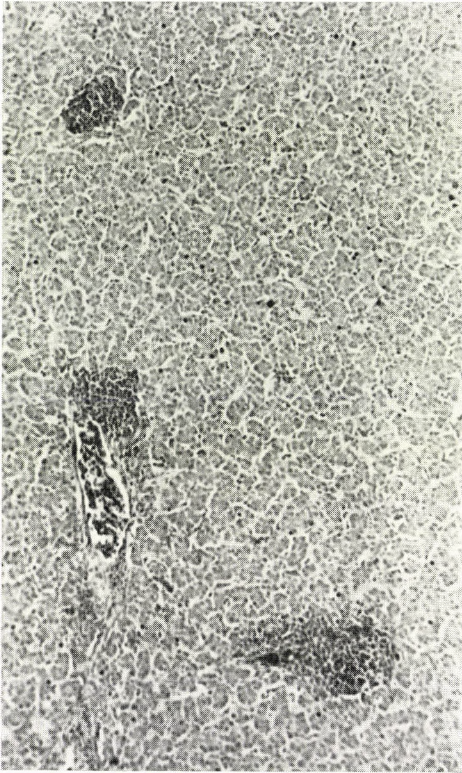

Fig. 13. Focal, lymphoreticular tissue proliferations in the liver parenchyma 8 days after inoculation. Haemalaun-eosin. ×95

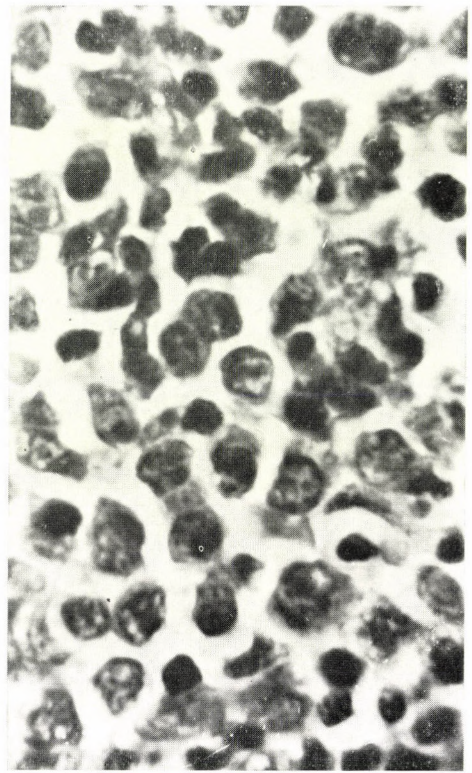

Fig. 14. Detail of Fig. 13. ×1400. Partly neoplastic proliferation of the lymphoreticular tissue cells

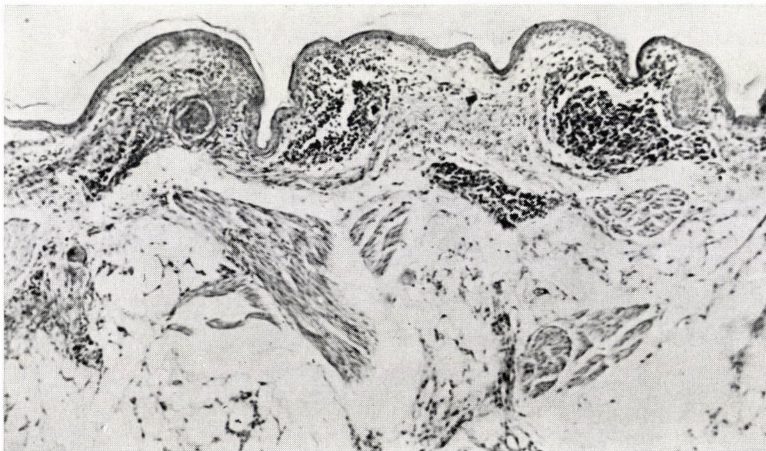

Fig. 15. Focal cell proliferations in the corium and subcutis of skin 8 days after inoculation. Haemalaun-eosin. ×95

Fig. 16. Lymphoid cell vessel wall infiltrations in the white substance of the cerebellum in the granular and molecular layer 8 days after inoculation. Haemalaun-eosin. ×95

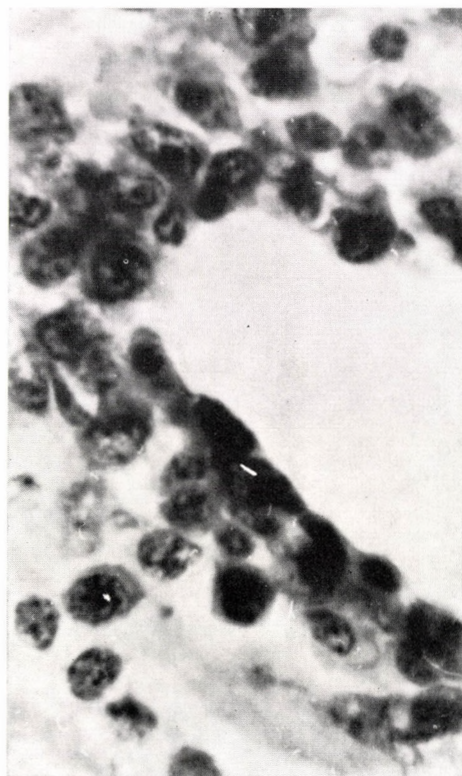

Fig. 17. Detail of Fig. 16. ×1400

plastic, partly neoplastic virus-induced proliferation of the lymphoreticular tissue cells early development of the lesions in the liver and heart of the inoculated chickens can be explained by the large number of the virus injected into the abdominal cavity. From there the pathogenic agent was quickly disseminated. Since the orally taken virus in naturally infected animals spreads slowly in the organism, the lesions appear 3—4 days later.

The normal lymphoid tissue of the birds is to be found in the spleen, thymus, bursa of Fabricius, in the mucous membrane of various extraneural organs as well as in certain parts of the body in the cellular fibrous tissue in form of dispersed lymph follicles. These consist of three cell types of the normal poultry lymphocytes (small, medium and large lymphocytes) which can be recognized in the form of regular follicles or diffuse lymphoid infiltrations.

Lymphoid cell infiltrations or cuffings which almost characteristically appear in certain areas of the cerebrum and spinal cord occurred around

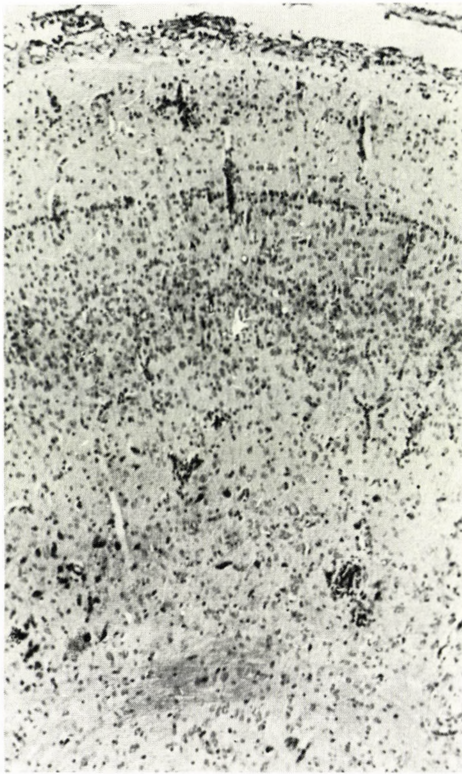

Fig. 18. Lymphoid cell infiltrations in all layers of the optic lobe around certain vessel walls 8 days after inoculation. Haemalaun-eosin. ×95

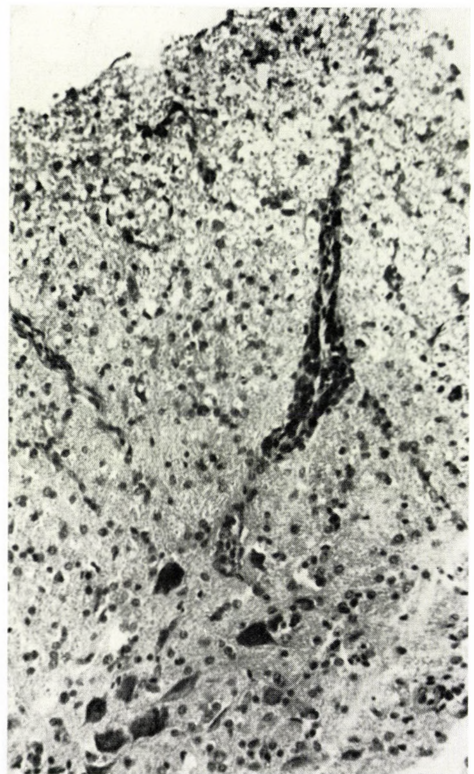

Fig. 19. Lymphoid cell vessel wall infiltration in both substances of the cervical intumescences of the spinal cord 8 days after inoculation. Haemalaun-eosin. ×95

the wall of blood vessels from the 8th day after inoculation in both substances of the CNS, the inoculated chickens. Similar lesions do not even occur by the 13th day in naturally infected chickens.

The lesions caused by MD can be easily differentiated from those due to viral infectious encephalomyelitis for in the latter case they always occur in the predilectional sites in the white substance of the CNS. In this case the small number of lymphoid cuffings is always accompanied by strong glia-proliferation and severe nerve cell damage. No cell infiltrations are observed in case of this disease in the leptomeninx covering the cerebrum.

In case of Newcastle disease the lymphoid cell vessel wall infiltrations to be found in both substances of the cerebrum and spinal cord are accompanied by gliaproliferations of different degree as well as by usually severe, alternative lesions of the nerve cells.

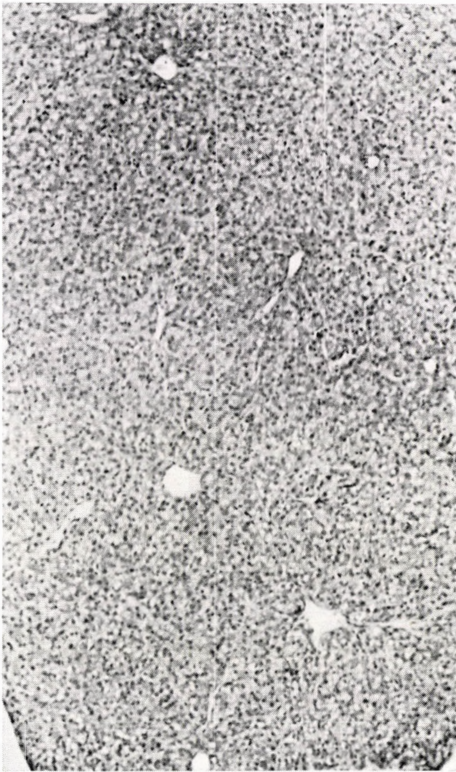

Fig. 20. Blood vessels without infiltration in the liver of a 3-day-old control chicken. The liver parenchyma is of dense structure, the lobules and the liver trabeculae do not differentiate, since the sinusoids are contracted. Haemalaun-eosin. ×95

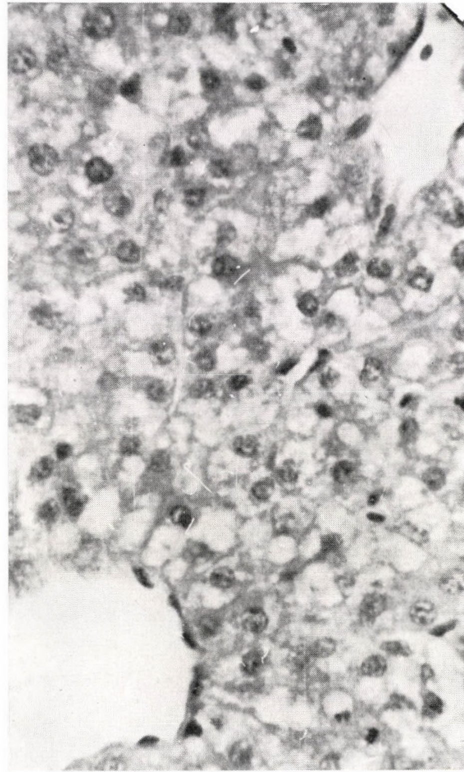

Fig. 21. Detail of Fig. 20. ×600. The cytoplasm of densely arranging liver cells is vacuolized because of the dissolved lipids, the blood vessels are free

SUMMARY

The development of histological lesions and the time of their appearance were studied in acute Marek's disease.

