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A COMPARISON OF THE BIOLOGICAL PROPERTIES AND PATHOGENESIS OF HOG ENCEPHALOMYELITIS AND HUMAN POLIOMYELITIS*)

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As broad a comparison as possible of hog encephalomyelitis virus with the viruses of human poliomyelitis is substantiated mainly for two reasons. Firstly from the theoretical point of view, it is essential if we are to attempt to classify the virus originally discovered by Klobouk, according to the current classification of viruses, with the precision that only a few years ago was not possible. The second reason, of more practical importance, which has guided us in our work since 1949 is the detailed investigation of new and hitherto unknown properties of the hog encephalomyelitis virus, on the basis of then known analogies between both viral groups and utilizing techniques developed during the study of human polio. Methods according to the human model systematically employed in our work brought and are bringing fruitful results. Immense progress has been recorded, especially since 1955, when tissue cultures of the same type as used in human polio were introduced into our studies of Klobouk's virus, into the studies of Larski, of Mayr and Schwöbell, and of workers in France and Great Britain in rapid succession and independently of each other. This new phase will most probably lead to important practical results, for it is already quite apparent which method will be both effective and economical in the production of antigen for active immunization, and later even for the direct virological or serological diagnosis of latent or inapparent forms of the disease. Lastly, it is not improbable that, with the combined implementation of tissue cultures and animal experiments hitherto unanswered questions concerning the pathogenesis of hog encephalomyelitis may be elucidated at least to the extent that has been achieved in the case of human poliomyelitis.

We had a precedent in our comparative studies. The first and then comprehensive report of its kind was the paper of Kaplan and Meranze (1948), which inspired us in our work. Fortner's lecture (1956) is relatively incomplete. We have already presented papers twice on this subject, i. e. in 1954 at a meeting of the Czechoslovak Academy of Sciences in Prague, and a year later at a virology seminar in Budapest. Since then many newly described properties were

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supplemented, enabling a more complete comparison of both viral groups to be made.

Data giving the size of the human poliomyelitis virus vary according to their type and especially according to the method of determination. In the three types the diameter is estimated to be 12—20 mμ on the basis of ultrafiltration, and 25—40 mμ by electron micrography.

The diameter of hog encephalomyelitis virus was first estimated by us (1951) to be 20—30 mμ on the basis of ultrafiltration, Horstmann (1952) reported the size to be about 10—15 mμ and later (1954), on the basis of electron micrographs, we estimated the diameter to be greater than 30 mμ.

On electron micrographs polio I and II appear as spherical formations, polio III as elongated and ovoid.

Our purificates of hog encephalomyelitis virus of a high infectious titre from infected spinal cord disclosed the presence of spheroid particles which were absent in control preparations of healthy tissues.

From this it follows that the size of both viral species is of the same order and, so far as can be judged, of analogous shape.

Viruses of human poliomyelitis may be purified from infected emulsion of CNS by precipitation of balast, either with protamines or with a suitable preparation of bentonite. We followed the same procedure successfully with the hog encephalitis virus, though with some loss of activity, using a cord suspension from a pig sacrificed in the early stages of paralysis.

The three typos of polio viruses are marked by their almost absolute resistance to ether.

In common with other authors, especially Horstmann and Sven Gard, we have demonstrated the same degree of ether resistance in the hog encephalitis virus as in polio.

Quite typical of the human polio viruses is their sensitivity to present freeze drying procedures, which in most viruses serve as the optimal mode of preservation. Lately, however, ways have been found of increasing the resistance of certain strains of Type I and also perhaps of Type III to freeze drying.

We have compared the activity of a lyophilized spinal cord suspension of Klobouk's virus with an untreated suspension. The activity of the freeze dried suspension diminished by more than 1 logarithm.

Another typical property of human polio viruses is their incapacity to agglutinate erythrocytes of various animal species following usual techniques.

Neither we nor anyone else has, as yet, succeeded in agglutinating any erythrocytes by the hog encephalomyelitis virus using highly active purifications from infected hog spinal cord or virus propagated in tissue cultures, not even when such a delicate method, as the one which gives positive results with one of the mouse poliomyelitis viruses (GDVII), was followed.

It is long common knowledge that human polio viruses are highly resistant to physical and chemical agents. Low temperatures preserve them very well. Partially purified human polio viruses are killed in a few minutes at 55^0 C. Alcohol is a relatively weak antiseptic, quartery ammonium bases are almost

completely ineffective. Oxidizing agents are highly effective as disinfectants. The action of an incredibly wide pH range of 4 to 10 for 24 hours is tolerated by polio viruses without significant loss of activity.

In our experiments with hog encephalomyelitis virus a 10% cord suspension from a pig sacrificed in the height of infection was inactivated only after 20 min. at 65° C, while a parallel protamine purificate was inactivated already at 50° C after 20 min. The resistance of the virus from tissue cultures, as observed by Mayr, falls somewhere in between that of our experiments, for 56° C was tolerated for more than 1 hour. The temperature of dry ice, and somewhat less —20° C, preserves the activity of both infected spinal cord and virus propagated in tissue cultures at least for a period longer than 1 year. Chloramine and chloride of lime in our experiments proved to be antiseptics of choice. In our studies a viral suspension tolerated exposure to an almost improbable pH range of 2.5 to 13 for 3—5 hours without loss of activity.

As in human polio, where in routine diagnostic isolation of the viral agent from stool is usual during infection, the hog encephalomyelitis virus was also demonstrated repeatedly in the faeces by several workers and by us after experimental infection. This points to the necessarily high resistance of both viral groups to digestive enzymes of the macro-organism and metabolites of intestinal bacteria.

Keeping in mind Koprowski's recent discovery of polio I in calves, it seems, nevertheless, that the principal natural host of all three types of human polio viruses in nature is man. In so far polio viruses have been recovered elsewhere, or contact of some animals with human polio has been demonstrated (usually in certain rodents dependent on human habitations, or insects, and, recently in calves, rarely by direct isolation of virus, more commonly by antibody response), we are most probably dealing with a mechanical transmission of the human polio viruses through an animal organism, following ingestion of human excreta containing virus, or antibodies which may in part be non-specific. The human polio viruses are experimentally adaptable, utilizing various routes of inoculation, to a few animals, particularly the monkey. The *Maccacus cynomolgus* and of course the chimpanzee seem to be the most susceptible. In these animals, however, the direct instillation of the virus into the lower alimentary tract rarely leads to a paralytic form of the disease. Polio Type II was adapted to rodents long ago, first to the cotton rat, then to the mouse, recently to new born mice, and newly (especially after treatment with cortisone) to the hamster. Only recently Types I and III, after criss-cross passages and by intraspinal inoculation, were propagated in rodents.

