Foot-and-mouth Disease - Vesicular Stomatitis - and "Vesicular Exanthema of Swine"


The differential diagnosis of foot-and-mouth disease from certain other diseases such as, e.g., vesicular stomatitis, when based on clinical observations alone, is notoriously unreliable. Although points of difference such as the degree of generalisation in the infected animal and the rate of spread of the disease from one animal to another or from one set of premises to another may provide useful suggestive evidence as to the nature of the disease under investigation, a reliable differentiation based on these observations is not possible.

Cattle have been found to be infected under natural conditions with the virus of vesicular stomatitis and, experimentally at least, swine can also be infected with the virus of this disease. The lesions in both these species of animal are indistinguishable from those occurring in infection with foot-and-mouth disease. Recently reports have come from America of the occurrence of another disease in swine which was clinically indistinguishable from foot-and-mouth disease or experimental vesicular stomatitis in this species (see Traum, 1936). In test animals this disease differed in certain respects from both of the latter infections, and this suggested that it should be considered as a separate entity; hence it has been termed "vesicular exanthema of swine." Up to the present, it has not been found possible to infect cattle or guinea-pigs with the virus obtained from swine infected with "vesicular exanthema", and, of course, guinea-pigs can be infected experimentally by inoculation with the viruses of foot-and-mouth disease and vesicular stomatitis, the lesions produced by these being clinically indistinguishable.

Horses are infected under natural conditions with the virus of vesicular stomatitis and have also been infected experimentally by inoculation. There are no accurate experimental observations on record which prove that horses contract foot-and-mouth disease under natural conditions, although this has been suggested by clinical observations [see Report of the Foot-and-Mouth Disease Commission of the United States Department of Agriculture (1928) p.115, "Foot-and-Mouth Disease in the Horse"], and although the horse may be very resistant to infection, the evidence available is insufficient to determine whether or not some sort of a lesion may not sometimes be produced by inoculation of the virus directly into the mucous membrane of the mouth or tongue. It has been found possible with the virus of "vesicular exanthema of swine" to produce mild localised lesions in the orae of the tongue of some horses (16 out of 28) by experimental inoculation. Usually these lesions were unaccompanied by any severe systemic disturbance such as is observed in infection with the virus of vesicular stomatitis. In this connection, it is interesting that Traum has suggested that, although no cases of "vesicular exanthema of swine" have so far been observed in horses under natural conditions, it is suspected that cases may have occurred and that perhaps this would explain some of the failures to transmit the disease experimentally from swine to horses.
The differential diagnosis of a disease simply by consideration of animal species susceptibility is obviously fraught with many possible sources of error. It is known, for example, that in Germany strains of the virus of foot-and-mouth disease have been recovered from swine which could only with great difficulty be transmitted to cattle, and some strains of virus recovered from cattle have only with difficulty been transmitted to guinea-pigs.

Again, although the problem does not immediately concern European or American workers, it is of interest to refer here to the fact that until quite recently it had been accepted that "blue-tongue", or catarrhal fever of sheep, was a disease confined to sheep. Attempts to produce this disease in cattle had generally failed and no records of the disease occurring in cattle under natural conditions had appeared. Since 1932, however, cases of blue-tongue have been proved to exist among cattle [see Bekker, J.G., de Kock, G.v.d.W., and Quinlan, J.B., "The occurrence and identification of "blue-tongue" in cattle, the so-called pseudo foot-and-mouth disease in South Africa" - Onderstepoort J., 1934, 2, 393]. In practically every instance when the disease was reported, the owners suspected foot-and-mouth disease. In one instance the disease occurred on a farm very near another premises where foot-and-mouth disease had actually been diagnosed. Lesions were observed on the mouth and feet, and from the reports it is extremely doubtful whether the disease could be diagnosed with accuracy at all stages of the infection by the clinical symptoms alone. Cattle have also been experimentally infected with "blue-tongue" virus, although they appear to be less susceptible than sheep.

To return to the diseases, foot-and-mouth disease and vesicular stomatitis, it has been made sufficiently clear from what has been said above, that it is extremely useful to have at our disposal rapid, certain and relatively economical methods of differentiating between these two diseases. Bearing in mind also the suggestion made originally by the American Foot-and-Mouth Disease Commission in 1928, that the two diseases might occur in the same herd, or indeed in the same animal, simultaneously, and the possibility that under experimental conditions one virus might become accidentally contaminated with the other, if these methods could be applied to the resolution of mixtures the advantages are obvious. Since the fact has been established that there are at least three types of the virus of foot-and-mouth disease and two of the virus of vesicular stomatitis, cross-immunity tests as a possible means of differentiating the two diseases have become very complicated. The question is not simplified when it is realised that there appear to be some strains, believed to be foot-and-mouth disease, which cannot be definitely typed, and there may exist other unrecognised types of the virus of vesicular stomatitis.

Experiments made on large animals such as horses and cattle are costly and require a long time and especially if possible mixtures of the two viruses were being investigated the results obtained might easily lead to false interpretations unless a sufficiently large number of test animals was employed.

Galloway and Elford (1933) showed that the virus of vesicular stomatitis has a diameter of about 70-100 µ and that this size value, which is the same for the immunologically different strains, "New Jersey" and "Indiana", is about eight times that previously estimated for the virus of foot-and-mouth disease, 8-12 µ. Galloway and Elford (1931).

Later Burnet and Galloway (1934) reported that it is possible to propagate the virus of vesicular stomatitis in the chorioallantoic membrane of the developing hen's egg and the most important fact that the inoculation of eggs renders possible the
detection of small amounts of the virus of vesicular stomatitis which may produce no reaction by inoculation into guinea-pigs. Employing a standard technique which is described in the present paper, the virus of foot-and-mouth disease was found not to survive even for 24 hours in a total of 56 developing hen's eggs injected. It has been found possible to resolve mixtures of the viruses of foot-and-mouth disease and vesicular stomatitis, irrespective of their relative concentrations, by a method combining filtration with egg inoculation tests. The technique provides a straightforward and relatively economical method of rapidly and accurately distinguishing the two viruses and resolving a mixture of them. It has also been shown that, under experimental conditions at least, it is possible for an animal to be infected simultaneously with the virus of vesicular stomatitis and the virus of foot-and-mouth disease. Both viruses were passaged together successfully in guinea-pigs and each virus was then recovered in guinea-pigs and each virus was then recovered in the pure state by the method referred to above.

During the course of this work it was found that vesicular stomatitis virus which has been passaged in eggs is found to filter more easily than virus which has been passaged only in guinea-pigs. This suggests that the virus undergoes some slight modification, either in size or surface properties, by passage in eggs. This modification, although definite and not easily reversible, is not of a sufficient magnitude to affect adversely the writers' original conclusion regarding the size of the virus (Y. supra).

A study was made also of the relative stabilities of the virus of foot-and-mouth disease and the virus of vesicular stomatitis at different hydrogen-ion concentrations. The results obtained confirmed in general the observations of Fyl and his co-workers. The virus of foot-and-mouth disease remained infective over a considerable period at 0°C, at about pH 2.5-3.5, i.e., under conditions more acid than the generally accepted pH stability limit, 6.5-8.0. Generally this virus rapidly becomes inactivated within the zone pH 4.5 to 6.2.