Examinations were carried out on different organs and on the CNS of 42 day-old chicks. The animals were divided into 3 groups. 8 chicks were inoculated intraperitoneally with 0.2 ml of MD virus marked MD 7/11, 55,300 PFU. Another 8 were exposed to natural infection. One animals of each group was killed 1, 2, 3, 4, 6, 8, 11 and 13 days after inoculation. Twenty-six chicks — from infection-free farms — were used as controls. Control animals were killed — two at a time — on the same days as infected ones and besides on days 12, 14, 16, 18 and 20. The heart, the two lobules of the liver and kidney, the glandular stomach, the spleen, the red bone marrow, the skin, the brain and the intumescences of the spinal cord of all chickens were histologically examined.

The lesions manifested themselves in the liver and heart of intraperitonally inoculated animals on the first day, more pronouncedly on the 2nd day in the wall of certain blood vessels by the proliferation of the active mesenchymal cells (reticular cells), on the 3rd day by the appearance of lymphoblasts and on the 4th day by the formation of foci consisting of mature lymphocytes. From the 6th day on an ever increasing neoplastic proliferation

of the lymphoreticular tissue was observed, especially in the liver and, later, in other extraneural organs. Lymphoid cells or the neoplastic forms of the reticulohistoicytes were characteristic. From the 8th day on lymphoid cell vessel wall infiltrations and cuffings appeared around certain blood vessels in both substances of the CNS in these animals. Similar lesions were observed in the same extraneural organs of naturally infected chickens, but 3—4 days later. No lesions had appeared in the CNS by the 13th day.

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MUCOID ENTERITIS IN THE RABBIT ASSOCIATED WITH *E. COLI* CHANGES IN WATER, ELECTROLYTE AND ACID-BASE BALANCE

By

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(Received April 13, 1973)

Mucoid enteritis is the most common disease in young rabbits. The economic loss to the large rabbit-farms caused by this single disease is very great. In Hungary more rabbits are lost annually from mucoid enteritis than from all other rabbit diseases combined. Mucoid enteritis seems to be largely associated with certain serotypes of non-haemolytic *E. coli* strains which multiplicate chiefly in the large intestine. The disease is characterized by its relatively short course and the excretion of large amount of jelly-like faeces. The commonest form of mucoid enteritis affects rabbits soon after weaning (one week or so) when they are 4—11 weeks old. Recently the disease caused serious loss in rabbits at the age of 2—8 days too.

In a previous communication (VETÉSI, 1970) it was assumed that severe metabolic abnormalities (e.g. disturbance of electrolyte equilibrium) occurred in the affected animals due to the profuse diarrhoea. Unfortunately in the literature little information is available on this field. The earlier reports described mostly the clinical signs and post-mortem findings of the disease (GORDON, 1943; MUIR, 1943; MARCATO and SJÁBAN, 1967; POUT, 1971) or bacteriological examination of the intestinal flora (GREENHAM, 1962: LÖLIGER et al., 1969). Only in the last year were published some data based on physiological and biochemical investigations. PROHÁSZKA (1972) studied the susceptibility of rabbits to mucoid enteritis. From the results of laboratory investigations he concluded that the decreased secretion of gastric acid promoted the invasion of *E. coli* bacteria by oral route. Since the clinically healthy rabbits of infected herds had higher serum bicarbonate levels than those from healthy herds, it was assumed that alkalosis was responsible for the hypochlorhydria. Most recently, KRUININGEN and WILLIAMS (1972) examined rabbits with mucoid enteritis, both spontaneous cases and cases developed after transmission of mucoid enteritis inoculum. The laboratory analysis of the body fluids showed dehydration, moderate leukocytosis, azotaemia, serum globulin alterations and electrolyte imbalance. From the results of these studies it was concluded that the enterotoxin induced hypersecretion of intestinal epithelium plays the most important role in the pathogenesis of disease.

The pathophysiology of mucoid enteritis is incompletely understood. This is probably a result of the lack of pathophysiologic research into the problem. The present work is undertaken to study the water, electrolyte and acid-base balance in the affected animals, since these mechanisms are of vital importance in the maintenance of homeostasis. It is excepted that the knowl-

edge of biochemical events which occur during the course of disease will bring about a better understanding of the pathogenesis of mucoid enteritis. At the same time, the following studies are intended to answer the question of whether the severe disturbances of fluid and electrolyte balance described in enteric colibacillosis of other domestic animals can also be observed in rabbits with mucoid enteritis.

Materials and Methods

Animals

A total of 35, weaned cross-bred ("Fehérgyöngy") rabbits of both sexes were used in this study. The 4—11 weeks old animals originated from a closed herd of large rearing farm which was reported to have a high incidence of mucoid enteritis. Among the rabbits studied, 20 animals were affected with naturally occurring mucoid enteritis. At the time of examination they showed profuse diarrhoea for 12—24 hours. 15 healthy rabbits served as controls. The animals were weaned at 4 weeks of age and fed on a commercial granulated fattening diet.

Collection of samples

The rabbits with mucoid enteritis and controls were transported to the laboratory and blood samples were drawn anaerobically from the jugular vein for the determination of haematocrit value and parameters of acid-base balance. After this, the rabbits were sacrificed by severance of the carotid artery. The heparinized blood samples collected in this way were centrifuged and the plasma was used for determination of other constituents.

The urine sample necessary for the determination of pH was obtained immediately after extermination from the bladder.

Laboratory analysis

Most of the methods used for determination of blood constituents have been described elsewhere (Kutas and Szabó, 1971). Plasma was analysed for sodium by conventional flame photometry (Zeiss type). Urine pH was measured on a Radiometer pH meter (type PHM27) and Micro Electrode Unit (E 5021a), immediately after taking of samples. The pH of caecal content was determined by the same method. In some cases, "Stuphan" (Feinchemie K–H Kallies KG) papers were used in measuring the approximate pH of the pasty contents of caecum of normal rabbits.

Necropsy procedures

All the rabbits were necropsied as soon as possible after death. Various organs of 6 control and 18 affected animals were examined histologically. Tissues for histological examination were fixed in 8% buffered formalin. In some cases, absolute ethanol and Sanomiya's sulfosalycilic acid mixture were also used for fixation. The tissues were embedded in paraffin, sectioned and stained by haematoxylin and eosin. From some organs frozen sections were also prepared and stained with Sudan III.

Bacteriological procedures

In the case of each rabbit bacteriological examination was performed from the spleen, liver, heart's blood, content of small and large intestine. The applied media were: common agar, ovine blood agar and Drigalski's agar. The incubation was carried out at 38 °C for 24 hours.

Statistical evaluations were carried out by the t test on selected groups.

Results

Clinical symptoms

The rabbits with mucoid enteritis developed a profuse diarrhoea. The watery faeces contained mucus in large quantities. In some cases the mucous faeces was reddish. The affected animals showed general listlessness, their skin was less pliable and a matting of the hair was noticed around the hind-quarters. Often a feeling of thirst could be observed and the animals excreted less urine. In some of the affected rabbits no excretion of urine was to be noted during the period of observation. Some animals showed the symptoms of muscular debility and shocklike condition: their skin became cool, the body-temperature was lower with 2—3 °C than that found in healthy rabbits. A weak and irregular pulse and signs of cyanosis were also noticed. From time to time the symptoms of shock became moderate and later relapsed again. Some rabbits showed salivation and convulsions.

Post-mortem findings

At the necropsy the appearance of affected animals was similar to that published in an earlier paper (VETÉSI, 1970). The colon and caecum showed the most characteristic changes: oedema in the wall (Fig. 1), in some places desquamation and destruction of epithelium. In certain cases in the superficial layers of caecal mucosa an acute focal inflammation was noted. Symp-

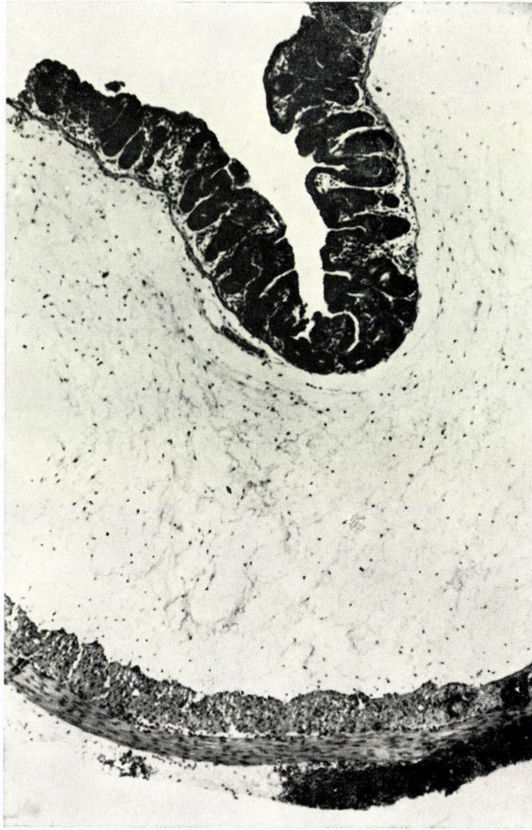

Fig. 1. Caecum. The thickness of submucosa and subserosa is increased (interstitial oedema). H E ×50 approximately

toms of hypersecretion of intestinal glands could be observed: like excessive amount of mucous (sometimes reddish and watery) content in the large intestine; mucus-rich Lieberkühn's glands etc. Vacuolar degeneration in the hepatic cells (Figs 2 and 3), tubulonephrosis in the kidneys, and occasionally necrosis of the muscle fibres in the heart were also observed. In 12 of the affected rabbits the bladder was empty. In the other animals the bladder contained a small amount of urine.

At the bacteriological examination pure cultures of non-haemolytic *E. coli* could be isolated from the intestine of each affected animal. In 3 of 20 rabbits with mucoid enteritis cultures of the same *E. coli* strains were isolated from the liver too. In 7 of control rabbits no culture of *E. coli* was found in the intestinal contents. From the intestinal samples of the other controls some coli colonies were isolated. The examination of the parenchymal organs for *E. coli* was negative in each control.