Provisionally, the most prominent difference between the polio viruses and the hog encephalomyelitis virus lies in the incapacity of the latter to be adapted to another animal outside the suidae, i. e. the hog, wild boar, and *Potamocherus larvatus* from Madagascar.

As to the possible routes of experimental infection of polio, any of the commonly employed routes of inoculation may lead to the paralytic form of the disease: intraspinal injection is the most successful, then intrathalamic, intra-

nasal, intraperitoneal, intramuscular, intravenous, subcutaneous, even intradermal, and by far the least succsessful is instillation of virus into the lower alimentary tract.

In hog encephalomyelitis, in accordance with other workers, we found intrathalamic and intranasal inoculation to be most advantageous, while intramuscular and subcutaneous injections lead to overt disease in only a small percentage. Rarely and under special experimental conditions did intraperitoneal and intravenous inoculation lead to clinical manifestations of the disease. As has been demonstrated in recent experiments of Mayr and Schwöbel, and which we in part can confirm from our own experience, the virus from tissue cultures (in addition to the fact that it appears in greater quantities than in the CNS) is capable of much greater penetration into an organism. This explains why in cases where originally the least effective routes of inoculation with virus from CNS (i. p. & i. v.) were employed and were not followed by either clinical symptoms or a demonstrable antibody response, on inoculation of virus propagated in tissue cultures latent disease with a high state of immunity was produced. The feeding of swine, especially of young animals, with a viral suspension leads most frequently to paralytic forms of the disease. The same occurs when virus from tissue cultures is used. This mode of infection, of course, does not preclude the oropharyngeal mucosa as the portal of entry. In a series of very exact experiments, where infected cord emulsion was administered directly into the stomach, even after increasing the permeability of the intestinal wall, neither Košťanský nor we were able to produce a paralytic form of the disease and, so far as we investigated, not even an antibody response. It will be necessary for us to repeat the last series of experiments employing a virus from tissue cultures, although it may already be asserted that the lower alimentary tract in swine is relatively resistant to infection by hog encephalomyelitis virus, as is the case in the chimpanzee with polio.

Infectious titres of the three polio viruses in man and experimental animals are of all organs by far the most prominent in the nervous system. Titres in the former are determined, for obvious reasons, with difficulty. In experimental animals the virus rapidly diminishes in the CNS, following the onset of paralyses and with convalescence, so usually, within a week the virus is hardly demonstrable experimentally.

The same is true of the hog encephalomyelitis virus which we were not able to recover from swine sacrificed a week following the onset of paralysis, not even by bithalamic inoculation of other pigs.

A comparison of the polio titres in the CNS of experimentally infected monkeys with, for example, those found in viral encephalitis reveals relatively low titres, usually varying from 10^{-3} (dilution of cord suspension) to $10^{-4.5}$, rarely attaining 10^{-5} in adapted strains. The amount of virus found in the CNS depends on its localization, the maximum is to be found in the spinal cord (especially the cervical and lumbar), much less in the brainstem.

We and other workers found titres of from $10^{-2.8}$ to (rarely) 10^{-4} in the CNS of experimentally infected swine. Horstmann reported the extreme value of

10^{-5}. We have investigated individual parts of the CNS and found that the highest titres, as in human polio, are to be met with in the cervical and lumbar spinal cord, much less in the cerebellum, and far less in the brainstem.

It follows from the presented studies that analogies between human poliomyelitis and hog encephalomyelitis are very striking, although the virus of the latter in its limited adaptability is one of the most specifically host selective of all known viruses.

Propagation of polio viruses in chick embryos long seemed an impossibility. Only much later was it found that certain strains of Type II may be induced to multiply in the whole embryo after treatment with cortisone (injected into the yolk sac), following inoculation into the allantoic cavity. Further, another method, after 100 passages through the hamster, led to the development of a virus cabaple of propagation in chick embryos. Finally, some viral growth in all three types was achieved when an explant of monkey kidney was placed on chorioallantois and inoculated with a drop of viral suspension.

None of these methods are of practical importance save for the possible exception of the second described.

Experiments with hog encephalomyelitis virus were far less successful. No propagation of virus was attained in chick embryonated eggs, even after treatment with cortisone. Harnach described interference of some strains with the development of chick embryos. Brauner reported viral multiplication in tissue explants on chorioallantois, as has been achieved with polio, but with the difference that instead of kidney tissue explants of CNS were employed.

A great chapter in the investigation of human poliomyelitis viruses is the detailed and very precise elaboration of tissue cultures in which all types of polio viruses propagate effectively, in unusually high titres, and in a relatively pure state, as has been demonstrated in the fundamental experiments of Enders, Weller and Robbins. Their original method, utilizing human embryonic fibroblasts, was subsequently elaborated in detail in innumerable studies. The elaboration of monolayer cultures from trypsin-dispersed cell suspension by Dulbecco and Vogt, and modified by others, revealed new possibilities of methods for very delicate diagnostics of polio. These possibilities are given by the cytopathogenic phenomenon which is caused by the polio virus and can be inhibited by specific antisera. Finally, production of pure virus in tissue cultures made sufficient quantities of antigen available for mass vaccination and serological reactions, namely complement fixation.

At the present time it is possible to exploit tumour cells of human origin and tissues from different organs of various animals, for the cultivation of poliomyelitis viruses, not only for primocultures, but also in subcultures of stable line cell strains capable of repeated passage. Recently, it has been reported that propagation of the three types of polio viruses was attained in epithelial cells of a special line from rabbit kidneys, and mention has been made of successful cultivation of polio even in pig kidney epithelium cells.