The zone of relative stability of the virus of vesicular stomatitis, on the other hand, appears to be limited on the acid side to about pH 4.5 and, in contrast to the virus of foot-and-mouth disease, no second zone of stability is found in a still more acid region. In view of the fact that variable experimental conditions may lead to erratic results, it is essential that every test shall be accurately controlled in any attempt to use these observations as a means of differentiation. Even so, a differentiation based on them cannot be wholly reliable. Fyl and Kleck (1933) [in Z.Bakt. Abt. I., Orig.128, 161] and Fyl (1934) [in Hoppe-Seyler's Z., 226, 18], for example, were of the opinion that the type of buffer systems used did not affect the results, but Galloway and Elford's observations show that the results may be affected by the different methods of adjusting the pH value.

Fyl and his co-workers have advanced what has been termed a "realkalinization" test for differentiating between the two viruses, foot-and-mouth disease and vesicular stomatitis. It is based on the observations made on the relative pH stabilities of the two viruses referred to above. Galloway and Elford, however, give reasons for their conclusion, drawn from experimental observations, that no accurate differentiation of the two viruses can be based on this "realkalinization" test.

Work with the virus of "vesicular exanthema of swine" is hampered at present by the fact that no small experimental animal has as yet been shown to be susceptible to the virus, swine and horses being the only animals so far infected with it experimentally. It would be extremely useful to be able to determine the size
value of the virus, but until a susceptible small experimental animal is found, extensive filtration experiments are not possible. It would also be useful to know if the vesicular exanthema virus can be propagated in eggs. Information on these points would be useful as a basis of accurate means of differentiating the virus from that of vesicular stomatitis or foot-and-mouth disease. Reppin and Pyl (1934) [in Arch. wiss. Tier., 68, 183] concluded that there is an immunological relationship between the viruses of vesicular stomatitis and "vesicular exanthema of swine", but Traum states that cross-immunity tests in horses failed to show this relationship, and further suggests that there may be different types of the virus of "vesicular exanthema of swine" as in the cases of vesicular stomatitis and foot-and-mouth disease. The interpretation of cross-immunity tests, therefore, is very difficult. Reppin and Pyl also stated that in their "realkalinization" test the virus of vesicular exanthema of swine behaved like the virus of vesicular stomatitis and not like that of foot-and-mouth disease.

Summarising the results of the experiments reported on in the papers by Galloway and Elford and Traum, the most rapid and certain methods of differentiating between the viruses of vesicular stomatitis and foot-and-mouth disease are filtration through standard collodion membranes to estimate the size value, combined with egg inoculation tests, and until further work has been done on the virus of "vesicular exanthema of swine", this disease can only be differentiated from the other two viruses by the fact that it has not yet been transmitted to either cattle or guinea-pigs.

I.A. GALLOWAY.

(Reprinted from The Veterinary Record, 1936, 48, 709.)
ANNOTATION

"Vesicular Exanthema of Swine."


Reference has already been made (see annotation in Vet.Rec., 1936, 48, 709) to the occurrence in California during 1933 and 1934, and probably also in 1932, of an infectious disease in garbage-fed swine, clinically indistinguishable from foot-and-mouth disease or vesicular stomatitis. Traum (1936, J.Amer.Vet. Med.Ass., 88, 316) in some preliminary experiments with material collected from diseased pigs, succeeded in infecting swine, and sometimes horses, by inoculation. His failure to infect cattle and guinea-pigs appeared to him sufficient evidence to justify the consideration of the disease as a separate entity and he termed it "vesicular exanthema of swine".

The subject matter now under review deals chiefly with experimental work carried out respectively at the Animal Disease Station, Beltsville, Maryland, U.S.A. (Crawford) and at the Research Station of the Ministry of Agriculture, Pirbright, Surrey, England (Andrews and colleagues), with strains of virus obtained originally from California. As far as can be ascertained, so far "vesicular exanthema of swine" has not been reported elsewhere than in California. As regards symptoms, vesicles of varying size may appear on the snout, nose, lips, tongue, or on the feet between the digits, around the coronary band, on the ball of the feet or on the dew-claws. Lesions on the udder and especially the teats have been observed in a large proportion of nursing sows. These eruptions are usually preceded by, and accompanied by, a febrile reaction. Later the vesicles rupture and heal. In some outbreaks there was a predominance of snout lesions, whilst in others foot lesions were more common. In general, all investigators, who have studied the disease closely, appear to be agreed that the disease cannot be distinguished clinically from naturally occurring, or experimental, foot-and-mouth disease in pigs, or from experimental vesicular stomatitis in pigs.

As regards species susceptibility, in different research centres in America, England and Germany, more than 100 cattle of different ages have now been inoculated by different routes, intra-dermally into the mucous membrane of the mouth, and/or intramuscularly or intravenously with 'strains' of virus of this Californian disease of pigs, with entirely negative results. Similarly, negative results have attended attempts to infect several hundreds of guinea-pigs. Mclaren in America (cited by Traum) failed to infect a European hedgehog with the virus of "vesicular exanthema of swine", although this animal appears to be highly susceptible to infection with foot-and-mouth disease. Andrews and his colleagues at Pirbright state also in reference to hedgehogs that no definite infection has resulted in these animals from inoculation with vesicular exanthema virus, nor in healthy hedgehogs placed in contact with those inoculated.

The last-named workers state that in a limited number of
experiments they failed to infect wild rats, sheep and goats. All these species of animal have, at one time or another, been infected with the virus of foot-and-mouth disease. (Andrews and his co-workers state, however, (p.72, Section III) that "up to the present time no evidence has been obtained of the existence of strains of foot-and-mouth disease with particular virulence for sheep.")

It was pointed out in the previous annotation on this subject that Traum stated that he had produced mild localised lesions on the dorsum of the tongue of some (16 of 28) horses by inoculation. The German workers at the Insel Riems have also reported that they were able to produce lesions in horses by inoculation. Andrews and his colleagues failed to observe any lesions in five horses inoculated intradermally into the tongue or lips with a strain of virus received from the U.S.A. Bureau of Animal Industry's Experimental Station. The annotator has no information as to whether this strain was the same as that employed by Traum in his original experiments, and/or the same as that sent to Germany for investigation. However, Traum stated in a letter to Crawford, accompanying three samples of virus collected from different sources during the 1933 outbreak, that only one of these was found to be capable of producing lesions in horses. Crawford also tested the susceptibility of horses to inoculation with three other "strains" of virus recovered from pigs during outbreaks of the disease during 1934. With only one of these three strains could horses be infected. This particular "strain" caused less severe lesions in pigs than the other two. These differences in the behaviour of "strains" of virus when inoculated into horses may be more apparent than real, but if the results of these comparative tests are valid they might serve to explain the seeming discrepancies in the observations of different investigators. In this connection, however, attention should again be drawn to the suggestion of Traum that although up to the present no cases of "vesicular exanthema of swine" have been observed in horses, under natural conditions, nevertheless cases may have occurred and perhaps this would explain some of the failures to transmit the disease experimentally from swine to horses.

Suggestive evidence emerges from Crawford's experiments as to the existence of more than one immunological type of the virus of "vesicular exanthema of swine". It is suggested that four types, A, B, C and D probably exist. Although the evidence for this would have been more convincing if the experimental pigs had been retested with an "homologous" strain before being re inoculated with an "heterologous" one, yet it was shown that as a general rule recovery from infection with one "type" did not confer immunity to inoculation with another "type". Further, in other groups of pigs, each "type" appeared to immunize the animals against re-infection with the homologous "type".