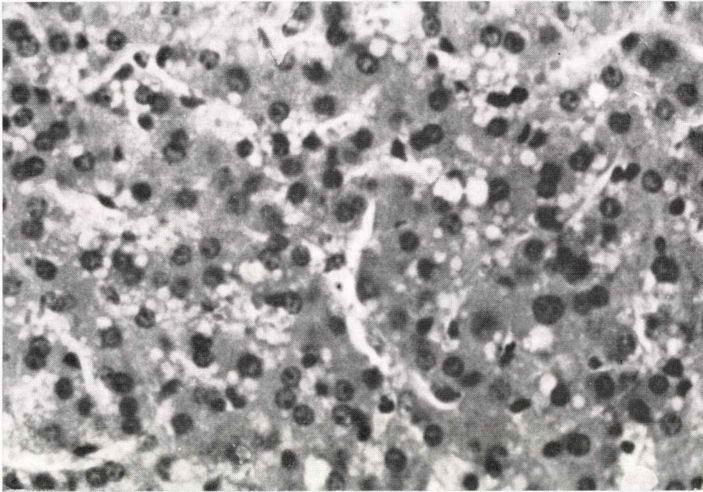

Fig. 2. Liver. Severe vacuolar degeneration of hepatic cells (intracellular oedema). H E ×400 approximately

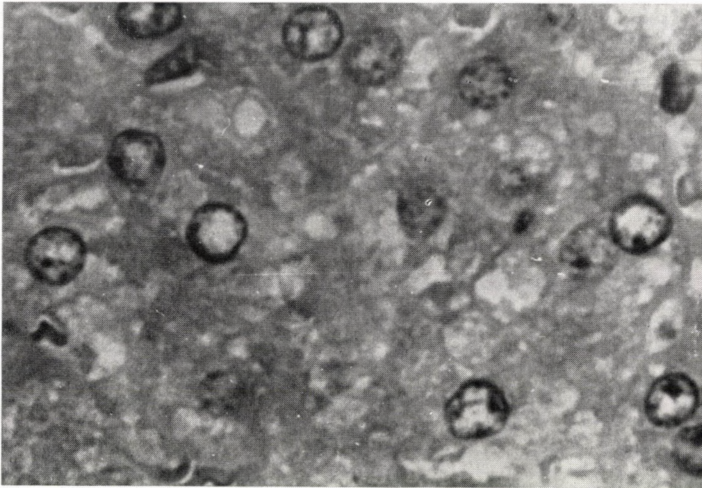

Fig. 3. Liver. Vacuolar degeneration (intracellular oedema). H E ×1000 approximately

Changes in water and electrolyte balance

Since there is no routine method for the determination of the volume of body fluids, according to the practice generally accepted in the clinical laboratory, the changes in plasma volume were followed by measuring the actual concentration of haematocrit and plasma proteins. From the results shown in Table I it can be seen that the haematocrit value and the level of total plasma proteins were significantly greater in rabbits with mucoid enter-

itis than those in control animals. These changes indicate the contraction of plasma volume which is symptom of dehydration. In order to determine the type of dehydration, the concentration of sodium in plasma was also measured, since it is known that sodium ions play a predominant part in maintaining normal osmotic pressure of extracellular fluid. The mean sodium level in the plasma of affected rabbits (152.03 mEq/L) was below the value observed in

Table I

Comparison of blood and urine constituents in groups of normal rabbits and rabbits suffering from mucoid enteritis

Group		Blood	Plasma				Blood		Urine
		Haematocrit value	Total protein	Na^+	Cl^-	Urea	pH	Standard HCO_3	pH
		%	g/100 ml	mEq/l		mg/100 ml		mEq/l	
Mucoid enteritis	n	19	18	12	13	14	10	14	8
	Mean	44.63	7.94	152.03	87.85	308.29	7.056	10.33	4.94
	S.E.	±1.26	±0.24	±2.575	±2.198	±38.531	±0.041	±0.763	±0.04
Control	n	15	15	10	11	11	11	14	13
	Mean	37.80	5.38	165.9	100.09	36.00	7.298	20.49	6.62
	S.E.	±1.36	±0.24	±1.779	±1.856	±5.191	±0.023	±0.62	±0.30
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

the control group (165.90 mEq/L). The hyponatraemia is an indication of hypotonic dehydration when excessive salt relative to water is lost from the body. There was a similar decrease — about 13 mEq/L — in the mean concentration of chloride when the data of rabbits with mucoid enteritis were compared to those of normal animals.

The histological examinations also indicated the disturbance of water balance. In the hepatic cells intracellular oedema (vacuolar degeneration) was observed. The oedema may be explained with the penetration of water into the cell from the hypotonic extracellular fluid. All the animals studied showed oedema in the wall of large intestine, especially caecum.

It was characteristic that the urea concentration reached very high level in the plasma of rabbits with mucoid enteritis (azotaemia). The mean value was about ten times larger than that in controls.

Changes in acid-base balance

The mean value of standard bicarbonate in the plasma of affected animals (10.33 mEq/L) was about half that of the controls. The severe base deficit and consequently the relative excess of hydrogen ions were compensated

by renal regulation: the urine of the diarrhoeic rabbits showed an acid reaction (average: pH 4.94). In contrast, the mean pH of urine was significantly higher in the control animals (pH 6.62). Since the compensation was no longer adequate to keep the equilibrium, a characteristic reduction in blood pH was to be observed. The mean value of pH 7.056 indicated decompensated metabolic acidosis in the blood of the affected animals.

The pH of the caecal content of rabbits with mucoid enteritis was usually alkaline (mean pH 8.09 ± 0.05; $n = 18$), while that of control animals was acid (mean pH 6.45 ± 0.09; $n = 15$).

Discussion

It is known that the caecal fermentation in healthy rabbits produces volatile fatty acids from the breakdown of complex polysaccharides. It was established that the quantity of fatty acids produced in caecum is equivalent to 10 to 12% of the daily basal caloric requirement (Hoover and Heitmann, 1972). The intensive production of volatile fatty acids make acid the caecal content which contains no or only small number of coli bacteria (Smith and Crabb, 1961; Matthes, 1969; Vetési, 1970). There is a quite different situation in the animals affected with mucoid enteritis. As a first step, *E. coli* bacteria proliferate in the large intestine (Greenham, 1962; Löliger et al., 1969; Vetési, 1970). It is still obscure which factors facilitate the multiplication of bacteria. Enterotoxin produced by the bacteria acts on the intestinal mucosa of the rabbit impairing the normal transport between the lumen of the intestine and the extracellular fluid. This results in the hypersecretion of mucus, water and electrolytes (sodium, bicarbonate etc.) into the intestinal lumen. At the same time, the reabsorption of the alkaline digestive secretions is also disturbed. Consequently, the accumulated content of the large intestine becomes alkaline and it is excreted in the form of watery, mucous faeces.

The loss of water, electrolytes and base causes severe disturbances in the body fluids. Hypotonic dehydration develops which is characterized by the decreased plasma volume and fall in plasma sodium concentration. The base deficit leads to decompensated metabolic acidosis. The serious alterations of electrolyte and water balance, the fall in the circulating blood volume are followed by decreased cardiac output, a decline in blood pressure and inadequate circulation. All this results in insufficient supply of oxygen to the tissues and soon the clinical symptoms of hypovolaemic shock can be observed. At the same time, the *E. coli* endotoxins and toxic end products of intestinal putrefaction are resorbed through the desquamated mucosa of large intestine. The toxicosis which is indicated by the high urea level, further aggravates the condition of the animal and also contributes to the fatal end of disease.

The present studies strongly suggest that the pathophysiological changes observed in the mucoid enteritis of rabbits are in many respects similar those described in the enteric colibacillosis of piglets (KUTAS and SZABÓ, 1971).

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The authors gratefully acknowledge the very able technical assistance of Miss L. KOVÁTS and Mrs. T. KÁDÁR.

SUMMARY

The effects of mucoid enteritis on the water, electrolyte and acid-base balance were investigated on 20 weaned rabbits, 4—11 weeks of age. 15 healthy rabbits of the same age served as control. The water and electrolyte balance was studied by the determination of haematocrit, total plasma proteins, urea, sodium and chloride concentrations; the acid-base status was followed by the Astrup's micromethod.

It was found that rabbits with mucoid enteritis developed hypotonic dehydration, azotaemia and decompensated metabolic acidosis. It was concluded that severe disturbances of fluid, electrolyte and acid-base balance play a significant part in provoking the shocklike syndrome and finally cause the death of animal.

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PATHOLOGICAL ANATOMIC EXPERIENCES IN THE AFRICAN SWINE FEVER OUTBREAK IN CUBA IN 1971

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African swine fever was first observed in East Africa (Montgomery, 1921). Later the disease spread in several areas of the African continent. In Europe it has occurred in the sequence in Portugal (Riberio, 1957), Spain (Jover and Botija, 1960), France (1964) and Italy (Vittoz, 1967). In the spring of 1971 it was introduced into Cuba under not yet fully understood circumstances.

The gross lesions caused by swine fever has been described by many authors. Descriptions from the time of the early European outbreaks (Riberio et al., 1958; Jover and Botija, 1961; Kovalenko et al., 1965; Boldrini, 1967; Lucas et al., 1967; Vittoz, 1967; Moulton and Coggins, 1968) agree well with those from the African outbreaks and correspond esentially to the pathological picture of the classical swine fever. Certain authors, however' noted a marked frequency and severity of some of the lesions and presence of certain organic changes (splenomegaly, oedema of the cholecyst wall) which were uncommon in classical swine fever, but could be regarded as characteristic of the African disease. In more recent European outbreaks, these characteristic lesions gradually became less frequent and less severe so that classical and African swine fever have become grossly indistinguishable. The microscopic lesions associated with the two conditions also are essentially similar, but many authors have pointed out the occurrence of characteristic regressive changes of the lymphoreticular tissue in African swine fever (Mauer et al., 1958; Kovalenko and Sidorov, 1965; Petisca, 1965; Scott, 1965: Heuschele, 1967; Mirri, 1967; Moulton and Coggins, 1968; Burba, 1969; Colgrove et al., 1969). Others (Konno et al., 1971a, b, 1972) showed in specimens originating from experimental infection that, especially in the chronic form, proliferative phenomena also occur in addition to the regressive changes.

Material and Methods

Carcases of 33 naturally diseased and 7 experimentally infected swine were examined for gross and microscopic lesions. Of the spontaneously died swine, 23 were free from a secondary septicaemic bacterial infection, whereas five each of the remaining 10 animals had a secondary bacteraemia due to salmonellae or pasteurellae. The seven experimentally infected animals originated from the group which had previously been used for the diagnostic biological test.

The following organs were examined histologically: spleen, regional and organic lymph nodes, liver, gallbladder, kidney, brain, spinal chord, lung,

heart, pancreas, adrenal, skin and skeletal muscles. The specimens were fixed in 10% formaldehyde, Zenker and Bouin solution, and embedded in paraffin. Most sections were stained with haematoxylin and eosin. In addition, Weigert, Nissl and toluidine blue stains, Gömöri's silver impregnation technique and the PAS reaction were employed.

Gross lesions

Neither the gross nor the microscopic lesions differed significantly between naturally diseased and experimentally infected swine. Presence or absence of a secondary bacteraemia had no influence on either type of lesion.

Grossly 23 (57%) of the 40 swine showed spots of cyanosis and haemorrhages in the skin. The violet-reddish cyanotic spots had indistinct margins and varied in size; they chiefly occurred in ear skin, often in association with cutaneous oedema, but were also found on the snout, cervical, thoracal and abdominal skin, in the inguinal region, inner surface of the thighs, at the tail base and — in females — in the vulvar region. Petechial or small circumscribed haemorrhages were regularly encountered on the limbs and lower-lateral parts of the abdominal skin. Extensive haemorrhages were found in 8 animals (20%), of which 6 had a secondary bacteraemia. The cutaneous lesions were equally frequent in swine with pigmented and light skin but were, of course, more conspicuous in the latter case.

The external visible mucous membranes were generally hyperaemic and cyanotic. A slight oedema was often found in the subcutaneous connective tissue, especially around regional lymph nodes. Minor haemorrhages were frequent in the subcutaneous connective tissue and skeletal muscles, especially beneath the fascias and less often large haematomas, some up to 5—10 cm in diameter, were found.

The submandibular, retropharyngeal, antescapular, praefemoral, popliteal and mesenteric lymph nodes were always swollen and hyperaemic, and in 24 cases (60%) they showed different degrees of haemorrhage and red blood cell infiltration. In the cortical parts of the lymph nodes streaks of extravasated red blood cells were less frequently found than haemorrhages. The haemorrhages were often petechial and seldom involved the entire lymph node.

In nearly every case, haemorrhages were milder in the regional lymph nodes than in the portal, retroventricular and perirenal ones of one and the same individual. Nine animals (22.5%) had small amounts of yellow, in two cases reddish, slightly turbid exudation in the abdominal, thoracal and pericardial cavities. Small subserous haemorrhages were often seen in great numbers.