Uncontestably, Larski was the first to exploit tissue cultures in studies of hog encephalomyelitis. Somewhat later Mayr and Schwöbel described a technique

of monolayer cultures from young pig kidneys for the cultivation of the hog encephalomyelitis virus, and performed extensive biological and immunological investigations with it. Practically simultaneously and independently of them, we elaborated an analogous method. In our first experiments we not only worked with pig kidneys, but also with other pig embryonic tissues. Later, reports on this subject by workers in France (Lépine, Atanasiu, Jacotot), and finally by workers in Great Britain (Chaproniere, Done, Andrewes) have appeared. The propagation of the hog encephalomyelitis virus is followed by a cytopathogenic effect of the same nature as in human polio and may be just as effectively inhibited by specific neutralizing antibodies. The virus readily multiplies in tissue cultures in large quantities and may attain infectious titres of 10^{-5} to 10^{-6}. A cytopathic effect is produced with even greater dilutions, at least by 1 logarithm, but even by 3 log, according to the degree of its adaptation to tissues. Mayr and Schwöbel were the first to point out that, by inhibition of the cytopathic effect neutralizing antibodies, which are otherwise hardly detectable in vivo, may be demonstrated in titres up to 1 : 1024 in convalescent animals. This laid the foundation for the possible serological diagnosis of inapparent infections. We were probably the first to use a formalin-killed virus from tissue cultures (original infectious activity exactly determined, inactivation followed by safety tests) in a model experiment intentionally as a vaccine for active immunisation, with, however, only partial success, but nevertheless indisputable (an unnaturally high intranasal infectious dose was used in the challenge). Only recently, Mayr described a virus that was passaged 91 times through tissue cultures, and lost its infectious activity while retaining its immunogenic properties.

It is not our purpose to compare the delicate differences in the histological picture produced by human poliomyelitis and hog encephalomyelitis. It is generally known that histological changes in the anterior horns of the spinal cord in certain phases of both diseases are almost indistinguishable. The involvement of the cerebellum and cerebral cortex is, however, more extensive in hog encephalomyelitis. The selection of Purkinje cells in the cerebellum resembles similar involvement in Japanese B encephalitis, changes in the cortex may be roughly compared with lesions induced by GDVII of mouse poliomyelitis (Horstmann, Manuelidis, Sprinz).

As to antigenic structure, at the present time, three different types (I, II, III) of human polio viruses are recognized which, in view of their partially different biological properties, are in reality biotypes. Investigations of the antigenicity of pure, concentrated virus from tissue cultures revealed that even in such a small virus a common basic antigen identical with viral particles, and a soluble antigen of smaller dimensions can be presupposed. This antigen may be the cause of occasional cross reactions in complement fixation of Type I with Type II and III. Other cross reactions in complement fixation were also described, usually, however, in newly isolated strains. It has already been mentioned that the complement fixation test for the diagnosis of paralytic and subclinical cases of poliomyelitis is already a useful diagnostic tool. Polio type specific neutraziling antibodies are an indicator of immunity or at least a sign of past infection, per-

sisting apparently throughout life. Formerly, their demonstration required tedious and expensive testing on animals; this now has been superseded by a far more delicate test for inhibition of cytopathic effect. These antibodies can be concentrated into the gamma-globulin fraction of convalescent serum. Gamma-globulin prophylaxis confers reliable, though short-term passive protection against paralytic poliomyelitis.

In hog encephalomyelitis only one antigenic type has been encountered by us and other workers. Recent reports by Mayr and Schwöbel indicate that no substantial differences have been detected by the method of inhibition of cytopathic effect among individual strains. Since, to date, neither we nor, as far as we know, anybody else has succeeded in elaborating a complement fixation test for this disease, a final judgement as to the very delicate differences among viral strains from different localities cannot be pronounced. As opposed to Horstmann, who was not able to demonstrate neutralizing antibodies in vivo, already in 1953 we demonstrated them in titres up to 1 : 32, and even 1 : 64, by using small infectious doses in convalescent or actively immunized animals. We then, and later concentrated virus neutralizing antibodies into the gamma-globulin fraction of blood plasma. Although, in our experiments immune gamma-globulin was administered prophylactically in relatively high amounts, neither we, nor in later studies, workers in Italy, were able to detect the slightest evidence of passive protection of animals. Our original demonstration of neutralizing antibodies in immune animals was, as already mentioned, confirmed by other workers in Germany, France and Great Britain by the far more delicate test in tissue cultures which gave values very similar to those in human polio, once again showing beyond doubt the usefulness of this test for the detection of past infection or the state of immunity.

Until now, no antigenic relationship between of the hog encephalomyelitis virus and any of the three types of polio, or any of the known animal encephalitic viruses has been found. (The question concerning the identity of the hog encephalomyelitis virus with that of Talfan-ill has not as yet been conclusively resolved.)

Active immunization against paralytic infection with human polio can be induced artificially by the well-known Salk vaccine, containing the three types of polio viruses produced in tissue cultures and inactivated by formalin. The vaccine is well tolerated and creates regular titres of virus neutralizing antibodies, which in school children usually persist in sufficient protective quantities for at least 3 years. Some workers claim that the persistence of these antibodies is of substantially shorter duration, especially in younger children. Extensive preparations have, therefore, been undertaken to introduce a live vaccine (Sabin) which is at present administered orally and consists of non-pathogenic, immunogenic variants of the poliomyelitis virus so attenuated that intraspinal inoculation into monkeys does not produce manifest disease. The virus of this vaccine multiplies in the human body and thus propagated is excreted. It induces intensive production of antibodies and remains non-pathogenic. It seems that by this

second vaccination procedure the problem of poliomyelitis as a paralytic disease will be finally solved.

Already for a number of years a vaccine, consisting of formalin inactivated nervous tissues of artificially infected pigs, has been in use for active immunization against hog encephalitis with relatively good results. The vaccine has been employed in a larger scale not only in our country, but also abroad (e. g. in Yugoslavia), and according to reports is capable of reducing the incidence of paralytic cases to a minimum. We also tested a live vaccine some years ago, likewise from tissue emulsions with lipoid adjuvants. Its immunizing effect was prominent, but we admit that in a very limited number of cases it did produce transient paralysis, which means that this procedure carries with it a risk. Recently, on a model experiment we have demonstrated the immunizing power of a completely safe and economical formalin-treated vaccine of very constant properties, produced with the aid of tissue cultures. It is certain that, after some technical adjustments, this vaccine can be exploited in practice, as is also indicated by other immunological studies furthering other ends. At the present time, as has already been mentioned, Mayr has succeeded in what we are also engaged with. He obtained an avirulent, immunogenic variant of the hog encephalomyelitis virus by multiple passage through tissue cultures, which, should it prove to possess constant properties, will, in our opinion, be the best and most economical means for active immunization against this disease.

Pathogenesis of human poliomyelitis, though long the subject of very intensive investigations in man and experimental animals, is not as yet wholly and satisfactorily explained. At the present time two conceptions are recognized, that of Bodian and that of Faber. The former considers the site of primary multiplication of the virus to be the lymphatics of the alimentary tract, while the latter the neural ganglia of the oropharynx, possibly the intestine. Both conceptions differ in a number of points, but both presuppose viraemia for the development of paralysis. Inapparent forms of polio to a certain measure or probably only occasionally coincide with the enteric phase of polio. The enteric stage does not perhaps always lead to viraemia and thus much less to the introduction of the virus into the CNS, especially in infections by less neurotropic strains (the existence of which has been demonstrated experimentally). The exact ratio of inapparent and paralytic forms of the disease is still not known, but undoubtedly inapparent cases are far more numerous.