A more complete examination of the three "types" of virus, A, B and D (C has now apparently been lost), taking the precautions suggested above, would appear to be indicated, and it would be useful if this was supplemented by cross-neutralisation tests with antisera. If Crawford's results are accepted on their face value they would suggest that the differences between the "types" of virus of "vesicular exanthema of swine" are more clear cut than in the case of foot-and-mouth disease. In the latter disease, although it is easy to distinguish between different immunological types in guinea-pig tests, when experiments are made in cattle and pigs in which species the disease occurs under natural conditions, there appears to be an overlapping, or extension of the resistance to infection produced by strains of different types, in some instances.

Reference has been made previously to the cross-immunity experiments carried out by Reppin and Fy1 (1934) [in Arch.wiss.prakt.Tierhik., 68, 183] in the Insel Riems, Germany, with a
sample of "swine vesicular exanthema" virus from America. These authors concluded that the virus differed immunologically from the three standard types, Vallée A, Vallée O, and Waldmann C, of the foot-and-mouth disease. Suggestive, but not conclusive, evidence to support this opinion is now forthcoming from the experiments made by Andrews and his co-workers at Pirbright.

It may be recalled that the German workers concluded that there is an immunological relationship between the viruses of vesicular stomatitis and "vesicular exanthema of swine". This conclusion was based on the results of experiments, one in which seven pigs recovered from the New Jersey type of vesicular stomatitis virus were inoculated with the virus of "vesicular exanthema of swine", and only two showed lesions; and another in which four pigs recovered from the Indiana type of vesicular stomatitis virus were inoculated with the virus of "vesicular exanthema of swine", and only one developed lesions. On the other hand, Traum stated that cross-immunity tests in horses failed to show this suggested relationship between the virus of vesicular stomatitis and the virus of "vesicular exanthema of swine".

Reppin and Pyl (1934) [in Arch.wiss.prakt. Tierhlk., 68, 183], as previously pointed out, also stated that in their "real al karinization" test the virus of "vesicular exanthema of swine" behaved like the virus of vesicular stomatitis and not like that of foot-and-mouth disease. This information is only of a limited interest since all the different viruses so far examined by the real al karinization test by Pyl behave like the virus of vesicular stomatitis and not like that of foot-and-mouth disease. Further, Galloway and Elford (1935) [Brit.J.exp.Path., 16, 588] have given reasons for their conclusion drawn from experimental observations that no accurate differentiation of two viruses can be based on this "real al karinization test" alone. Up to the present, therefore, there is insufficient evidence to permit the statement which has been made that "vesicular exanthema of swine" appears to be "nearly related" to vesicular stomatitis.

Crawford submits the results of certain experiments which have led him to the conclusion that, unlike foot-and-mouth disease, "vesicular exanthema of swine" does not appear to be transmitted readily by "indirect exposure" under experimental conditions. However, he hastens to add that there is evidence that under field conditions the disease may spread as a true epizootic. Galloway and Elford (1935) [Brit.J.exp.Path., 16, 588] have similarly pointed out that although it has been stated that vesicular stomatitis spreads less easily than foot-and-mouth disease, yet it may sometimes assume the characteristics of a severe epizootic disease. However, even if "vesicular exanthema of swine" and vesicular stomatitis did not, at least on occasion, behave as severe epizootic diseases, the suggested differentiation of foot-and-mouth disease from these two exanthemata, which it resembles so closely clinically, solely on the basis of its greater contagiousness and more rapid spread by indirect means, is not sound. Even in European countries (e.g., England) and in circumstances where prevailing nutritional and/or climatic conditions and/or particular breeds of cattle could not be considered as factors possibly affecting the issue, outbreaks of foot-and-mouth disease have occurred in which the disease has shown no tendency to spread rapidly by contagion or by indirect methods although the strain of virus concerned in these outbreaks proved to be capable of infecting susceptible animals by inoculation (personal communication, Andrews). Further, Waldmann and Reppin [Z. Infektkr. Haustiere (1935) 47, 283-282], working in the Insel Riems, Germany, have referred to a strain of foot-and-mouth disease virus which after laboratory manipulations would no longer infect susceptible cattle by contact although it produced a severe disease in such animals by inoculation.
Crawford states that a differential diagnosis of the three diseases, foot-and-mouth disease, vesicular stomatitis, and "vesicular exanthema of swine" requires the inoculation of cattle, horses, pigs, and guinea-pigs. In the case of an unknown virus, if only pigs or pigs and horses show characteristic lesions, the diagnosis is "vesicular exanthema of swine", if all four species of animals react the diagnosis is vesicular stomatitis, and if pigs, cattle and guinea-pigs react and horses fail to react, the diagnosis is foot-and-mouth disease. In the last case, on account of the seriousness of the occurrence, supplementary inoculations would also be made.

The annotator has already stressed the fact that quite apart from the cost and time necessary for such experiments, the differential diagnosis of a disease, exclusively by consideration of animal species susceptibility, especially if a limited number of animals is used, is obviously fraught with many possible sources of error. It must again be emphasized, e.g., that it has been reported that in Germany strains of the virus of foot-and-mouth disease have been recovered from swine which could only with great difficulty be transmitted to cattle, and some strains of virus recovered from cattle have only with difficulty been transmitted to guinea-pigs.

Andrews and his colleagues (5th Progress Report (loc. cit), Section III p.63-72) have brought further evidence on this point. They have given examples of "viruses occurring naturally in pigs which were found to be almost devoid of virulence for cattle, and also of bovine strains which would infect pigs only with great difficulty." 

"In no instance have we failed completely to induce lesions of foot-and-mouth disease in the species of low susceptibility, although in connection with the porcine virus R/1 a three out of five inoculated cattle gave no reaction at all, one gave an indefinite reaction that would not have permitted a positive diagnosis, and the fifth gave only a poor and limited reaction." 

"In connection with three of the viruses (one bovine, two porcine) the earlier inoculation gave negative results, and although later inoculation caused characteristic but limited reactions, the strain still failed to infect the resistant species by contact." They also state that although "these viruses have not been characterised by an unusually low pathogenicity for the guinea-pig, one virus R/V1 (see Section IV) was adapted to the guinea-pig with considerable difficulty."

The reader is referred to the previous annotation (loc. cit) for a fuller discussion of this question, and also the possibility of the occurrence, simultaneously in the same epizootic or in the same animal, of more than one virus, which would lead to further complications.

It must be stressed that the most rapid, economical and certain method of differentiating between the virus of foot-and-mouth disease and that of vesicular stomatitis is filtration through standard collodion membranes to estimate the size value, combined with egg inoculation tests. As regards the virus of "vesicular exanthema of swine", the position has not yet been modified, it can only be differentiated at present from the other two viruses by the fact that it has not yet been transmitted to either cattle or guinea-pigs.

Traum (1936, loc. cit) stated that the virus of "vesicular exanthema of swine" had been received by the annotator with a view to carrying out filtration experiments, but that no information had yet been published. Work of this nature has been hampered by the failure of the different investigators who have studied the disease to find a susceptible small experimental animal. Extensive filtration and other experiments are only possible when such an animal is available. However, if small susceptible
animals cannot be found, it may be possible to carry out a limited number of experiments on swine which will provide sufficient data to estimate the size value of the virus. It would also be useful to know if the virus can be cultivated on the chorio-allantoic membrane of eggs. It is considered that experiments of this nature are now indispensable as they might well provide more direct evidence as to the distinct individuality of the disease, and might thus form the basis of a simple, rapid and economical technique of distinguishing it from the other two exanthemata which it resembles so closely clinically.