Twenty swine (50%) had petechial haemorrhages in the lung parenchyma and beneath the visceral pleura. Nine animals (22.5%) had a con-

spicuous lung oedema; the serous-jellinaceous oedema fluid caused thickening of the interlobular septa. A haemorrhagic croupous pneumonia was only found in 5 naturally diseased swine (12.5%), out which two had a secondary salmonellosis, one a secondary pasteurellosis. Hyperaemia and haemorrhage frequently occurred in the epiglottal and oesophageal mucosa. Haemorrhages varying in dimensions and form were often found beneath the epicardium and pericardium, less often in the auricle. Myocardial haemorrhages were also frequent. Fourteen animals (35%) had enlarged spleen and out of them 11 were free from a secondary bacterial infection. Splenomegaly appeared in two forms. One form was characterized by an enlargment to about 1.5 times of the original size, dark brownish-red colour, rounded edges and a consistence more compact than usual in hyperaemic inflammation of the spleen. The dark red pulp of the bisected spleen bulged above the cut surface, but its consistence was characteristically rigid, jelly-like rather than soft. The Malpighian bodies could be recognized in places. The other form was characterized by moderate enlargement and moderate rounding of the edges and a rigid-consistence reminding of hyperplasial enlargement of spleen. The brownish red pulp bulged only slightly above the cut surface and its substance was slightly friable in those cases in which there was an extensive infarction. The Malpighian bodies were scarcely visible. Circumscribed infarctions were only sporadically found. The liver was often hyperaemic and in a few cases there was a nutmeg-liver. Nine animals (22.5%) showed a characteristic serous hepatitis and 18 (45%) had conspicuous liver haemorrhages, localizing chiefly beneath the capsule, mainly on the visceral surface, nearby the gallbladder. Typical changes of the portal lymph nodes were found in 26 cases (65%), appearing either in the form of a serous or hyperplasial inflammation, if the course of the disease was rapid, or in the form of haemorrhages of varying severity.

Extremely enlarged lymph nodes reminded of a haematoma rather than of their original self (Fig. 1).

The gallbladder was usually dilated and filled with thick bile. A serous-jellinaceous infiltration of the cholecyst wall and of the loose connective tissue around the bile duct was found in 15 carcases (37.5%). In 9 cases (22.5%) haemorrhages were present in the mucosa of the gallbladder. In nearly every case, mild to severe hyperaemia was found in the kidneys and 30 swine (70%) had haemorrhages in the renal cortex and medulla and in the mucosa of the renal pelvis as well (Fig. 2). Circumscribed hyperaemic areas and petechial haemorrhages were often found in the deep cortical layers, medulla and pelvic mucosa also in these cases in which the surface of the renal cortex showed little or no haemorrhages. In a few cases coagulated blood was found in the renal pelvis and in the ureter. Despite the hyperaemia, present in practically all cases, the renal cortex often had a faded brownish-red colour

Fig. 1. Severe haemorrhage in the retroventricular and portal lymph nodes

and could be torn easier than normally. Red blood cell infiltration and haemorrhagic changes of the perirenal lymph nodes corresponded in severity to the renal haemorrhages. Twenty-seven carcases (67.5%) showed petechial haemorrhages or injected vessels in the urinary bladder.

Small petechial haemorrhages were often present in the gastric mucosa, small and large intestine, and the retroventricular lymph nodes of 15 swine (37.5%) showed lesions reminiscent of those observed in the portal lymph nodes. Six swine (15%) had circumscribed ulcers in the colon, but no boutons

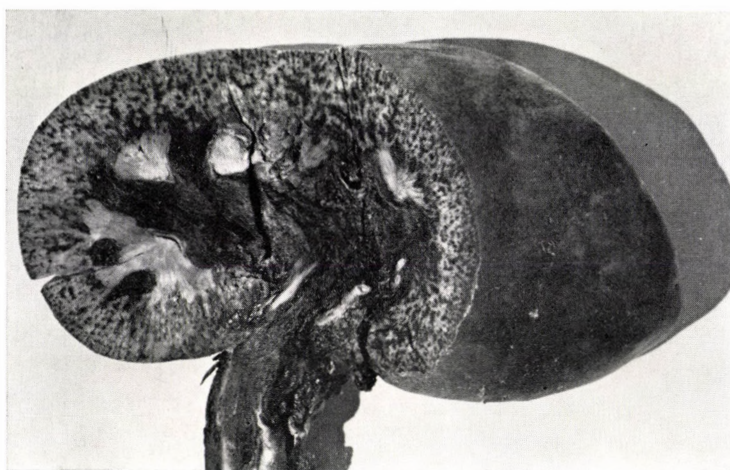

Fig. 2. Severe haemorrhages in the kidney

Fig. 3. Many red blood cells and nuclear debris have replaced a Malpighian body (pulverization). H. & E., × about 400

were observed throughout. Haemorrhage and red cell infiltration were found in most of the mesenteric lymph nodes. Four swine (10%) had haemorrhages in the pancreas and pancreatic lymph nodes. The meninges were usually hyperaemic and oedematous.

Microscopic lesions. The Malpighian bodies of the spleen showed typical regressive lesions, chiefly manifested as necrosis of lymphocytes in different stages of maturation. Many cells showing signs of karyopycnosis and karyorrhexis were found in the central part and, chiefly, in the marginal parts of the Malpighian bodies. The necrotic processes were frequently so severe that single or several adjacent Malpighian bodies had completely disintegrated (Fig. 3), only a mass of nuclear debris being found in their place (pulverization). Neighbouring Malpighian bodies in one and the same spleen showed mild to severe regressive changes. Lymphocyte counts were generally low in the red pulp and many cells showed signs of karyorrhexis and karyopycnosis.

Severe circulation disorder and regressive-type vascular wall lesions were also found in the spleen. There was frequently a marked hyperaemia, above all around Malpighian bodies showing no regressive lesions, but once necrosis had advanced, haemorrhages of different degrees appeared around the Malpighian bodies, sometimes surrounding them in an annular fashion (Fig. 4).

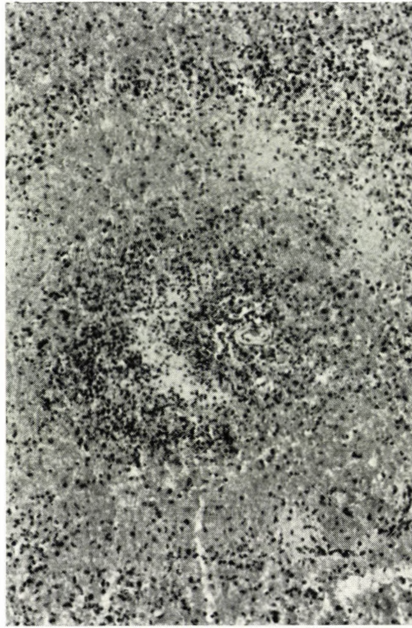

Fig. 4. Extensive perifollicular haemorrhage and necrotic processes in a Malpighian body. H. & E., × about 460

Infrequently haemorrhages were seen within the Malpighian bodies themselves, more often in other areas of the red pulp. In a few cases an extensive haemorrhagic infarction of the spleen was found: the tissular structure lost definition in such areas and only masses of red blood cells and, in smaller areas, nuclear debris, were recognized. The red blood cells usually stained pale. The reticular

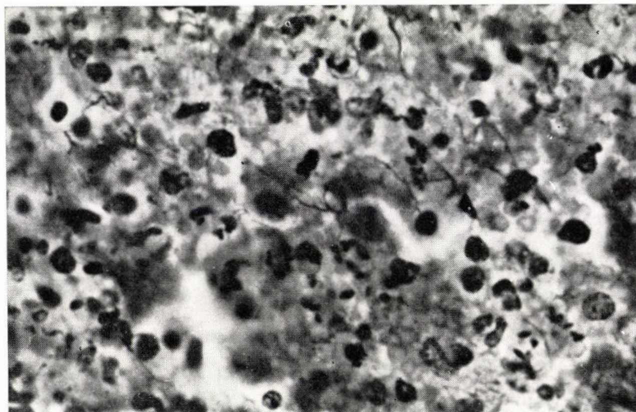

Fig. 5. The reticular fibre structure is broken and deteriorating in the area of a severe regressive splenic lesion. Gömöri's silver impregnation technique. H. & E., × about 1000

fibre structure of the spleen was loose and broken in the areas of the regressive lesions and in severe cases only traces of it could be recognized over large fields (Fig. 5).

Regressive vascular wall lesions were chiefly found in the central and sheath arteries. The endothelial cells of the central arteries were swollen, showed a cytoplasmic vacuolization and, occasionally, karyopycnosis. Intramural deposition of a finely granular, Pas-positive substance, taking on a homogeneous eosin stain, was found in the wall of numerous vessels (Figs 6 and 7), causing a considerable mural thickening. Rarefication and vacuolization were often seen in the vascular wall itself. The sheaths of the brush arteries frequently showed a loose structure and an increased number of red blood cells as well as a homogeneous substance taking on a pale eosin stain accumulated between cells of the Schweiger–Seidel sheaths. The sheath arteries of severely changed spleens completely lost structural definition and even their contours were scarcely recognizable. Apart from the above regressive lesions, proliferative phenomena were also observed in the spleen, but these were limited to minor areas. The dividing cells increased in number in several Malpighian bodies and both lymphoid and reticulum cells were more numerous. Many eosinophilic granulocytes were often found in the splenic parenchyma. The lymph nodes chiefly showed regressive changes and circula-

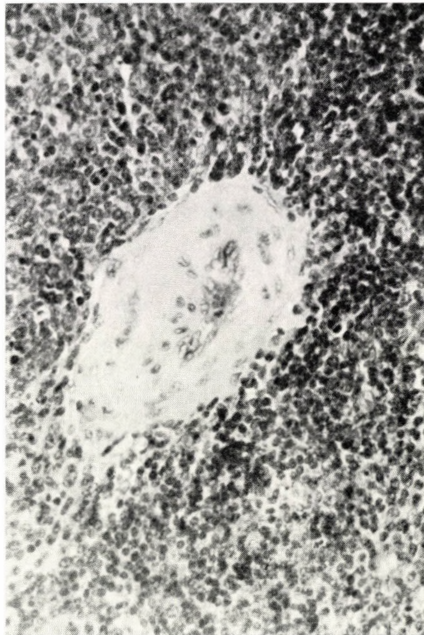

Fig. 6. Marked thickening of arterial wall owing to intramural accumulation of a homogeneous substance. Splen. H. & E., × about 400

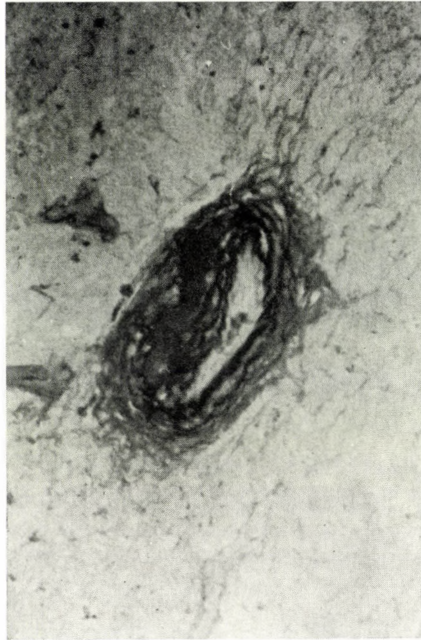

Fig. 7. Deposition of a considerable amount of PAS-positive substance in the wall of the vessel shown in Fig. 6. PAS staining × about 400

tion disorders, but occasionally there were also proliferative phenomena. The regressive processes were chiefly manifested as necrosis of the lymphoid elements, above all of the lymphocytes and as degenerative changes in vessel walls. In milder cases karyopycnosis and karyorrhexis were limited to single cells; in such cases phagocytized cell debris were abundantly present in the cytoplasm of several large macrophages (Fig. 8). In severe cases, the cell necrosis extended over large areas, including the central and peripheral cortical zones (Fig. 9) and the medulla (Fig. 10). Very severe cases, in which almost the entire lymphoid tissue had necrotized and transformed into a homogeneous eosinophilic mass containing powder-like nuclear debris, were also encountered.