The old belief that the olfactory bulbus is the portal of entry of the virus into the CNS is valid only in the case of artificial inoculation of monkeys, and is not epidemiologically substantiated in man.

Our knowledge of the pathogenesis of hog encephalomyelitis is not by far as complete as to warrant analogies with one or the other conception on polio. As far as we know, viremia in the course of hog encephalomyelitis was demonstrated directly only once or twice. The elimination of the virus in the faeces has been repeatedly proven by Czech and other authors, including ourselves. Its epidemiological significance is obvious. Since in our experiments elimination of virus in

the stool did not occur until several days after intranasal inoculation, usually prior to or at the onset of paralyses, it is certain that we were dealing with a virus propagated in the animal. It remains a mystery why elimination of the virus in the stool was and is not as yet demonstrated as regularly as in human polio. Another series of our investigations, and the very illustrative experiments by Košťanský, indicated that the lower alimentary tract of the pig is at least as resistant to the hog encephalomyelitis virus, as that of certain monkeys in experiments with human polio. Nevertheless, it cannot be excluded that the virus is absorbed by the intestine, for Hecke demonstrated the virus with the aid of tissue cultures not only in tonsils and laryngeal lymph glands, but also in mesenteric and hepatic nodes, and in addition, exceptionally in the liver, spleen and kidneys of pigs infected orally. In a recent series of experiments we found excretion of the virus by the nasal and oropharyngeal mucosa. Finally, we succeeded by chemical blocking of the nasal and partially of the oropharyngeal mucosa in protecting animals against a lethal paralytic outcome of the disease, which followed intranasal instillation of the virus in all controls. From all this we conclude that in swine natural infection is accomplished most frequently by means of feed contaminated by stools or by nasopharyngeal secretions containing virus. The greater part of the virus, following such an infection, is most probably absorbed by the mucosa of the tonsils and upper alimentary tract. Whether the virus multiplies in these parts with ensuing viraemia, or whether the virus progresses along neural axons and is propagated in the Gasserian or other ganglia (Röhrer), is not, as yet, certain. Likewise, it is not known how regularly viraemia occurs in natural infection. The lower alimentary tract as a portal of entry certainly plays a minor role in hog encephalomyelitis. The existence of viraemia is probably also confirmed by successful immunization with inactivated virus, inducing production of antibodies only. The specific manner of intake of feed by the pig does not of course preclude the possibility that the upper nasal mucosa can occasionally be massively infected. From there infection may ascend directly through the olfactory bulbus without necessarily producing viraemia. Therefore, it will not be possible to prevent a paralytic course of the disease by direct infection via the olfactory portal, with a vaccine consisting of inactivated virus, but, in our opinion, only with a live, avirulent virus of constant properties. We have not demonstrated an inapparent infection with hog encephalomyelitis virus in direct experiment, but we are convinced of its occurrence, and that it certainly is more frequent than paralytic infection. Our assertion is based not only on analogies with human polio, but also on experience from our first experiments: when a greater number of pigs was kept for longer periods near experimentally infected ones, regardless of meticulous care and upkeep of the stalls, the animals became resistant to usual infectious doses. The epidemiological significance of these inapparent and subclinical infections is undoubtedly of primary importance. Many fundamental questions concerning the pathogenesis of hog encephalomyelitis remain to be answered. Moreover, further investigations will perhaps be more tedious and will encounter greater difficulties than in human poliomyelitis.

CONCLUSIONS

A comparison of our and other available data concerning the human poliomyelitis viruses and Klobouk's virus of hog encephalomyelitis (Teschen disease), demonstrating their similarity with due respect to their diffrences, warrants us in grouping them close together taxonomially.

Similarity of the viruses is most marked in the following points:

1. The size of both species of viruses is of the same order and, so far as determined, they are of the same shape.
2. Both are highly ether resistant, and tolerate temperature, antiseptics and digestive enzymes to the same degree.
3. They both withstand a wide range of pH, even over longer periods of time.
4. They are very sensitive to lyophilization procedures.
5. So far, following usual techniques, the agglutination of mammalian erythrocytes has been unsuccessful with both viruses.
6. At the present time, it seems that in nature each virus has only one principal natural host, in the case of Klobouk's virus the pig is also the only known experimental animal.
7. Both species of viruses attack the anterior horns in the spinal cord. Neither of them, however, are strictly neurotropic. The histological picture of the involved spinal cord is almost identical.
8. In natural and in experimental infection titres of the viruses in the CNS are relatively low, the highest level is attained in the cervical and lumbar spinal cord.
9. Propagation of the viruses in the chick embryo is slight, exceptional, and may take place only under special experimental conditions.
10. Both viruses readily multiply in homologous (polio also in heterologous) tissue cultures (kidney epithelium), in which both attain similar high titres and produce a prominent cytopathogenic effect of similar sequence and appearance.
11. In both cases cultivation in tissue cultures may well be employed in the demonstration and titration of virus neutralizing antibodies.
12. Active immunization may be achieved with either active or formalin-killed virus (obtained to advantage from tissue cultures).
13. Both viruses are excreted in the oropharyngeal secretions and in the faeces of infected organisms.
14. It already seems apparent that the pathogenesis of the human disease and of hog encephalomyelitis have identical fundamental characteristics. Inapparent infection has been proved to be especially frequent in the case of human polio, and it is most probable that the same is true with hog encephalomyelitis.

We are well aware of certain differences between the three types of polio viruses and the hog encephalomyelitis virus. Some of them may be explained by our hitherto incomplete knowledqe of Klobouk's virus, which only further studies may clarify. The prominent points of distinction in the viruses are the following:

1. Attempts to adapt Klobouk's virus to another experimental animal outside the suidae, i. e. its natural host, were hitherto unsuccessful.

2. Histological changes induced by Klobouk's virus in the brain are substantially greater than in polio.

3. Prevention of infection in pigs with gamma-globulin, although the gamma-globulin contained distinct amounts of specific neutralizing antibodies, was unsuccessful.

4. Hitherto employment of complement fixation reaction in the serological demonstration of Teschen disease has been unsuccessful.