I. A. G.
Louping-ill in the Horse.

Laboratory Report by I.A. Galloway.
(National Institute for Medical Research, London, N.W.3.)

Mr. J.M. Fletcher, M.R.C.V.S., Lochgilphead, Argyll, Scotland, sent me a sample of serum taken from a horse which had shown a febrile disturbance, associated with symptoms suggestive of nervous involvement, two or three months previously (see clinical report). The finding of some ticks on the animal and the resemblance of the clinical picture to that observed in sheep affected with louping-ill led him to the conclusion that the horse in question may have become infected with the virus of that disease. At the same time Mr. Fletcher sent a sample of serum taken from another horse which I understand had been grazing on the same pasture but which apparently had not itself shown any symptoms.

It was found that both the serum from the horse which had shown symptoms, as described by Mr. Fletcher, and that from the "contact" horse contained neutralising antibodies to the virus of louping-ill in a considerable titre; in fact, both sera contained neutralising antibodies in a titre equivalent to that of sera from sheep and monkeys convalescent from louping-ill or to that of the highest titre human serum which I have examined (v. infra), i.e. they neutralised between 1000 and 10,000 minimal infective doses of virus. "Control" horse sera included two samples of serum taken from horses in a district where no louping-ill is known to exist [viz. Rhodes Farm of the Medical Research Council, Mill Hill - sera kindly collected by Mr. Balfour Jones], a tetanus antitoxin serum, an anti-pneumococcus serum, an anti-staphylococcus-toxin serum [these three standard sera, which contained no preservative, were kindly supplied by my colleague, Dr. P. Hartley], and an anti-influenza virus serum given to me by colleague Dr. C.H. Andrews. None of these "control" horse sera showed any neutralising properties against the virus of louping-ill.

I have now tested a large number of sera from humans, monkeys and sheep against the louping-ill virus (see also Proc.Roy.Soc.Med., 1933, 267, 710.).

Human Sera.

The human sera tested were taken (1) from laboratory workers engaged on investigations into the disease, (2) from children and adults recovered from infection with poliomyelitis or different types of encephalitis and meningitis, and (3) from adults and children not known to have been in close contact with the virus of louping-ill or to have suffered from any nervous disease. The only human sera containing neutralising antibodies against louping-ill virus were those obtained from laboratory workers who had been engaged on studies on the disease and who may or may not have shown symptoms of a non-fatal "influenza-like" disease. These results are in agreement with those of Rivers and Schwentker (1933).

Monkey Sera.

The monkey sera examined were taken (1) from monkeys recovered from infection with the virus of louping-ill (see Galloway, I.A. and Ferdrau, J.R. (1935) in J.Hyg., 35, p.344), (2) from monkeys recovered from infection with the virus of poliomyelitis, and (3) from un inoculated monkeys in the Institute stock of experimental animals. Only the sera from monkeys recovered from louping-ill have contained neutralising antibodies against the virus of that disease.
Sheep Sera.

In the same way sera have been tested (1) from sheep recovered from or immunized against louping-ill [sera kindly supplied by Mr. W. S. Gordon, Ph.D., M.R.C.V.S., of the Moredun Research Institute, Gilmerton, Scotland], (2) from sheep in districts in which no louping-ill is known to occur. Only the sera from sheep immune to louping-ill have contained neutralizing antibodies against the virus of that disease.

There is therefore no doubt as to the specificity of the virus neutralization test in this disease and the presence of antibodies capable of neutralising the virus of louping-ill in the serum of an animal or human is strong evidence that the individual has been previously infected with that virus whether definite symptoms of disease have been observed or not. The results of the tests on the horse sera submitted for examination by Mr. Fletcher demonstrate that his assumption that at least one of the animals had been infected with louping-ill was correct. Mr. Fletcher has also informed me that in his district, where louping-ill among sheep is very common, he has frequently observed nervous symptoms in cattle very like those occurring in sheep and he has already stated [see Vet. Rec. (1936) 46, 458] that he is of the opinion that cattle can also be infected with louping-ill.

The fact that horses and possibly cattle can become infected with the virus of louping-ill under natural conditions is of considerable interest and affords confirmation of the experimental findings of Alexander, R.A. and Neitz, W.O. (1935) [in Onderstepoort Vet. Sci., 5, 15]. These authors succeeded in infecting one horse with louping-ill virus by allowing R. appendiculatus nymphs, which had fed as larvae on a sheep during a louping-ill reaction, to feed on it. No nervous symptoms were observed but the virus of louping-ill was recovered from the blood of the horse on the eighth day after the tick infestation and at the height of a febrile reaction. Samples of serum taken from the horse on the 57th and 140th day contained neutralising antibodies to the virus. The authors also showed that cattle can be infected experimentally either by tick infestation or inoculation without necessarily showing any symptoms other than a febrile reaction.

A considerable amount of further work is necessary to determine the pathogenesis of the disease in bovines and equines, but there is sufficient evidence to show that these animals can be infected with louping-ill and thus may serve as reservoirs of the virus. Apparently horses and cattle, like humans, may have an "infection inapparente" (Nicolle) as a result of infection with the virus and that such an infection has occurred can subsequently be demonstrated by testing the serum for neutralising antibodies.
NOTES ON THE HYDROGEN-ION CONCENTRATION OF THE URINE OF HERBIVORA*

I. A. GALLOWAY

[FROM THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH, HAMPSTEAD, N.W.3.]

During the course of some experiments made in attempts to recover virus from the urine of animals infected with, or convalescent from, foot-and-mouth disease, records were kept of the reactions of the different samples examined. Similar estimations were made on the urine of un inoculated guinea-pigs and cattle for comparison. The observations made are here placed on record. On referring to published statements, it is clear that it is not generally realised that there are quite a number of factors which may influence the hydrogen-ion concentration of the urine, quite apart from any pathological condition. These factors are also discussed here with special reference to the urine of herbivora.

It is generally stated that the urine of herbivora is alkaline. This statement requires some modification. It was shown by Claude Bernard (1859) that the urine of herbivora was alkaline and that of carnivora acid on account of their diet, and that this reaction was not dependent on the species of the animal, for while the urine of dogs fed on a non-nitrogenous diet became alkaline, that of rabbits and horses fed exclusively on oats, and of calves on a highly nitrogenous diet became acid. The change in reaction was due to the chemical composition of the diet independently of whether it came from a vegetable or animal source. He also explained that the urine of fasting animals was acid because they were living on their own substance. Smith (1912) also states that the urine of horses fed only on oats and bran is acid, and that the reaction of the urine of pigs may be acid or alkaline according to the diet.

Slight changes in reaction also occur during the course of the day, associated with the processes of digestion. In man definite "acid and alkaline tides" are generally recognised, the "acid tide" coming after a fast and the "alkaline tide" occurring during digestion, but, as has been pointed out by Hopkins (1898), one may actually get a primary increase of acidity in the urine after a meal due to increased protein metabolism, and while the secretion of the gastric juice into the stomach tends to lead to a change to alkalinity in the urine, so must secretion of the pancreatic and duodenal juices lead to a change to acidity, so that variations are also to be expected during the course of the day. It may be assumed that in herbivora such as cattle, similar variations occur.