Vessels traversing the lymph node proper showed mural changes similar to those encountered in the spleen (Figs 11 and 12). Markedly dilated capillary vessels whose lumen was filled by nuclear debris embedded in a homogeneous, acidophilic substance were often seen (Fig. 13). Hyperaemia, haemorrhagic lesions in various parts of the lymph node and extravasation of red blood cells into the sinuses were nearly always observed. In the hematoma-like lymph nodes the mass of red blood cells completely masked the basal tissue which, on closer examination, showed regressive lesions. Proliferative phenom-

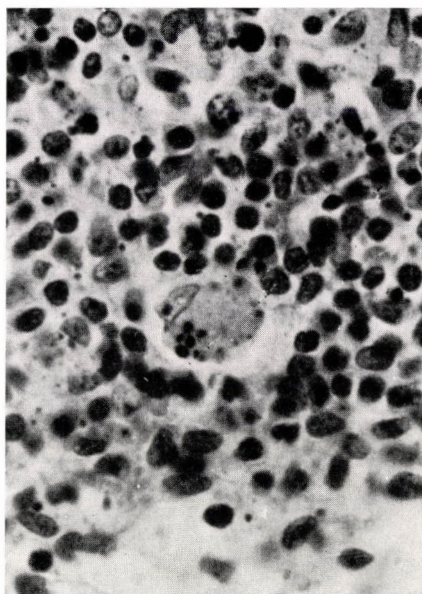

Fig. 8. Phagocytized cell debris in the cytoplasm of a reticulum cell. Lymph node. H. & E., × about 400

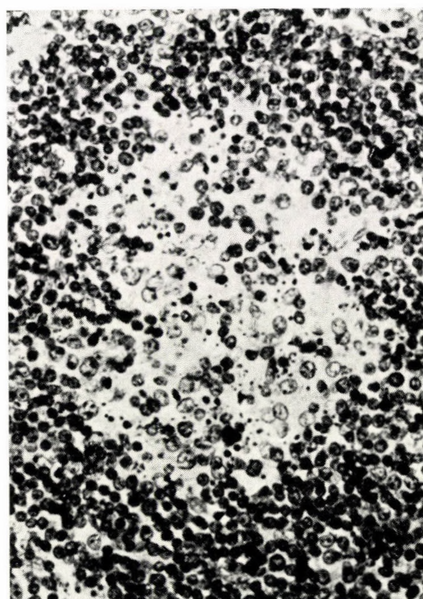

Fig. 9. Early regressive changes in the central area of a lymph follicle. Lymph node. H. & E., × about 400

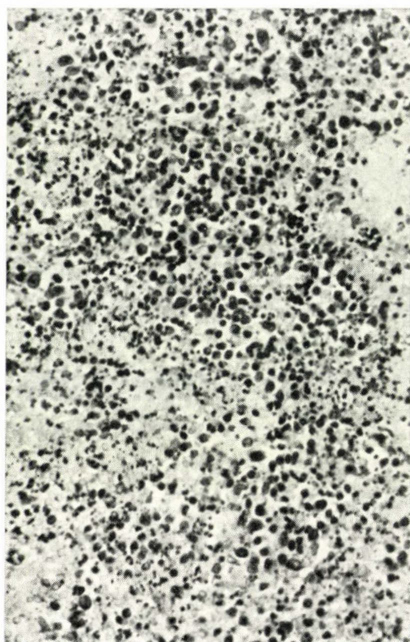

Fig. 10. Advanced cell necrosis in a medullary bundle. Lymph node. H. & E., × about 400

ena were chiefly observed in those cases in which the regressive cell lesions were limited to a few single cells or cell groups. These phenomena were accompanied by an increase in numbers of lympho-reticular elements and dividing cells. In some cases the cortical elements and myeline bundles were enlarged and their margins were indistinct. Eosinophilic granulocytes appeared in the lymph node tissue in a substantial proportion of the cases.

The liver parenchyma chiefly showed regressive lesions, which were limited to single liver cells (Fig. 14) or cell groups (Fig. 15) and RHS cells. The main changes were hyperchromasia of the nuclear membrane, nuclear pycnosis and rhexis, acidophilia, turbid swelling and, less often, fatty infiltration of the parenchymal cells. Although the regressive phenomena were seen in all parts of the liver lobules, their main seat was the medial zone. In these cases in which the regressive lesions were inconspicuous, swelling of the RHS cells and formation of small, granuloma-like foci from proliferating cells was observed in the sinusoids. Proliferative changes were always found in the liver and seemed to be unrelated to the presence or absence of a secondary bacterial infection. The interlobular septa were infiltrated by lymphocytes, histiocytes and, frequently, by many eosinophilic granulocytes. Many inflammatory cells showed necrotic phenomena (Fig. 16).

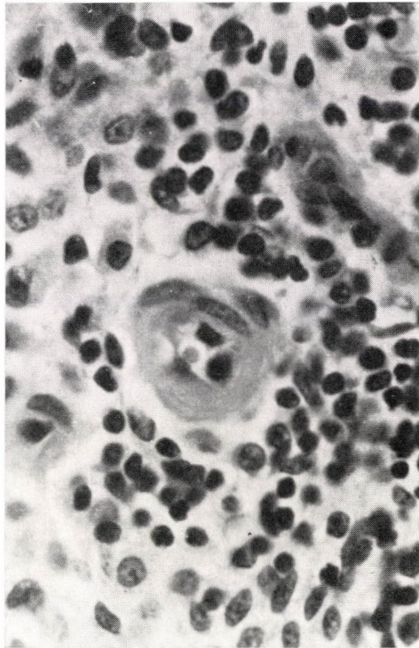

Fig. 11. Thickening of the wall of a small vessel owing to intramural accumulation of a homogeneous substance and swelling of the endothelial cells. Lymph node. H. & E., × about 1000

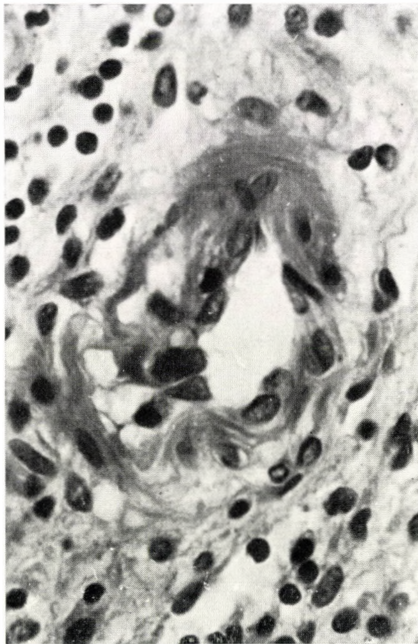

Fig. 12. Vacuolization and structural rarefication in a vessel wall. Lymph node. H. & E., × about 1000

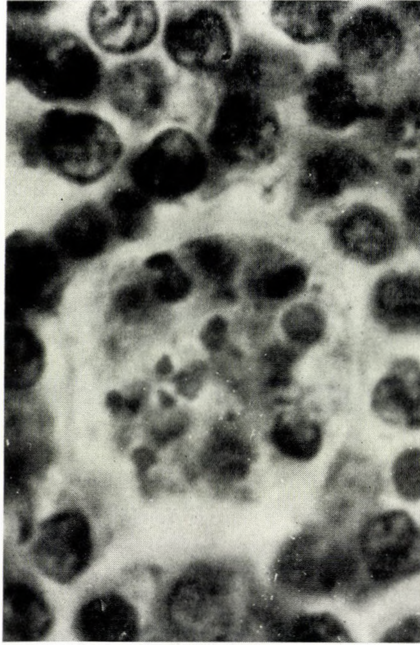

Fig. 13. Capillary thrombus in the lymph node parenchyma. H. & E., × about 2500

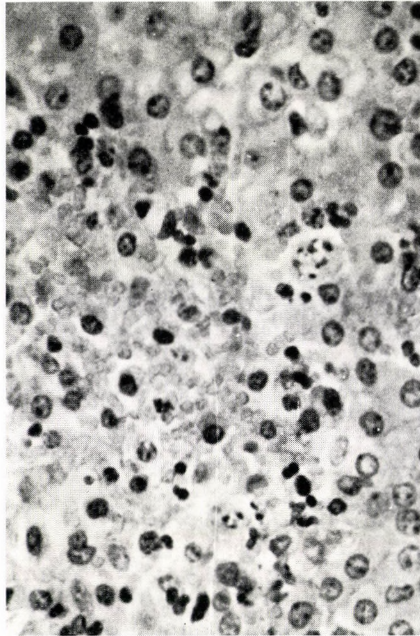

Fig. 14. Regressive lesions of single liver cells and circumscribed haemorrhage in the liver parenchyma. H. & E., × about 400

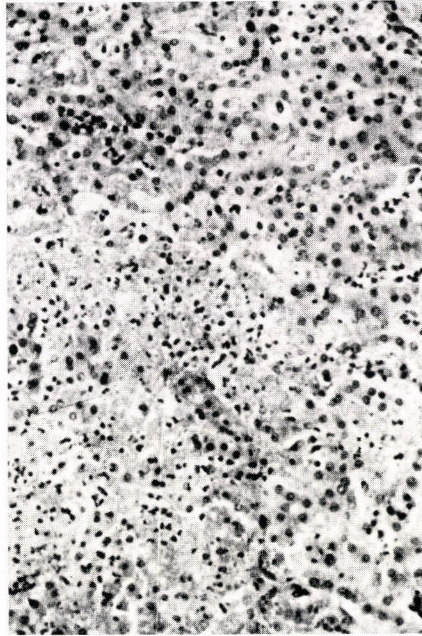

Fig. 15. Confluent areas of necrosis in the liver. H. & E., × about 160

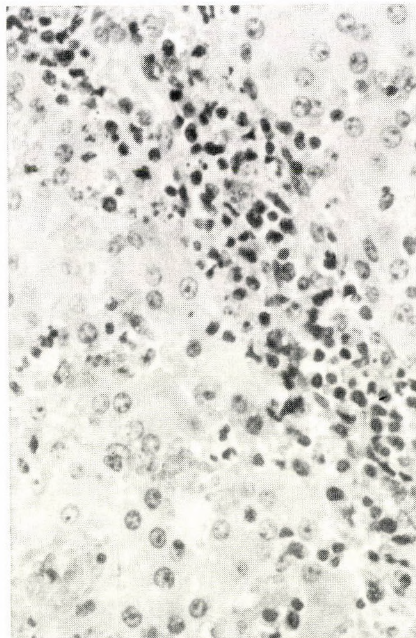

Fig. 16. Histio-lymphocytic infiltration in the interlobular septa and regressive changes in inflammatory cells. Liver. H. & E., × about 400

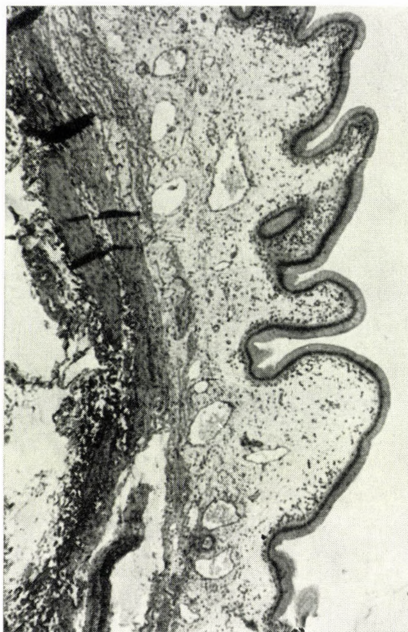

Fig. 17. Oedematous infiltration in the gallbladder wall. H. & E., × about 65

The grossly visible thickening of the cholecyst wall was due to oedema of the propria mucosae and of the subserous tissues (Fig. 17). In the oedematous areas haemorrhages and erythrophagocytic macrophages were seen. The lymph follicles of the mucous membrane were enlarged and the vessels traversing the various layers of the cholecyst wall were markedly dilated. Lesions similar to those in the gallbladder were found in the extrahepatic bile ducts.