5. So far, of all the known strains of Klobouk's virus only one antiqenic type has been demonstrated, which has no antigenic relationship with any of the three types of poliomyelitis viruses.

With due respect to the described differences, it may in our opinion, nevertheless be concluded that sufficient data, analogies a similarities between the viruses of human polio and Klobouk's virus of hog encephalomyelitis have been accumulated to justify these viruses, including the three murine viruses of Theiler, being grouped together under a common and more general heading of poliomyelitis-like viruses.

CONCLUSION

Si nous recapitulons concisément tous les faits enumerés ci-dessus d'un qrand nombre de ressemblances, dejà moins concernant les différences éventuelles des deux virus — et cela des 3 types de la poliomyélite humaine comparé point par point avec le virus de la paralysie infectieuse d'après Klobouk — il est clair que nous sommes autorisés à les classifier dans une proximité taxonomique relative. Des analogies étendues existent surtout dans les qualités suivantes:

1) Les deux virus sont approximativement de la même grandeur et d'après ce qui est connu jusqu'à présent, de la même forme.

2) Les deux sont également résistents contre l'éther et de même contre la température, les antiseptiques et les ferments de digestion.

3) Ils supportent à peu près les mêmes larges délimitations pH pour de longues durées.

4) Ils sont très sensibles à la lyophilisation.

5) En employant la méthode courante ils n'hémagglutinent jusqu'à présent d'erythrocytes d'aucun mammifère.

6) Jusque maintenant il semble que dans la nature chacun d'eux n'a qu'un hôte, qui chez la paralysie inféctieuse des porcs est aussi l'unique animal d'expériment connu.

7) Les deux éspèces du virus ont lors de l'attaque du système nerveux central une affinité spéciale vers les cornes antérieures de la moelle. Mais aucune d'elles n'est pas absolument neurotrope. Le tableau histologique de la moelle attaquée est presque identique.

8) A l'occasion d'une infection naturelle ou expérimentale les titres du virus dans le système nerveux central sont rélativement bas, les plus hauts dans la moelle cervicale et lumbale.

9) La propagation dans l'embryo d'une poule est minimale a lieu exceptionellement et se fait seulement à conditions spéciales d'expériment.

10) Les deux virus se propagent très bien sur des explantates des tissus homologues (epithèles des reins), la poliomyélite aussi sur des explantates hétéroloques, où tous les deux atteignent des hauts titres semblables et forment un phenomène cytopathogène d'un cours et d'une apparence ressemblants.

11) La cultivation dans le tissu peut très bien être usité dans les deux cas pour la preuve et la titration des anticorps neutralisant le virus.

12) Avec le virus actif et formalisé (surtout de cultures dans tissu) on peut immuniser avec succès.

13) Les deux virus s'éliminent de l'organisme infiqué de la muqueuse oropharyngeale et des féces.

14) Il semble déjà, que la pathogenèse de la maladie humaine et, tant que connu, aussi celle des porcs ont des traits fondamentaux semblables. Des inféctions inapparentes ont surtout maintes fois été prouvées pendant la poliomyélite humaine et il est vraisemblable que tout aussi souvent elles sont prouvées chez la paralysie des porcs.

Entre les trois types du virus de la poliomyélite et le virus de la paralysie inféctieuse il éxiste des différences, dont quelques sont probablement du à un manque dans nos recherches et études concernant le virus des porcs, et il est possible que pendant les recherches ultérieures nous arrivions aux mêmes accords, que chez tous les qualités jusqu'à présent notées.

Ces différences jusqu'à présent sont les suivantes:

1) L'impossibilité d'adapter le virus de la paralysie inféctieuse sur des autres animaux expérimentaux, exceptés les suides, les hôtes naturels du virus.

2) Une étendue sensiblement plus grande des altérations histologiques dans le système nerveux central que chez la poliomyélite.

3) L'impossibilité d'une prévention par le gamma-globuline, même si le globuline appliqué contient des quantités distinctes d'anticorps spécifiques neutralisants.

4) L'impossibilité jusqu'à nos jours d'employer la réaction de la fixation du complément pour prouver sérologiquement la maladie.

5) L'unité antigène des toutes les souches du virus de la paralysie infectieuse des porcs connues jusqu'à présent et avec cela leur complète différence de tous les types connus du virus de la poliomyélite.

Malgrè toutes les différences nous nous croyons de droit à conclure, que des analogies et des ressemblances entre les virus de la poliomyélite humaine et celui du virus d'après Klobouk ont été trouvées et resumées en tant dans notre matériel, que d'après notre opinion nous pouvons ranger le virus de la maladie de Těšín et cela communément avec les 3 types du virus, des souris, ainsi nommé d'après Theiler, dans le groupe commun des ainsi nommés virus de la poliomyélite.

SCHLUSSFOLGERUNG

Falls wir kurzgefaßt alle oben angeführten Fakta über eine große Reihe von Analogien, die sich aus dem punktweisen Vergleich der 3 Typen menschlicher Polio mit dem Kloboukschen Virus ergeben, rekapitulieren, und die schon weit weniger hervortretenden Differenzen in Erwägung ziehen, sind wir berechtigt, sie in eine verhältnismäßige taxonomische Beziehung zu bringen.

Weitgehende Analogien bestehen insbesondere hinsichtlicht dieser Eigenschaften:

1. Beide Virusarten haben annähernd die gleiche (Reihen-) Größe und nach den bisherigen Erkenntnissen auch eine annähernd gleiche Form.

2. Beide sind gleicher Weise resistent gegenüber Aether und ähnlich gegenüber Wärme, Antiseptika und Verdauungsfermenten.

3. Sie vertragen lange Zeit hindurch in ungewöhnlich weitem Ausmaß beinahe die gleichen pH.

4. Sie sind äußerst empfindlich auf Lyophilisierung.

5. Bei Anwendung einer laufenden Methodik hämagglutinieren bisher keine Erythrozyten bei Säugern.

6. Es hat bisher den Anschein, daß in der Natur jede von ihnen nur einen hauptsächlichen natürlichen Gastgeber hat, welcher bei der infektösen Schweinelähmung auch das bisher einzige bekannte Versuchstier darstellt.

7. Beide Virusarten haben bei Befall des ZNS eine specielle Affinität zu den Vorderhörnern des Rückenmarks. Absolut neurotrop ist jedoch keine von ihnen. Das histologische Bild des befallenen Rückenmarks ist beinahe identisch.

8. Bei natürlicher und auch Versuchsinfektion sind Virustiter im ZNS verhältnismäßig gering, am höchsten im zervikalen und lumbalen Rückenmark.