In the present series of experiments it was found that the different samples of urine collected over a considerable period from healthy un inoculated cows or heifers were never less alkaline than about pH 7.6. The cows or heifers from which the urine was obtained were found on later enquiry to have been on a mixed diet of mangles, pasture grass, some bran, hay and a few oats. Later, samples of urine were obtained from these same animals which were definitely acid, even as acid as pH 4.8 on one occasion. In all experiments the pH values were estimated both electrometrically and colorimetrically. The results obtained by the two different methods were found to be in good agreement. On enquiry it was found that, coincident with the change in the reaction of the samples of urine, there had been a change in the diet of the cows; the mangles were no longer given and were replaced by linseed cake and more oats. Examination of the urine from several different cows during the course of the day showed that great variation in the reaction of the urine from the same animal occurred, as wide as between pH 5.0 and pH 8.0. Such variations did not appear to bear any strict relationship to meal or milking times, but owing to the complicated process of digestion in these animals, this was to be expected. It should be pointed out that the estimations of the pH values were made very shortly after collection of the urine. There is, of course, a tendency for urine to become more alkaline on standing at room temperature even for relatively short periods. This initial change of reaction is due to loss of carbon dioxide as in the case of other body fluids. The rapidity with which the change takes place is controlled by a number of factors, such as whether the urine is in a closed or open vessel. The surface area of the urine exposed to the air also plays a part in controlling the change, as does the question of whether the urine is in a large bulk or not. The rate of this primary change of reaction of the urine can be increased by employing a vacuum pump.

The urine of guinea-pigs and cows placed on various diets was next examined. It was found that the feeding of oats with a minimum quantity of hay rendered the urine definitely acid, pH 5.4 or less. The change in acidity in the urine varied in different guinea-pigs, but generally took place very rapidly. Usually all my guinea-pigs under experiment are fed on hay and cabbage, or similar greenstuff, alone, and on this diet the urine has been found to remain consistently alkaline [i.e., pH 7.6 to 8.2]. The urine of guinea-pigs which were fed on a mixed...
diet of oats, bran, hay and cabbage was definitely alkaline, pH 7.6 or higher at one time of
the day, and definitely acid, even below pH 5.0 at another time. The urine of guinea-pigs which
had become acid on a diet deficient in hay or greenstuff rapidly resumed an alkaline reaction
when hay and cabbage alone were given. It was found also that the urine of cows remained
alkaline if they were fed on pasture grass, hay and bran. The urine of cows on a mixed diet of
oats, bran, hay, linseed cake and grass did not become more acid than pH 7.0 if 1.0 to 2.0 oz.
of sodium citrate were given in the drinking water twice a day. All the above observations
were made on healthy uninoculated animals. It has been observed, however, that in animals
infected with foot-and-mouth disease, if they are very severely affected and show signs of
being off their food, then the urine may become very acid even although the food supplied, hay
and cabbage, is such as generally maintains its alkalinity. It may be assumed that, as in the
case of starvation, the animals become in effect "carnivorous," i.e., they live on their own
tissues.

It was suggested to me that repeated catheterisation of guinea-pigs or cattle in the
recent series of experiments may have led to the occurrence of cystitis in some instances and
this would account for the urine becoming acid. It has been found, however, that although
repeated catheterisation of guinea-pigs on a diet of hay and cabbage was carried out during
many days, on no occasion was the urine found to be less alkaline than pH 7.6. Further, the
giving of hay or cabbage to guinea-pigs whose urine had become acid, pH 4.8 to pH 6.6, as a
result of feeding on oats and bran was found to be sufficient to bring the reaction of the
urine back to pH 7.6 or higher within 24 hours. Moreover, not all the samples of urine of cattle
examined were obtained by catheterisation.

In the study of a disease due to a virus easily affected by comparatively slight changes in
hydrogen-ion concentration, it is obvious that any observations made on the presence or
absence of virus in urine without noting its reaction are valueless.

In view of the fact also that diet may influence the reaction of the urine, due consideration
should be given to this factor in interpreting a change in reaction of the urine as an indication
of a pathological state.

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SUPPLEMENTARY MATTER


2. Annotation -
   "Foot-and-Mouth Disease" - "Vesicular Stomatitis" - "Vesicular Exanthema of Swine".

3. Annotation -
   "Vesicular Exanthema of Swine."


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8. "Ultrafiltration et ses applications a l'étude des virus."

9. "Modification de certaines propriétés d'une souche de virus d'encephalo-myélite enzootique."
   Nicolau, S. and Galloway, I.A.
Priby Council

MEDICAL RESEARCH COUNCIL

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BY

S. NICOLAUS, M.D., D.Sc., and
I. A. GALLOWAY, B.Sc., M.R.C.V.S.

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PREFACE

The present report, which was received for publication in October, 1927, gives the results of investigations made by Dr. Nicolau and Mr. Galloway, working as guests in the National Institute of Medical Research. These studies of the virus which is responsible for a dangerous communicable disease in horses, cattle and sheep have happily no immediate practical application in this country. Borna disease and its congeners are at present only known in continental Europe and in America. Our present immunity, however, may only be temporary, and in any case it is highly important that we should have the fullest knowledge of these epizootic diseases of other countries.

The scientific advantages gained by close association between studies of disease in animals and studies of human disease have long been obvious. The potential value to medical science of the work now presented lies in two directions. The accurate experimental study of the 'virus' which is the causal agent in this disease is part of the general study of disease viruses, and in this field of work great gain must come from the free exchange of ideas, methods and results among workers in different parts of it. Besides this, however, Borna disease has special points of interest to students of human neurology. The infective virus produces changes in the brain and spinal cord, the so-called encephalo-myelitis, which throw light upon analogous forms of encephalitis and myelitis which occur in sporadic or epidemic form in human beings.

It will be seen that the authors have extended or confirmed the observations of many previous workers, and have gained new knowledge at several points of detail by their experimental work. Fresh studies have been made of the immunity reactions of the virus, and it has been shown that animals may be successfully immunized against it.

Dr. Nicolau was enabled to conduct this work in the National Institute by a grant from the Roumanian Government. Mr. Galloway co-operated with him in the course of other work upon foot and mouth disease in cattle, which he is doing on behalf of the Ministry of Agriculture and Fisheries. The council are indebted to the Ministry for the facilities they have given for this co-operation.

28th July, 1928.

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BORNA DISEASE AND ENZOOTIC ENCEPHALO-MYELITIS OF SHEEP AND CATTLE

BY S. NICOLAU, M.D., D.Sc., AND I. A. GALLOWAY, B.Sc., M.R.C.V.S.

(National Institute for Medical Research, Hampstead.)

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I. ENZOOTIC ENCEPHALO-MYELITIS OF THE HORSE (BORNA DISEASE).

The disease has various designations: Enzootic Encephalomyelitis, Meningo-encephalo-myelitis of the Horse, Névraxite enzootique; Mal d’Azeau (after the region where a severe epizootic occurred in Belgium in 1909); Bornasche Krankheit, Genickstarre, Gehirnruckenmarksentzündung, Gehirnruckenmarkseuche, Nervenfieber; Encephalitis Lymphocyntaria Equi.

It is an infectious malady with a mortality rate reaching 90 per cent in some epizootics. The characteristic symptoms are due to lesions in the nervous system both central and peripheral, which are produced by an ultravisible and filterable virus.