In the renal cortex and medulla, marked vasodilatation and circumscribed haemorrhages were found. In some cases the haemorrhages were more numerous in the medulla than in the cortex. A marked hyperaemia and haemorrhage were often present in the mucosa of the renal pelvis. Glomerulas cells, endothelial cells and parietal cells of Bowman's capsula were swollen and showed necrotic phenomena. Bright acidophilic serum and droplets were frequently encountered in the cavity of Bowman's capsule (Fig. 18). Haemorrhages were rarely found in the glomeruli. Many homogeneous intra-cytoplasmic droplets, taking on much eosin stain and alternately showing Weigert- or PAS-positivity (Fig. 19) were present in most of the tubular epithelial cells and in the tubular lumen; sometimes the entire lumen was filled by a homogeneous mass of similar staining properties. Digestion with diastase did not alter the staining properties of the PAS-positive droplets or granules. The nuclei of the tubular epithelial cells rarely showed degenerative phenom-

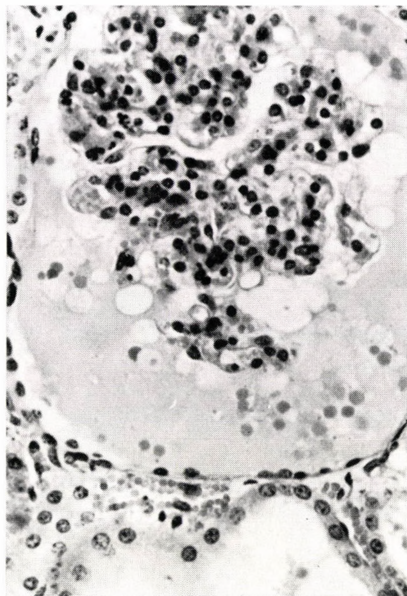

Fig. 18. "Hyaline droplets" in the cavity of Bowman's capsule. Kidney. H. & E., × about 400

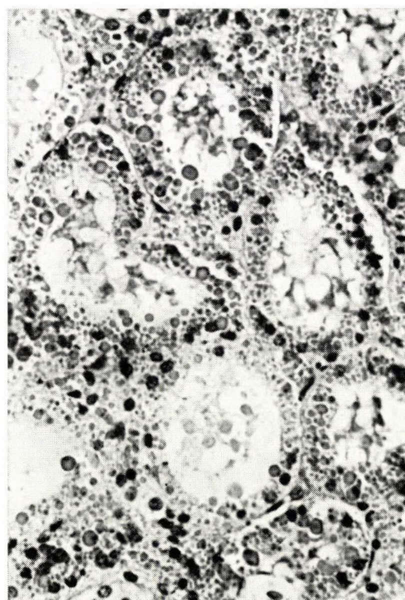

Fig. 19. Severe hyaline degeneration in many renal tubules. H. & E., × about 400

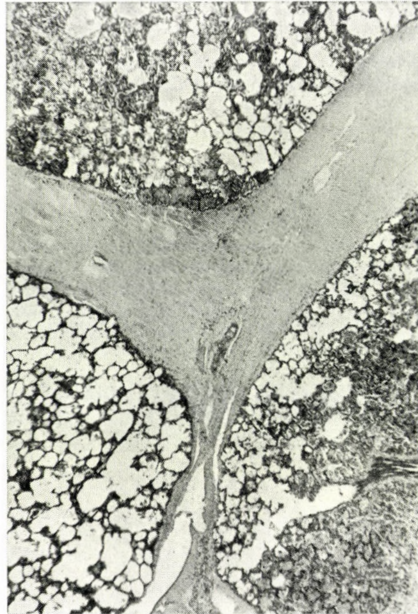

Fig. 20. Interstitial pulmonary oedema. H. & E., × about 65

ena. Circumscribed areas of lymphocytic and histiocytic infiltration were sometimes observed in the renal interstitial tissue. Karyopycnosis and karyorrhexis were frequent in the inflammatory cells. The small vessels of the renal parenchyma showed mural changes of similar type to splenic and lymph node vessels.

The lungs were usually hyperaemic, with small haemorrhages and enlargement of the interlobular septa due to serous oedema (Fig. 20). The lymphoreticular cell elements of the peribronchial lymph nodes often increased in number and showed necrotic phenomena. The degnerative vascular wall lesions resembled those found in the vessels of other organs.

Haemorrhages were often found in the epicardium, endocardium and myocardium. The small arteries showed marked regressive mural lesions both inside and outside the haemorrhagic areas. A PAS-positive substance loosened the structure of the vessel wall, the endothelial cells were enlarged and degenerated. Heart muscle dystrophy was but infrequently found in circumscribed areas.

No characteristic changes were observed in the pancreatic tissue. Minor haemorrhages were found in only a few cases and the excretory gland epithelium showed necrosis in two cases.

Hyperaemia and less often haemorrhages were found in both the medulla and the cortex of the adrenals.

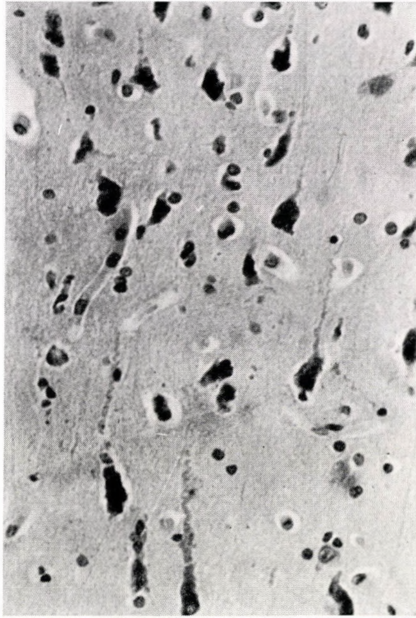

Fig. 21. Nerve cell pycnosis. Brain. H. & E., × about 160

In the grossly cyanotic appearing skin areas chiefly hyperaemia of the corium and, to a certain extent, also of the subcutis was observed. Degenerative lesions in vessel walls were rare and there was no thrombus formation.

In the central nervous system, characteristic lesions were found in the nerve cells, leptomeninges and blood vessels. The changes were predominantly regressive, inflammatory phenomena being less frequent. Degenerative changes

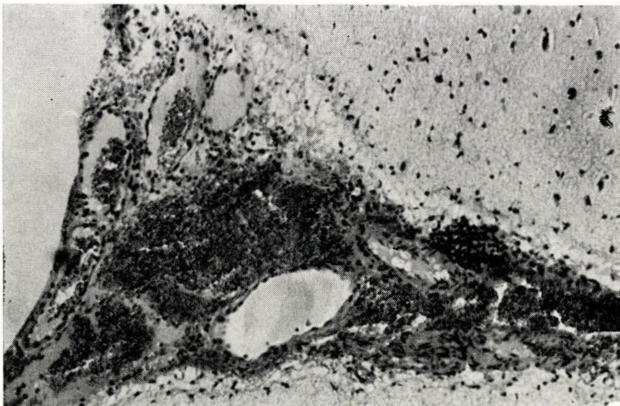

Fig. 22. Extensive haemorrhage in the leptomeninges. H. & E., × about 65

of different severity were observed in various cerebral nuclei, cortical nerve cells and Purkinje cells. The nerve cells were shrunken, showed a marked cytoplasmic basophilia and condensation of Nissl granules. The nuclei were either markedly swollen or pycnotic. Neurocytophagia was very rarely obser ved. The leptomeninges showed in most cases hyperaemia, serous infiltration and haemorrhages (Fig. 22), the latter were in some cases confluent over large areas, while in others they were limited to the inmediate surroundings of one or another vessel. A diffuse, perivascular inflammatory cell reaction was quite rare in the pia mater. The small cerebral arteries showed swelling and necrosis of endothelial cells, subendothelial vacuole formation, moderate thickening of the wall owing to intramural deposition of a PAS-positive substance and perivascular oedema. In the grey matter and white matter, there was usually a very slight histio-lymphocytic infiltration; in the few cases when it was severe, the inflammatory cells showed nuclear degeneration phenomena. There was also a glia cell infiltration, more often of the diffuse than of the focal type. Only few glia cells showed regressive changes (karyopycnosis and karyorrhexis). Lesions in the spinal medulla and meninges were of similar type to the cerebral changes, but less severe.

Discussion

The majority of lesions found in the examined carcased were characteristic of the hyperacute and acute forms of African swine fever; changes corresponding to the subacute form were found in only few instances. The main gross lesions were phenomena indicative of a general circulation insufficiency and haemorrhages. The main signs of circulation disorder were an extensive cutaneous hyperaemia and cyanosis, but similar lesions were also present in intestinal and upper respiratory mucosa as well as in certain organs (liver, kidneys, lungs, meninges). The haemorrhages apparent in all body regions were of the petechial type in acute cases and larger and more numerous if the disease took a longer course. The renal haemorrhages were not limited to the cortex; they appeared in the deeper layers and in the mucous membrane of the renal pelvis as well. The retroventricular, portal and perirenal lymph nodes usually showed severe haemorrhages, even in those cases in which bleeding and/or red blood cell extravasation were of a low degree in the regional lymph nodes of the subcutaneous connective tissue.

Other important gross lesions were the frequent enlargement of the spleen, serous-jellinaceous infiltration of the cholecyst wall and of the loose connective tissue between liver and bile duct, and the interstitial pulmonary oedema, which was not infrequently encountered in our series.

The main microscopic lesions were firstly, regressive changes of lymphoreticular cells, vessel walls, certain parenchymatous organs and brain, secondly

circulation disorders and above all haemorrhages which were very severe and/or characteristic in certain organs. Proliferative phenomena were also observed, but these were seldom characteristic.

In the lympho-reticular system, especially in the lymph nodes and spleen, lymphocytes in different stages of development and, to a less degree, various macrophages, showed regressive lesions which were chiefly manifested as karyopycnosis and karyorrhexis. Of the vessels the precapillary and small arteries showed characteristic changes in all organs, above all in the spleen and lymph nodes. The endothelial cells were swollen, vacuolized, and their nuclei were pycnotic. Accumulation of an acidophilic, PAS-positive, finely granular substance caused thickening of vascular wall and loss of its structural definition. Among the changes of parenchymatous organs, the regressive lesions of the liver and the hyaline droplet degeneration of the kidneys appeared to be most characteristic. Degenerative changes of the brain were chiefly manifested by shrinking of large nerve cells and condensation of Nissl granules in the grey matter and cerebral nuclei, without much indication of neurocytophagia. Unlike classical swine fever, perivascular inflammatory phenomena were rare and slight.

Circulation insufficiency was indicated in all examined organs by a usually severe hyperaemia, capillary thrombus formation and haemorrhage. These processes were the most severe in the spleen and lymph nodes.

Apart from the marked regressive lesions, less conspicuous proliferative phenomena were also observed. These were a histio-lymphocytic infiltration of the hepatic and renal parenchyma, proliferation of the RHS cells and hyperplasia of certain lymphoid tissues.

It may be concluded from literary data and our own experience that the viral agent of African swine fever bears the primary responsibility for the greater part of the above lesions. The severe regressive lesions of lymphoreticular cells and changes of the vascular wall and endothelium, resulting in a markedly increased permeability and, consequently haemorrhages in all body regions, are due to direct damage by the virus. In other regressive changes, such as liver and nerve cell lesions, hypoxia due to severe circulation insufficiency also seems to play a part. The appearance of hyaline droplets in tubular epithelial cells of the kidneys is indicative of proteinuria and/or of protein reabsorption from the glomerular filtrate. The appearance of hyaline droplets was clearly unrelated to a toxic damage, because the greater part of the tubular epithelial cells did not show regressive nuclear changes which, if present, appeared to be a sequel to hyaline droplet degeneration rather to any other cause.