9. Die Mehrung im Kückenembryo ist nur gering, ausnahmsweise erfolgt sie nur unter besonderen experimentellen Bedingungen.

10. Beide Virusarten mehren sich gut auf homologen (Polio auch auf heterologen) Gewebeexplantaten (Nierenepithel), wo beide ähnlich hohe Titer erreichen und ein markantes zytopathogenes Phänomen ähnlicher Folge und Erscheinung bilden.

11. Die Gewebekultivation kann in beiden Fällen zum Nachweis und zur Titrierung von virusneutralisierenden Antikörpern gut verwendet werden.

12. Mittels des aktiven und auch formolisierten Virus (insbesondere aus Gewebekulturen) kann mit Erfolg aktiv immunisiert werden.

13. Beide Virusarten werden aus dem infizierten Organismus durch die Schleimhäute des Oropharynx und durch den Stuhl eliminiert.

14. Es hat schon jetzt den Anschein, daß die Pathogenese der menschlichen Erkrankung und soweit bekannt auch der Erkrankung von Schweinen ähnliche Grunzüge aufweist. Inapparente Infektionen wurden als sehr häufige Erscheinung bei menschlicher Polio nachgewiesen und es ist wahrscheinlich, daß sie ebenso oft bei der infektiösen Schweinelähmung in Erscheinung tritt.

Zwischen den 3 Typen von Poliovirusarten und dem Virus der infektiösen Lähmung bestehen aber auch Differenzen, von denen wenigstens einige durch

unser bisher nicht völlig hinreichendes Studium des Schweinevirus verursacht sein dürften und die sich vielleicht im Verlauf des weiteren Experimentierens zur gleichen Einheit wie bei allen bisher angeführten Qualitäten herausbilden werden. Die bisherigen Unterschiede sind:

1. Die Unmöglichkeit der Adaptierung des Virus der infektiösen Lähmung an andere Versuchstiere mit Ausnahme der Gattung suidae, d. i. des natürlichen Virusgastgebers.

2. Ein bedeutend größeres Ausmaß von histologischen Veränderungen im ZNS als bei Polio.

3. Die Unmöglichkeit einer Gama-Globulin-Prävention auch wenn das verwendete Gama-Globulin offensichtliche Quanten spezifischer neutralisierender Antikörper enthält.

4. Die bisher dauernde Verwendungsunmöglichkeit von Komplementfixationsreaktionen zum serologischen Nachweis der Erkrankung.

5. Die antigene Einheit aller bisher bekannten Stämme des Virus der infektiösen Lähmung von Schweinen und dabei ihre absolute Verschiedenheit von allen bekannten Typen der Poliovirusarten.

Trotz all der angeführten Unterschiede sind wir berechtigt zu schliessen, daß in unserem Material soviel Analogien und Ähnlichkeiten zwischen dem Virus menschlicher Polio und dem Kloboukschen Virus gefunden und zusammengefaßt wurden, die unserer Ansicht nach den Virus der Tetschener Krankheit zusammen mit noch 3 bisher bekannten Mäusetypen, den sg. Theilervirusarten, in eine gemeinsame übergeordnete Gruppe der sg. poliomyelitischen Virusarten zusammenzufassen gestatten.

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A COMPARISON OF THE BIOLOGICAL PROPERTIES & PATHOGENESIS OF HOG ENCEPHALOMYELITIS & HUMAN POLIOMYELITIS

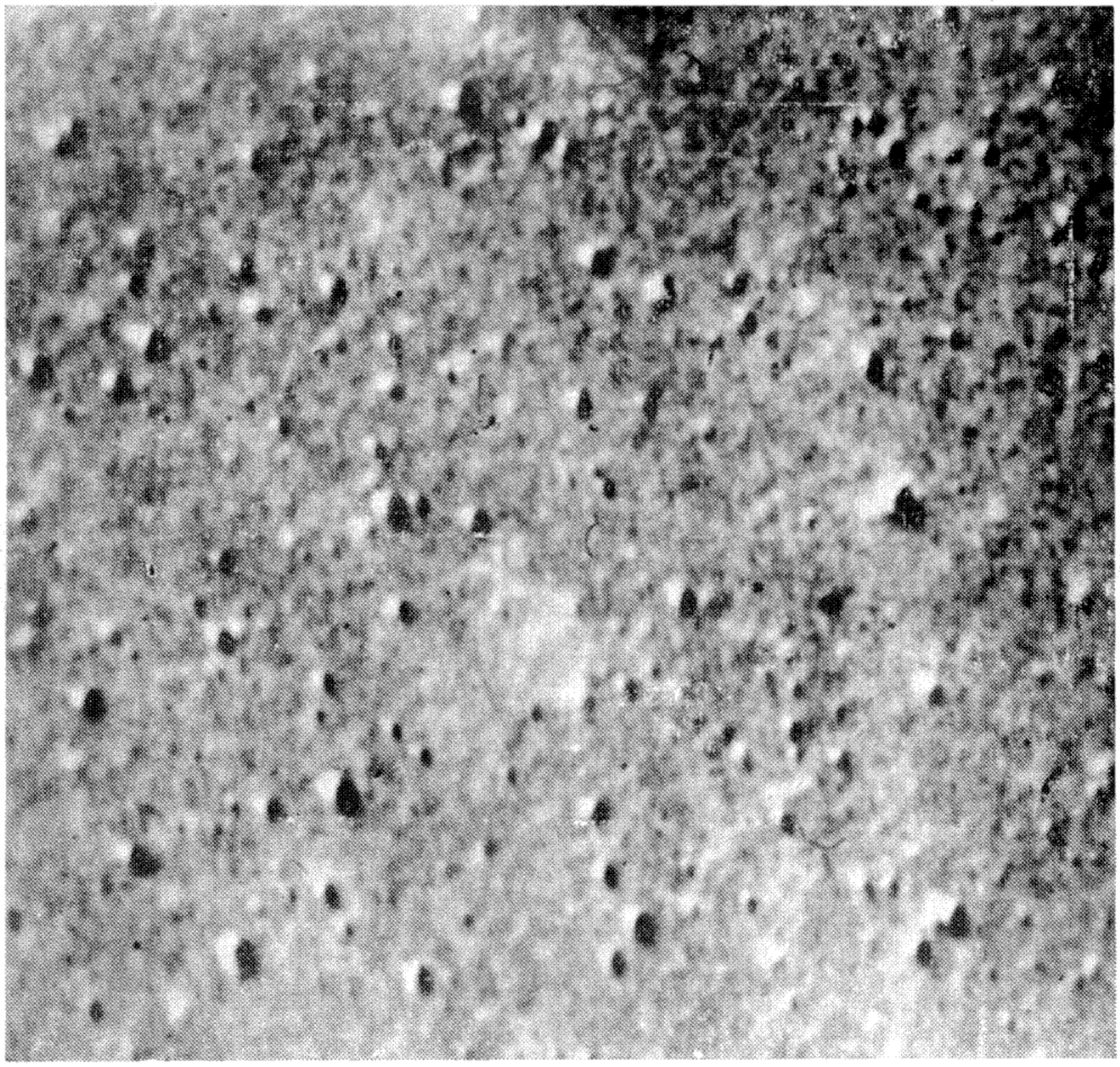

Fig. 1. Electron micrograph of hog encephalomyelitis virus, 1 : 100,000. (Photo Prof. Dr. J. Wolf.)