The name Borna disease, by which the disease is more commonly known, originated from the locality in Saxony where a severe epizootic occurred in the years 1894 to 1896.

Borna disease has been known for more than a century. It was first described by Wörz in Württemburg in 1813. Subsequently it was reported from Germany, North America, the Argentine, and Hungary. Since 1900 it has appeared in Russia, in the region of the river Don, in Belgium, France, Italy, Roumania, Germany, and South America.

The epizootics have been of varying severity. In 1896 the disease occurred in several districts of Germany, where 1,198 horses were reported to have contracted it; in the epizootic which occurred in the valley of the Colorado and the Brazos in America about 4,000 horses and mules succumbed. In other areas the disease has appeared sporadically.

Climatic conditions appear to have an influence on the spread of the disease, the incidence of which is greater when abnormally warm and moist conditions obtain. The diagram published by Zwick, Seifried, and Witte (1926) shows that epizootics generally begin in the month of May and decline later, the cases again becoming sporadic during the winter months.

The period of incubation in the spontaneously contracted disease is difficult to estimate and is therefore not definitely known. Noack (1908) considers 9 days to be an average.

Joest (1926) suggests that the portal of entry of the virus is through the nose, but the possibility of ingestion being the mode of infection cannot be excluded.

The onset of the disease may be sudden, but some authors have reported that fatigue, gastro-intestinal disturbances, and symptoms of affection of the upper respiratory passages precede the onset of characteristic nervous symptoms by as much as 14 days.

The first symptom which usually draws attention to the infected animal is lassitude: the horse is easily fatigued and appears depressed and indifferent to external impressions. A period of excitation follows, which may last with intermittence till the end of the disease. Tonic
contractions of diverse groups of muscles occur and there is difficulty in mastication and deglutition. During the stage of excitation external stimuli produce exacerbation of the spasms in certain groups of muscles; champing of the jaws is a common symptom, and saliva flows from the commissures of the mouth. The pupils are unequal in size. Soon paresis or paralysis sets in, affecting the hindquarters, the muscles of the tail, muscles of mastication, muscles of the tongue and of the back to a varying degree. Paraplegia or hemiplegia is not uncommon.

In some cases the symptoms of encephalitis are dominant, in some those of acute myelitis are more evident, while in others symptoms characteristic of affection of both brain and cord coexist. The temperature generally remains normal throughout, and according to Huitry and Marek (1922) persistent fever indicates secondary complications of a septic nature.

The frequency of the respiration remains little changed, except during periods of excitation, or when the nucleus of origin of the vagus nerve is involved. Towards the end of the disease, however, the respirations become superficial and approach the Cheyne-Stokes type.

The examination of the blood and urine does not indicate any constant change. The cerebrospinal fluid shows lymphocytosis occasionally. The duration of the disease varies from a few days to 6 weeks.1

Aetiology. As has been the case with a number of diseases subsequently proved to be due to a filterable virus, the disease was at first attributed to the pathogenic action of various cocci. Siedamgrotzky and Schlegel (1896) isolated a Gram-positive diplococcus and John (1896) a Gram-negative one. Other observers, Ostertag (1909), Christians (1909), Mareq (1909), Löhrr (1910), Lessage and Prise (1912), have found streptococci or diplococci, each organism isolated being considered by the investigator concerned as the aetiologic agent. More recently Kraus, Kantor, Fischer, and Quiroga (1929) isolated a diplococcus similar to that found by Siedamgrotzky and Schlegel, which they believed to be the aetiologic agent of Borna disease until the appearance of the work of Moussu and Marchand in France, and Zwick, Seifried, and Witte in Germany, indicating the encephalo-myelitis of the horse is produced by an ultraviolet or filterable virus. Although the virus isolated by Moussu and Marchand appears to differ from that isolated by Zwick and his collaborators it is possible that this may be explained by the fact that two strains of the same virus were being dealt with (see note on p. 28). Becker and Frobböse (1926) and Ernst and Hahn (1926) also showed that a virus isolated from the brain of horses affected with encephalo-myelitis is capable of infecting rabbits.

Joest in collaboration with Degen (1909) determined the consta 1

1 In the epizootics described by R. Moussu (1926) the duration of the disease is very short. According to this author, in those cases showing symptoms of an encephalitic type 'the evolution is rapid. In the cases we have studied death intervened in 20, 32, and 37 hours.'
presence of certain intranuclear ‘inclusions’ in the ganglion cells of the Ammon’s horn which they considered as reactions of the cell to the parasite and ‘similar to Chlamydozoa’. In the opinion of Joest these chlamydozoa, or at least certain forms of their cycle of development, are capable of passing through bacteriological filters.

Siedamgrotzky and Schlegel (1896), Lohr (1910), and Ostertag (1900 et seq.) made frequent attempts to infect laboratory animals by various methods of inoculation or by feeding them with cerebrospinal fluid, blood, emulsions of spleen, liver, kidney, or bone marrow from horses affected with the disease. The results were negative.

In résumé the work of Moussu and Marchand (1924 et seq.), Zwick, Seifried, and Witte (1926), Beck and Frohböse (1926), Ernst and Hahn (1926–7), has shown definitely that the aetiologic factor is a filterable ultraviscous virus, and the results obtained by Kraus and his collaborators (1920) and other investigators with their cultures of diplococci or diplo-streptococci must now be considered as due to what Nicolle called microbes de sortie.

II. ENZOOTIC ENCEPHALO-MYELITIS OF CATTLE.

The epizootiology and symptoms of encephalo-myelitis of cattle are similar to Borna disease of the horse. Hutrya and Marek (1922) refer to outbreaks of disease amongst cattle described by Meyer (1867), Schmidt (1888), Utz (1896), Röder (1896), and Manfredi d’Ercole (1896) which may be attributed to enzootic encephalo-myelitis. Pröger (1896) recorded the coexistence of this disease with Borna disease, and as Ernst and Hahn (1927) have suggested that the outbreaks of disease in Hungary referred to as ‘cerebrospinal meningitis’ may also have been encephalo-myelitis. G. Moussu, quoted by R. Moussu (1926), in 1906 studied an enzootic on a farm in the region of Orne which killed 10 cattle in a few weeks. The disease had a similar onset; periods of excitation were observed followed by depression of the jaws, salivation, loss of vision, and muscular twitches. The possibility of intoxication was excluded, and the symptoms were suggestive of Borna disease. Moussu also considers that the cases of ‘cerebrospinal meningitis’ of cattle reported by Kragerud and Gunderson (1921) were the same as encephalomyelitis of the horse, and the symptoms described by Causel (1924) in an enzootic of what he termed ‘infectious bulbar paralysis’ amongst cattle are consistent with the view that the animals suffered from encephalo-myelitis.

The symptoms of encephalo-myelitis in cattle are similar to those of Borna disease in the horse.

Aetiology. Moussu (1926), who described 31 cases of encephalo-myelitis of cattle on 18 different farms in France, attempted to transmit the disease to laboratory animals without success.

Ernst and Hahn (1927) found lesions in the brain of cattle dead of encephalo-myelitis analogous to those in the brain of horses dead of Borna disease; in the lesions the corpuscles of Joest-Degen were seen. With the virus which they recovered they succeeded in producing
symptoms in rabbits similar to those produced by Zwick with a virus from horses.