The question whether allergic processes might play a role in the establishment of the gross and microscopic lesions is a further matter of consideration. The severe circulation insufficiency, the marked interstitial lung oedema and

the numerical increase of eosinophilic granulocytes in certain tissues might well be attributed to allergy and it does not seem improbable that allergic phenomena play some role in the development of the vascular wall lesions. Certain protein-like substances which are released from the disintegrated lympho-reticular cells may probably induce an allergic response.

Some gross and microscopic lesions can be regarded as a diagnostic aid, especially if they are observed in a large group of animals, but sure diagnosis needs by virological examinations.

Gross differentiating features from classical swine fever are pronounced cutaneous cyanosis, severe haemorrhages in the retroventricular and portal lymph nodes, oedematous swelling of the cholecyst wall, interstitial pulmonary oedema, localization of renal haemorrhages (frequent occurrence in deep layers and renal pelvis) and swelling of the spleen in the cases free from a secondary bacterial infection. Microscopically, extensive karyopycnosis and karyorrhexis of lympho-reticular and RHS-cells (mainly in the lymph nodes and spleen) as well as the hepatic and renal changes are important criteria and generally more conclusive than gross lesions.

SUMMARY

Carcases of 33 naturally diseased and 7 experimentally infected swine were examined for the gross and microscopic lesions associating with African swine fever. The greater part of the animals showed lesions characteristic of the hyperacut and acute forms of the disease.

The main gross lesions were spots of cutaneous hyperaemia and cyanosis, and haemorrhages appearing in all regions of the body, indicating a general circulation insufficiency. Renal haemorrhages were found not only in the cortex, but also in the deeper layers and in the mucosa of the renal pelvis as well: the deeply seated lesions were often more severe than the superficial ones. Severe haemorrhages were also found in the retroventricular and portal lymph nodes, even in those cases in which the regional lymph nodes showed only slight bleeding and/or red blood cell extravasation. Enlargement of the spleen, interstitial lung oedema and cholecyst wall oedema, changes with the classical swine fever, were frequently observed.

The main microscopic lesions were of the regressive type. These occurred on the one hand in cells of the lympho-reticular organs, mainly in the spleen and lymph nodes, on the other in parenchymatous organs, above all in liver and kidneys, as well as in nerve cells of the central nervous system and in blood vessels. Vascular wall lesions were most marked in the lymph nodes and spleen: intramural deposition of a PAS-positive substance, vacuolization and endothelial cell injury were observed. Severe regressive lesions were also found in the reticular fibre system, especially in the spleen.

Circulation insufficiency was manifested by capillary thrombosis and haemorrhages in all body regions. The most severe haemorrhagic lesions were found in the lymph nodes and spleen, often causing infiltration of the organ with blood. Haemorrhages were also found in the liver parenchyma and in the leptomenings.

Slight proliferative phenomena were found in the not yet severely necrotized lymph nodes and spleen (esentially as a hyperplasial phenomena) as well as in the liver in which they were manifested as swelling and often proliferation of the RHS cells. Histio-lymphocytic infiltration was frequently found in the interlobular septa of the liver. The nuclei of the infiltrating cells often showed regressive changes. Perivascular infiltration of cerebral vessels was rarely found. A few infiltrating eosinophilic granulocytes were encountered in many spleens and lymph nodes.

It is concluded that the virus of African swine fever plays a primary role in the development of the lesions, but in certain changes hypoxia due to circulation insufficiency seems to be an additional causative factor. The probable role of allergic processes in the lesions is also discussed.

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ТОМ 23 — ВЫП. 4

РЕЗЮМЕ

САЛМОНЕЛЛЫ В ПИЩЕВЫХ ПРОДУКТАХ ЖИВОТНОГО ПРОИСХОЖДЕНИЯ В 1971 ГОДУ

Я. ТАКАЧ и ДЬ. НАДЬ

В 1971. году типизировано 1427 штаммов салмонелл, изолированных из продуктов животного происхождения в Венгрии. Обнаружено 43 вида салмонелл. Наичаще изолированы **S. anatum, S. cholerae-suis** и **S. typhi-murium.** Первый раз среди материалов из Венгрии обнаружены следующие виды — **S. epicrates, S. kilwa, S. menston, S. havanna** и **S. coeln.** Среди убойных животных наиболее богатым резервуаром салмонелл явились свинья и среди птиц — утка и гусь. Источником заражения этих животных салмонеллами явилось кормление концентратами животного происхождения. Заражение ведет к бессимптомному салмонелло-носительству и рассеиванию салмонелл и, таким образом, возникают салмонеллезные резервуары. Импортное замороженное мясо и печень являются дальнейшим источником заноса салмонелл. Ради снижения салмонеллезных инфекций необходимо урегулировать способ использования засоренных салмонеллами концентратов животного происхождения. Гигиену содержания животных, в том числе гигиену среды, необходимо удерживать на высоком уровне. Необходимо улучшить условия транспорта животных. Нужно обеспечить, чтобы о группах животных стояли к распоряжению доубойные информации. Информация должна касаться и таких вопросов, как например, не были ли в хозяйстве массовые вспышки салмонеллезного заражения. При выполнении предписаний боенных и мясообрабатывающих мероприятий в технологической процедуре надо соблюдать правила гигиены; в уходе за продуктами как в производстве, так и в доставке к конзументу нужно соблюдать строжайшую гигиену.

ФОРМИРОВАНИЕ ПОКАЗАТЕЛЕЙ ТИТРА ПРИГОТОВЛЕННЫХ РАЗНЫМИ МЕТОДАМИ ГЕМОЛИЗИНОВ

Г. НАДЬ, Д. ФОДОР и Й. САТМАРИ

Изучено формирование значений титра гемолизинов, приготовленных разными методами. Применяемые авторами методы повышения чувствительности можно группировать в три группы. В первую группу принадлежат методы, когда прививки проводились только разными дозами суспенсии овечьих зритроцитов; во вторую, когда прививки проводились взвесью эритроцитов овцы и сыворотками овцы и крупного рогатого скота соответственно; в третью, когда повышение чувствительности осуществлялось только разным сыворотками.

Полученные сыворотки подвергались индивидуальному титрированию согласно рекомендации **FAO/WHO.**

Результаты исследований показали, что наиболее высокий титр можно получить от применения сыворотки от разных овец и взвеси эритроцитов овцы, дальше, от применения сыворотки той же овцы и суспензии овечьих эритроцитов. Формирование титра при прививке кроликов только эритроцитами зависит от количества взвеси эритроцитов.

У привитых кроликов только сывороткой овцы тоже получен измеримый гемолизиновый титер.

На основании своих исследований авторы приходят к заключению, согласно которому гомологическая и чужая овечья сыворотка, применившаяся до или одновременно с обработкой суспензией овечьих эритроцитов, стимулирует образование гемолизина.

Сыворотка крупного рогатого скота не стимулировала образование гемолизина так, как сыворотка овцы.

ПРОДЛЕНИЕ ПЕРИОДА ВСАСЫВАНИЯ ПРОТЕИНА: ЭКСПЕРИМЕНТЫ НА КОТЯТАХ И ПОРОСЯТАХ

К. БАИНТНЕР мл.

Сделана попытка восстановления характерной гистологической картины кишечника новорожденных котят и поросят у животных возраста старше 4-х дней.

Выпоением крупной дозы коровьего молозива котятам возраста 4,5—14 дней удалось добиться переобразования вакуолей энтероцитов подвздошной кишки в эозинофильные капельки, характерные для активного всасывания протеина. Этот факт является дальнейшими доказательством того, что энтероциты подвздошной кишки до определенного времени не теряют способности всасывать протеин после того, как переваривание протеина уже началось. Нуждается еще в прямом доказательстве тот факт, что всосавшийся при таких обстоятельствах протеин попадает тоже в циркуляцию.

Не удалось вызвать образования эозинофилов в энтероцитах 4—4,5-дневных поросят отбитых от собственной метки и подставленных к опоросившейся свиноматке-кормилице, но обнаруживались морфологические признаки резидуального всасывания протеина во форме мельчайших эозинофильных телец — включений в энтероцитах подвздошной кишки как контрольных, так и экспериментальных поросят.

В контрольных животных обоих видов вакуолизация наблюдаласть только в энтероцитах подвздошной кишки, но никогда в энтероцитах тощей кишки. На основании изменчивости локализации вакуолей по отношению к ядру клетки можно считать, что они обладают внутриклеточной подвижностью.

ЧАСТОТА И КЛИНИКОПАТОЛОГИЧЕСКОЕ ЗНАЧЕНИЕ ПОРАЖЕНИЙ ОРГАНОВ ПРИ ОПРЕДЕЛЕННЫХ ТИПАХ ИНФЕКЦИОННОГО МАСТИТА КОРОВ

И. САЗАДОШ и И. КАДАШ

Сто коров с маститом после убоя изучено на наличие макро- и микроскопических изменений в разных органах и бактериологически. Как при острых, так и хронических формах мастита, вызываемых микробами **Pseudomonas aeruginosa**, **Klebsiella pneumoniae**, **Escherichia coli**, **Corynebacterium pyogenes**, **Staphylococcus** или их комбинациями часто обнаружены в разных органах соответствующие изменения, указывающие на гематогенный разнос заразного начала.

Частота обнаруживаемости разного рода изменений была следующей: набухание селезенки 43%, инфаркты в селезенкие 8%, набухание надвыменных лимфоузлов 50%, гнойное воспаление надвыменных лимфоузлов 12%, некроз надвыменных лимфоузлов 7%, метастазы актиномикозного типа в надвыменных лимфоузлах 3%, набухание региональных лимфоузлов 12%, некротические изменения в региональных лимфоузлах 5%, жирное перерождение печени 16%, некробиотические очаги в печени 20%, жирное перерождение и некробиотические очаги в печени 4%, холецистит 2%, свежий геморагический инфаркт в печени 1%, инфаркты в почках 8%, гнойные метастазы в почках 2%, жирное перерождение почек 2%, очаговый интерстицальный нефрит 17%, метастатические процессы в легких 4%, артриты 18%.

Патологическая картина при инфекционном мастите от **Kl. pneumoniae** и **Ps. aeruginosa** настолько характерна, что диагноз можно ставить без бактериологического исследования.

При мастите от **Kl. pneumoniae** анемические инфаркты кроме вымени могут быть в надвыменных и других лимфоузлах, в селезенке, в почках и в легких. Некротические воспалительные очаги с головки булавки по просяное зерно, как правило, одновременно обнаруживаются и в печени.

При актиномикозного характера мастите, вызываемом **Ps. aeruginosa** кроме очагов в вымени они часто имеются и в почках. При этом типе мастита некротические воспалительные изменения в печени невооруженным глазом едва ли видимы, но суставы обычно являются тоже пораженными.

ДАЛЬНЕЙШИЙ ТИП СЫВОРОТОЧНОГО ТРАНСФЕРИНА У КРУПНОГО РОГАТОГО СКОТА

П. ШООШ, Й. ШТУКОВСКИ, Г. ЧОНТОШ и Э. ГИППЕРТ

У венгерского пестрого скота обнаружен дальнейший тип сывороточного трансферина, провизорно названный Tf[I] **Hungary**. Он обладает слабшей электрофоретической подвижностью на крахмальном желе, чем **Tf**[E]

Данные авторов по изучению семейства подсказывают, что этот новый тип унаследуется как кодоминантный признак.