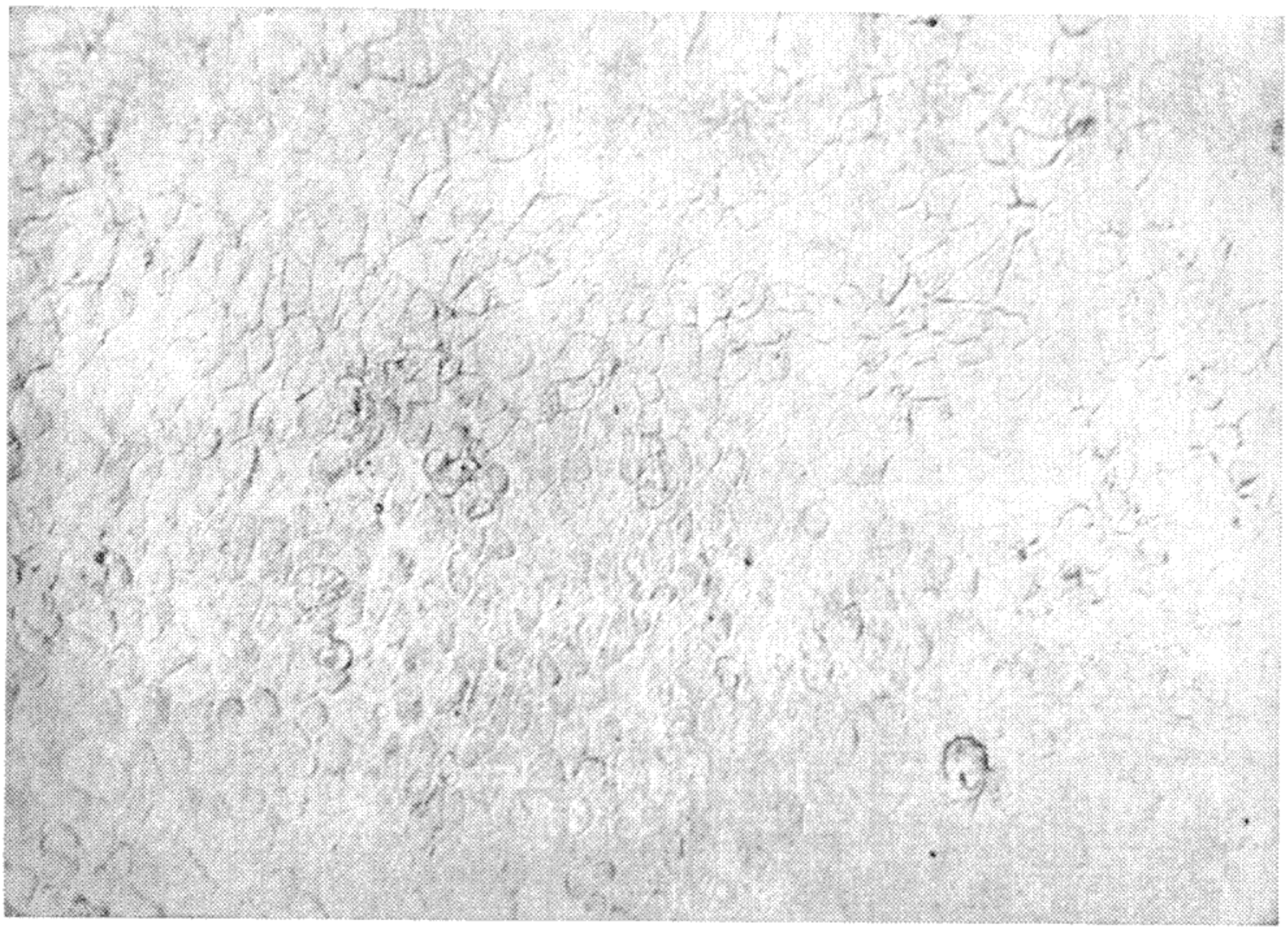

Fig. 2. Normal pig kidney epithelium culture. (Photo Dr. S. Gašpar.)