The general aspect of the lesions in the brain of rabbits infected with the two viruses and the similar presence of the corpuscles of Joest-Degen suggests that varieties of the same virus are the cause of encephalo-myelitis of horses and cattle.

III. Enzootic Encephalo-myelitis of Sheep.

Eichbaum, Stöhr, and Wilke (1865, 1866) recorded an enzootic of encephalo-myelitis among sheep, and Roloff (1868), who examined the brain of animals which succumbed to a similar epizootic, found perivascular infiltration in the pia mater. Schmidt (1870), about the same time, described an enzootic of a similar nature in Prussia. Later, Popow (1882) and Wischniotitsch (1889) described the disease in Russia. Prietsch (1896) referred to an enzootic among sheep, and suggested the possibility that the source of infection was the water in troughs contaminated by the virus of Borna-disease of horses. Walther (1899) reported an enzootic in two flocks of sheep in the district of Borna at a time when equine encephalo-myelitis was prevalent; the two diseases, equine and ovine, bore many resemblances to one another. Savigné and Leblanc (1897) also have described an enzootic in France.

The descriptions of these authors differ essentially and may not all refer to the same disease. More precise accounts of ovine encephalo-myelitis have been published within recent years by Spiegl (1922), Priemer (1925), Beck and Frohbose (1926), Moussu (1926), Miessner (1926), and others, from which it appears that in the spontaneous disease of the sheep the same succession of symptoms occur as in horses and cattle. The evolution of the disease may take as long as 2 to 12 days. Death generally supervenes. The temperature is variable. In certain cases the temperature may rise to 41° C, while in others no pyrexia is observed. According to Moussu (1926) the incubation period averages 27 days.

Aetiology. Beck (1925) studied the microscopic lesions in the brain of sheep dead of the disease, and emphasized their resemblance to those found in the brain of horses dead of Borna disease. In the ganglion cells of the Ammon's horn of sheep the characteristic oxyphilic intranuclear corpuscles of Joest and Degen were found. In collaboration with Frohbose, Beck (1926) transmitted the disease to rabbits, and from the similarity in the symptoms and in the lesions produced by the virus isolated by them from sheep with that isolated by Zwick from horses, considered that the two diseases were identical.

About the same time Moussu and Marchand also passed the disease to rabbits and transmitted it from sheep to sheep. Later Miessner (1926) and Ernst and Hahn (1927) confirmed the transmissibility of the disease of sheep to rabbits.

We shall describe later how the experimental study of enzootic encephalo-myelitis of sheep has shown that the disease is produced by a virus similar, if not identical, with that which produces Borna disease in horses.
IV. MALIGNANT CATARRHAL FEVER OF CATTLE.

Glamser (1928) and Dobberstein (1925) found perivascular and parenchymatous infiltration as well as alterations in the ganglion cells of the brain of cattle dead of a disease which they called malignant catarrhal fever. These lesions were similar to those described in Borna disease. Ernst and Hahn (1927) also draw attention to the similarity of the lesions in the brain of cattle dead of this disease with those found in encephalo-myelitis of the horse, and in 8 out of 5 cases they observed the intranuclear corpuscles of Joest-Degen in the ganglion cells of the Ammon's horn. Emulsions from the brain of one of these cases inoculated intracerebrally into rabbits produced a disease similar to experimental Borna disease and transmissible from rabbit to rabbit. They concluded that malignant catarrhal fever of cattle is produced by a virus which approaches very closely, if it be not identical with, that producing encephalo-myelitis in horses.

We might here mention that Ernst and Hahn (1927) also made an observation indicating that deer are susceptible to Borna disease. A sick deer was killed by a hunter under curious circumstances. The animal allowed the hunter to approach very closely, drew away in fear, and then rushed on him suddenly. The ears were seen to twitch and the animal turned round in a circle until the hunter, probably more frightened than the animal, killed it. The head was brought to Munich. The brain showed the presence of lesions similar to those of Borna disease of the horse, and the intranuclear corpuscles of Joest-Degen were observed. Their attempts at transmission of the disease to laboratory animals had given no results at the time of publication of these observations.

V. SUMMARY.

From the foregoing résumé of the literature of spontaneous encephalo-myelitis in different species, the following conclusions may be drawn:

1. The enzootic encephalo-myelitis of horses and cattle and of sheep appears to be the same disease. The symptomatology and the lesions found in the central nervous system are analogous, and the intranuclear corpuscles of Joest-Degen occur in the large ganglion cells of the Ammon's horn in all three species suffering from the disease in question.

2. From cases of all three diseases a virus has been recovered and shown to be responsible for the disease.

3. From the observations of Ernst and Hahn it would seem not improbable that, if the animals had not in addition to malignant catarrhal fever a concomitant infection with Borna disease, some of the cases described as malignant catarrhal fever of cattle were encephalo-myelitis.

4. Deer appear to suffer from a similar disease spontaneously.

5. The transmission of the disease under natural conditions is probably by the respiratory tract or by ingestion.
2. PROPERTIES OF THE VIRUS

I. INVISIBILITY.

The virus in ultravisible. Various methods of staining have been used to discover a parasite, but without success. Methods of impregnation with silver have not revealed the presence of parasites in the brain of animals dead of the experimental disease in our hands. The existence of the virus in the brain is, however, associated with the presence of intranuclear corpuscles, first described by Joest and Degen (1909). The interpretation of the nature of these corpuscles which we favour is discussed in the chapter dealing with the histopathology of the disease, and we are persuaded that they are of the same nature as the similar 'inclusions' found in other diseases produced by filterable viruses, such as fowl plague, fowl pox, distemper, and 'Virus III' disease of rabbits.

II. FILTERABILITY: EFFECT OF DILUTION.

Filtration of emulsions containing viruses through filters which hold back bacteria generally greatly diminishes the concentration of the virus. There are many reasons for this, apart from the size of the virus. If the virus is contained in or on cellular particles these may be retained, or the virus itself may be adsorbed on the filter. In such adsorption the hydrogen-ion concentration and the electrical charge carried by the virus exert a decisive influence. The pressure under which the filtration is carried out is also a factor of importance.

Further, the sensitiveness of the tissue into which a filtrate is inoculated may influence the decision whether a virus is filterable or not. When a sensitive reagent is employed, the passage of an extremely small quantity may be detected. For instance, the dose of neurovaccinia required to infect a rabbit if inoculated intracerebrally is 1/100th to 1/1000th of that required to produce pustules on application to the scarified skin. An experimenter using the former method might conclude that the virus passed a bacteria-proof filter, one using the latter that it did not. The sensitiveness of the tissue into which a filtrate is inoculated is therefore a factor of capital importance often neglected in interpretations as to the filterability or non-filterability of the virus.

The experiments of Moussu (1926), Zwick, Seifried, and Witt (1926), and Ernst and Hahn (1927) show that the virus of Borna disease can pass through ordinary bacteriological filters, but the filtration of the virus is not easily effected. Zwick carried out more than thirty filtration experiments with different filters and inoculated more than 100 rabbits with the various filtrates before he succeeded in infecting the animals with a filtrate.

We have carried out two filtration experiments, using the following technique: from the brain of Rabbit 275, which died on the 82nd day after cerebral inoculation and exhibited characteristic lesions throughout the nervous system, an emulsion 1:30 was made with
ENZOOTIC ENCEPHALO-MYELITIS

physiological saline. The emulsion was placed in the ice-chest for three hours to allow the coarser particles to deposit. The supernatant fluid was filtered by aspiration under low pressure through a Mandler filter.