ИЗУЧЕНИЕ ШТАММОВ ACHOLEPLASMA, ИЗОЛИРОВАННЫХ ИЗ СВИНЬИ

Л. ШТИПКОВИЧ, Д. ШИММЕЛ и Л. ВАРГА

Авторами изучено биохимически, серологически и на резистентность 8 штаммов **Acholeplasma**, изолированных из дыхательных путей свиньи. Один среди штаммов отличался от основных штаммов **A. laidlawii A. PG-8, A. laidlawii BF-8, A. granularum (Friend), A. axanthum (ATCC 25176)** и **A. sp. GP 49 (Squire)**. Остальные приняты за **A. laidlawii** хотя некоторые среди них отличались от основных штаммов как то, **A. laidlawii, A. PG 8** и **A. laidlawii B F-8** в способности гидролизации эскулина и разложения целлобиозы, некоторые — в отсутствии способности гидролизации эскулина, но в наличии целлобиозу разлагающей способности, некоторые — в отсутствии эффекта расщепления как эскулина, так и целлобиозы.

ИДЕНТИФИКАЦИЯ БЫЧЬИХ МИКОПЛАЗМОЗНЫХ ШТАММОВ, ИЗОЛИРОВАННЫХ ИЗ КРУПНОГО РОГАТОГО СКОТА

Л. ШТИПКОВИЧ

Проведено сравнительное биохимическое и серологическое изучение изолированного в Венгрии бычьего микоплазмозного штамма группы-6 (согласно Лич) при помощи основного штамма **A. sp. PG 49 (Squire)**. Результат исследований показывает не только идентичность двух штаммов, но и важнейшие свойства этой группы рода **Acholeplasma**.

УЛЬТРАСТРУКТУРАЛЬНЫЕ ИЗМЕНЕНИЯ В ПЕЧЕНИ АБОРТИРОВАННЫХ ЛОШАДИНЫХ ПЛОДОВ ПРИ РИНОПНЕЙМОНИТЕ

П. КАПП

Изучены электронно-микроскопические изменения двух лошадиных плодов после 7-недельного и 10-месячного внутриутробного развития, абортированных во время вспышки ринопнеймонита. В более молодом плоде имелся острый гепатит с обширными повреждениями клеток паренхимы (Сн. 1). Частыми были некроз печеночных клеток и внутриядерные включения Ковдры типа А, спорадически наблюдалось формирование гигантских клеток. Грубый эндопласматический ретикул часто был гипертрофированным, но и другие цитоплазматические органеллы были сильно поврежденными (Сн. 4 и 5). Явления воспаления присутствоали в синусоидах и щелях Диссе. Вирусные частицы с признаками вирусов герпес, имелись в поврежденных печеночных клетках, их ядрах и межклеточном пространстве (Сн. 2—5).

В старшем плоде, погибшем скоро после аборта, наблюдались хронические изменения печени. Междольчатые прослойки были инфильтрированы гистиоцитами и лимфоцитами (Сн. 6) при наличии большого количества фибробластов и коллагенных волокон, имелась очаговая и диффузная пролиферация клеток RSH, в щелях Диссе наблюдалось образование сплошной базальной мембраны (Сн. 8), обнаружено повреждение желчных ходов (Сн.

7, 10, 11), спорадическое повреждение печеночных клеток (Сн. 12), образование телец Коунцилмана (Сн. 9). Внутриядерные включения, указывающие на наличие вируса, в печеночных клетках и клетках RSH обнаружены и в этом плоде (Сн. 12).

Согласно раньшим и настоящим наблюдениям автора в плодах при ринопнеймоните возникает острый или хронический гепатит, вызывающий гибель плода. Данные сравнительных исследований показали, что морфологическая картина гепатита плодов очень похожа таковой при известных вирусных гепатитах. На основании морфологических исследований можно заключить, что ринопнеймонит является не что иным, как вирусным гепатитом лошадей.

УЛЬТРАСТРУКТУРАЛЬНЫЕ ДЕТАЛИ ВЕНЧИКА КОПЫТА СВИНЬИ

П. КАПП и А. Б. КОВАЧ

Образцы венчика копыта свиней возраста 2—80 дней изучены при помощи электронного микроскопа. Описывается соотношение эпидермиса и основы кожи (сн. 1—3) и составные части слоев призматических (сн. 1—4), шиповатых (сн. 5—7), гранулезных (сн. 8—11) и роговых (сн. 12) клеток. Авторы анализируют ультраструктуру и специализированное переобразование и следят за процессом ороговения согласно возрасту животных. Описывается трансформирование десмосом, связывающих клетки друг с другом в определенные слои (сн. 13a, b, c).

Ултраструктуральные детали напоминают таковые эпидермиса человека и животных, описанные другими авторами с той разницей, что венчик копыта свиньи проходит — благодаря увеличивающейся с возрастом механической нагрузке — специализированную структуральную трансформацию. Наиболее характерным для этого структурального изменения является усиленное образование эпителио-фибрилл (тонофибрилл). Авторы приходят к заключению, что нормальное течение ороговения и нормальное образование копытного рога зависит от механических факторов, взаимосвязанных с активным движением.

ИЗОЛИРОВАНИЕ ACHOLEPLASMA AXANTHUM ОТ СВИНЬИ

Л. ШТИПКОВИЧ, Л. ВАРГА и Д. ШИММЕЛ

На основании способа культивирования, морфологических, биохимических свойств (расщепление глюкозы, маннозы, маннитала, целлобиозы, сукрозы, ксилозы, эскулина, аргинина и мочевины; редуцирование тетразолия, метиленовой синьи, теллурита калия; продуцирование фосфатазы, формирование «пленки и пятна»; потребность в холестероле; резистентность к хлориду натрия, метиленовой синьи, Твин 80, азиду натрия, таврохолату натрия, полианетолу натрия, pH 5,5 и 9,5, дигитонину) и серологических свойств (торможение роста, торможение обмена веществ, косвенная гемагглютинация, иммунофлуоресценция) изолированных раньше два штамма идентифицированы как **Acholeplasma axanthum**.

ГИСТОГЕНЕЗ ОСТРОЙ ФОРМЫ БОЛЕЗНИ МАРЕКА

А. СЕКИ и А. ВАНИ

Изучены изменения при болезни Марека в хронологическом порядке их появления.

Изучались разные органы и центральная нервная система 42 дневных цыплят. Животных разбили в три группы. 8 цыплятам введено по 0,2 мл вируса болезни Марека, обозначенного MD 7/11, 55, 300 PFV. 8 цыплят второй группы оставлены, чтобы они заразились естественным путем. По одному цыпленку убито к 1-у, 2-у, 3-у, 4-у, 6-у, 8-у, 11-у и 13-у дню после заражения. Двадцать шесть цыплят благополучного хозяйства оставлено контрольными. Среди контрольных животных убито по два цыпленка вместе с экспериментальными животными и, кроме этого, еще к 12-у, 14-у, 16-у, 18-у и 20-у дню. Гистологически изучены сердце, две дольки печени и почек, железистый желудок, селезенка, красный костный мозг, кожа, головной мозг и утолщения спинного мозга всех цыплят.

У внутрибрюшно зараженных животных изменения в печени и сердце наблюдались уже на первый, более выраженно, на второй день в стенках определенных кровеносных сосудов в виде разрастания активных мезенхимальных (ретикулярных) клеток; на третий день в виде появления лимфобластов и на четвертый в виде образования очагов из зрелых лимфоцитов. С 6-го для обнаруживалось со временем усиливающаяся неопластическая пролиферация лимфоретикулярной ткани особенно в печени и позже в других экстранейральных органах. Лимфоидные клетки или неопластические формы ретикулогистиоцитов были характерными. С 8-го дня лимфоидная инфильтрация стенки кровеносных сосудов и муфты из лимфоидных клеток кокруг них имелись в обоих веществах центральной нервной системы. Подобные изменения наблюдались в тех же экстранейральных органах естественно заразившихся цыплят, но 3—4 дня позже. Изменений в центральной нервной системе раньше 13 дней не обнаружено.

КОЛИБАЦИЛЛЕЗНЫЙ МУКОИДНЫЙ ЭНТЕРИТ КРОЛИКОВ. ИЗМЕНЕНИЯ БАЛАНСА ВОДЫ, ЭЛЕКТРОЛИТОВ И КИСЛОТА-ЩЕЛОЧЬ

Ф. ВЕТЕШИ и Ф. КУТАШ

На 20 отбитых крольчатах возраста 4—11 недель изучалось действие мукоидного энтерита на баланс воды, электролитов и кислота-щелочь; 15 здоровых крольчат того же возраста служили контрольными. Баланс воды и электролитов определялся при помощи гематокрита, всего белка сыворотки, мочевины, концентрации натрия и хлорида. Соотношение кислота-щелочь проверялось при помощи микрометода по Аструп.

Обнаружено, что у кроликов с мукоидным энтеритом наступила гипотоническая дегидрация, азотемия и декомпенсированный метаболический ацидоз. Авторы приходят к заключению, что тяжелые перебои в балансе воды, электролитов и кислота-щелочь играют существенную роль в возникновении шоку подобного синдрома и в конечном итоге вызывают гибель животного.

ПАТОЛОГОАНАТОМИЧЕСКИЕ НАБЛЮДЕНИЯ ПРИ КУБИНСКОЙ ЭПИЗООТИИ АФРИКАНСКОЙ ЧУМЫ СВИНЕЙ

Э. ТУРИ, И. Р. РАМОС и Р. УРКИАГА

Патологоанатомические и патогистологические исследования проводились на материале от 33 естественно заразившихся и 7 искусственно зараженных животных. В большинстве животных имелись характерные изменения для подострой и острой формы болезни.

На переднем плане патологоанатомических изменений стояли таковые, указывающие на перебои в кровеснабжении (гиперимичность и цианоз кожи, кроветечения по всему организму). Кроветечения в почках имелись не только в корковом слое этого органа, но часто наблюдались они — в более тяжелой форме — в более глубоких слоях и в слизистой почечной лоханки. Заметными были сильные кроветечения в лимфоузлах желудка и ворот печени. Здесь они были заметными даже в тех случаях, когда в других лимфоузлах имелась только слабая инфильтрация эритроцитами, или только еле заметные кроветечения. Авторы обращают внимание на часто обнаруживаемое в их случаях набухание селезенки, эдематозную инфильтрацию стенки желчного пузыря и интерстициальный отек легких: эти изменения не принадлежат к атологоанатомической картине, обнаруживаемой обычно при классической чуме свиней.

Среди патологических изменений наиболее характерными явились регрессивные процессы, обнаруженные отчасти в клетках лимфоретикулярной системы, главным образом лимфоузлах и селезенке, дальше, в некоторых паренхиматозных органах, как то, печени и почках, в нервных клетках центральной нервной системы и кровеносных сосудах. Изменения кровеносных сосудов заметными были в лимфоузлах и селезенке: здесь они обнаруживались во форме разрыхления стенки сосудов, в накоплении в них ПАШ положительного материала, в образовании вакуолей и в повреждении эндотелиальных клеток. Имелись тяжелые регрессивные изменения и в ретикулярной ткани селезенки.

Перебои в кровеобращении регистрировались в виде образования тромбов и крове течений. Последние, особенно в некоторых лимфоузлах и селезенке, ведут к инфильтрации кровью. Сильные кроветечения наблюдались кроме этого в печени и мягкой оболочке мозга.

Слабые пролиферативные явления — по существу в качестве явлений гиперплязии — имелись с одной стороны в лимфоузлах и селезенке, непоказывающих еще некротических изменений, с другой стороны в печени, где они налицевствовали в виде набухания клеток РГС и разрастания. Часто наблюдалась гистио-лимфоцитарная инфильтрация в междольковых прослойках печени. В ядрах клеток, вызывающих инфильтрацию, часто имелись регрессивные изменения. В мозге авторы редко наблюдали периваскулярную инфильтрацию. Незначительная инфильтрация эозинофильными гранулоцитами часто наблюдалась в лимфоузлах и селезенке.

Согласно авторам непосредственной причиной описанных изменений является вирус африканской чумы свиней; но некоторые изменения могли наступить от гипоксического состояния, вызванного перебоями в кровеобращении. Поднимают вопрос о возможности роли аллергических процессов в возникновении патологоанатомической и патогистологической картин.

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