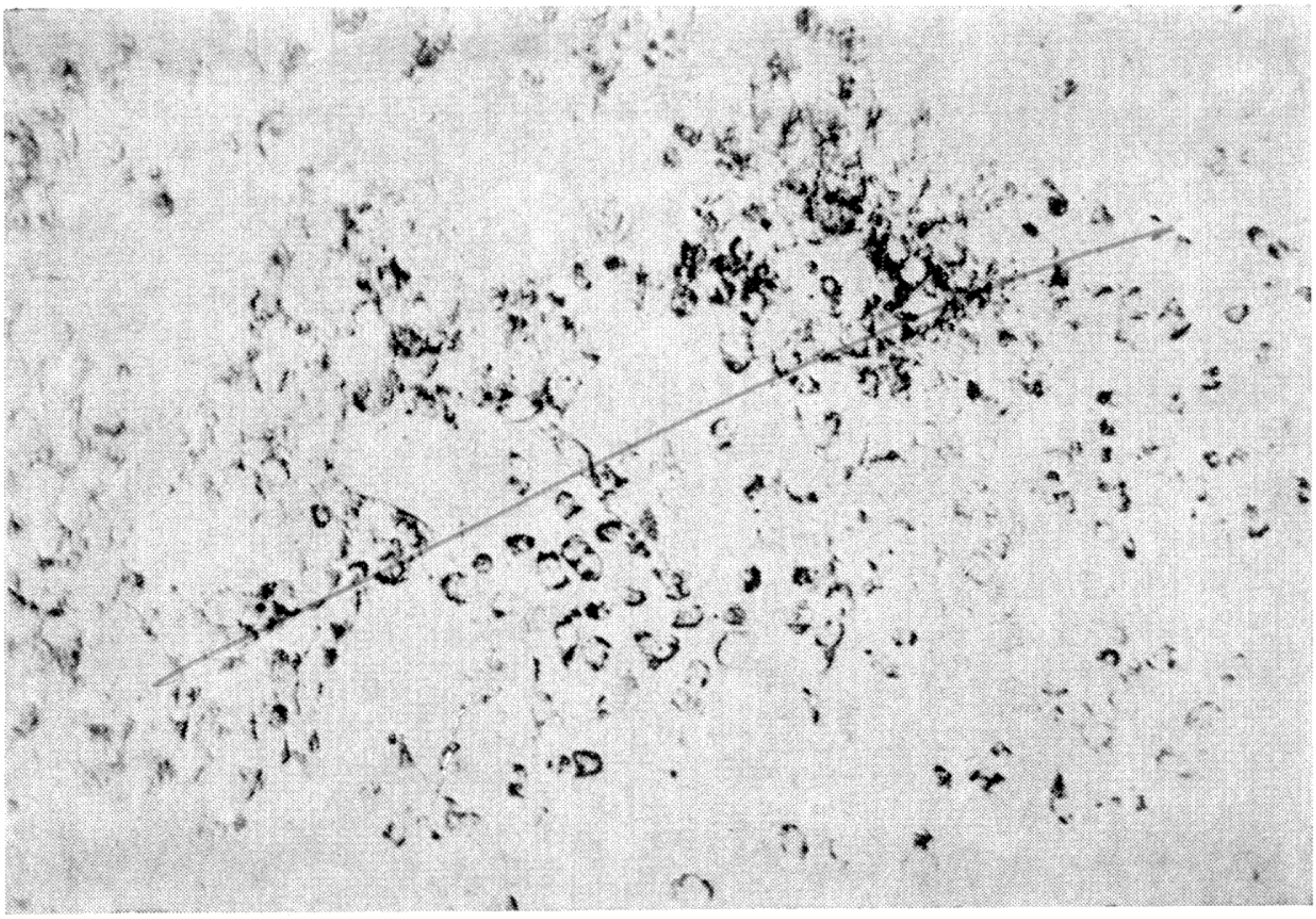

Fig. 3. Pig kidney, 24 hrs. after inoculation with hog encephalomyelitis virus.
(Photo Dr. S. Gašpar.)

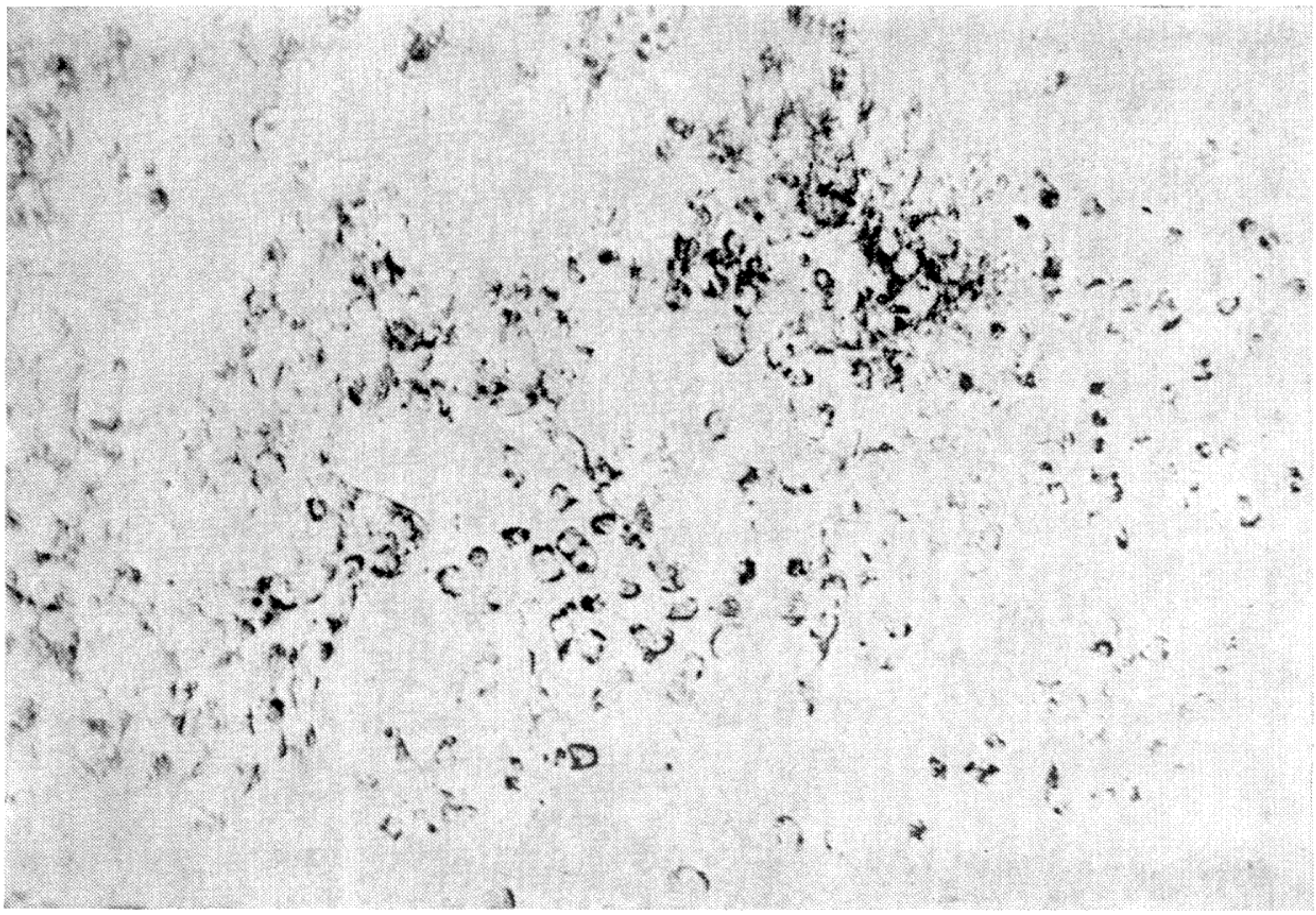

Fig. 4. Pig kidney, 48 hrs. after inoculation with hog encephalomyelitis virus.
(Photo Dr. S. Gašpar.)