Experiment 1.

The filtrate was inoculated intracerebrally into Rabbits 218A, 168A, 228A, and 201A, on the 15.3.27.

During a period of 174 days following the inoculation Rabbit 218A did not show any symptoms, and its weight increased in an even curve by 780 gms. After this period had elapsed the resistance of the animal was tested with fresh passage virus by intracerebral inoculation. It died in 37 days after showing typical symptoms of the disease. On microscopic examination of sections of the central nervous system characteristic lesions were found.

Rabbit 168A, weighing 700 gms., and Rabbit 228A, weighing 2,080 gms., also remained well for 174 days following the inoculation, and their weights increased to 1,330 gms. and 2,640 gms. respectively. They were both reinoculated intracerebrally with fresh passage virus on September 5, 1927 (174th day since inoculation), and proved not to be refractory to infection; Rabbit 168A dying 14 days and Rabbit 228A 37 days after inoculation with the test dose. The control rabbit, 25A, inoculated intracerebrally on the same date as the others, died 48 days after infection, and typical lesions were found in the central nervous system.

Rabbit 201A was inoculated intracerebrally with filtrate on the 15.3.27.

Observations.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,500</td>
<td>Normal</td>
</tr>
<tr>
<td>20.3.27</td>
<td>1,620</td>
<td>Normal</td>
</tr>
<tr>
<td>25.3.27</td>
<td>1,660</td>
<td>Normal</td>
</tr>
<tr>
<td>30.3.27</td>
<td>1,520</td>
<td>Animal appeared to be ill. Rabbit found dead 18 days after inoculation.</td>
</tr>
<tr>
<td>2.4.27</td>
<td>1,500</td>
<td></td>
</tr>
</tbody>
</table>

On the autopsy no abnormal condition of the organs was observed and the cultures of the brain remained sterile. On microscopic examination slight infiltration and perivascular lesions were found in the brain, mid-brain, and spinal cord, and, in addition, a marked pathological ‘satellites’ of the nerve-cells. Passage was made with the brain of this rabbit to a healthy rabbit, No. 260A, with the results given in detail below:

Rabbit 260A, weighing 1,740 gms., was inoculated intracerebrally with an emulsion of the brain of Rabbit 201A.

It developed a disease of a recurrent nature and succumbed after the second crisis, 161 days after inoculation.

Autopsy. All organs macroscopically normal.

Cultures in broth from the brain remained sterile.

Microscopic examination. Intense and characteristic lesions were found in the brain and in the cord. The intranuclear corpuscles of Joest-Degen were also found.
**Observations.**

**Date.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.27</td>
<td>1,700</td>
<td>Nothing abnormal in animal’s condition.</td>
</tr>
<tr>
<td>19.4.27</td>
<td>1,800</td>
<td>&quot;</td>
</tr>
<tr>
<td>28.4.27</td>
<td>1,900</td>
<td>&quot;</td>
</tr>
<tr>
<td>5.5.27</td>
<td>1,920</td>
<td>&quot;</td>
</tr>
<tr>
<td>12.5.27</td>
<td>1,600</td>
<td>Depressed, posterior paresis.</td>
</tr>
<tr>
<td>16.5.27</td>
<td>1,570</td>
<td>Placed on his flank the animal made several vain efforts to recover its normal position.</td>
</tr>
<tr>
<td>21.5.27</td>
<td>1,620</td>
<td>Condition ameliorated—slight paresis of hindquarters.</td>
</tr>
<tr>
<td>30.5.27</td>
<td>1,600</td>
<td>&quot;</td>
</tr>
<tr>
<td>13.6.27</td>
<td>1,640</td>
<td>Normal.</td>
</tr>
<tr>
<td>22.6.27</td>
<td>1,740</td>
<td>&quot;</td>
</tr>
<tr>
<td>30.6.27</td>
<td>1,770</td>
<td>&quot;</td>
</tr>
<tr>
<td>15.7.27</td>
<td>1,800</td>
<td>&quot;</td>
</tr>
<tr>
<td>1.8.27</td>
<td>1,700</td>
<td>&quot;</td>
</tr>
<tr>
<td>8.8.27</td>
<td>1,540</td>
<td>Animal ill.</td>
</tr>
<tr>
<td>15.8.27</td>
<td>1,300</td>
<td>Paresis of hindquarters.</td>
</tr>
<tr>
<td>24.8.27</td>
<td>1,180</td>
<td>&quot;</td>
</tr>
<tr>
<td>31.5.27</td>
<td>1,000</td>
<td>&quot;</td>
</tr>
<tr>
<td>9.9.27</td>
<td>980</td>
<td>Head depressed, paresis of hindquarters, typical symptoms of the disease.</td>
</tr>
<tr>
<td>12.9.27</td>
<td>820</td>
<td>Placed on the flank, the rabbit made vain efforts at recovering the normal position.</td>
</tr>
<tr>
<td>13.9.27</td>
<td>940</td>
<td>Found dead 161 days after inoculation.</td>
</tr>
</tbody>
</table>

**Experiment 2.**

A second filtration experiment was carried out with the same technique. The filtrate through a Mandler filter was inoculated into the brain of four rabbits, 150A, 152A, 210A, and 231A.

Rabbit 231A died accidentally 5 days after inoculation.

Rabbit 210A remained unaffected and gained progressively in weight.

The protocols of Rabbits 150A and 152A are given below.

**Rabbit 150A.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,800</td>
<td>&quot;</td>
</tr>
<tr>
<td>20.3.27</td>
<td>1,880</td>
<td>&quot;</td>
</tr>
<tr>
<td>31.3.27</td>
<td>1,860</td>
<td>&quot;</td>
</tr>
<tr>
<td>15.4.27</td>
<td>2,260</td>
<td>&quot;</td>
</tr>
<tr>
<td>28.4.27</td>
<td>2,320</td>
<td>&quot;</td>
</tr>
<tr>
<td>5.5.27</td>
<td>2,520</td>
<td>Slight paresis behind.</td>
</tr>
<tr>
<td>12.5.27</td>
<td>2,420</td>
<td>2,620</td>
</tr>
<tr>
<td>21.5.27</td>
<td>2,340</td>
<td>2,680</td>
</tr>
<tr>
<td>30.5.27</td>
<td>2,340</td>
<td>Condition improved.</td>
</tr>
<tr>
<td>5.6.27</td>
<td>2,460</td>
<td>Normal.</td>
</tr>
<tr>
<td>29.6.27</td>
<td>2,640</td>
<td>&quot;</td>
</tr>
<tr>
<td>15.7.27</td>
<td>2,900</td>
<td>&quot;</td>
</tr>
<tr>
<td>1.8.27</td>
<td>2,970</td>
<td>&quot;</td>
</tr>
<tr>
<td>29.8.27</td>
<td>3,000</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

**Rabbit 152A.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>2,200</td>
<td>&quot;</td>
</tr>
<tr>
<td>20.3.27</td>
<td>2,440</td>
<td>Normal.</td>
</tr>
<tr>
<td>31.3.27</td>
<td>2,440</td>
<td>&quot;</td>
</tr>
<tr>
<td>15.4.27</td>
<td>2,500</td>
<td>&quot;</td>
</tr>
<tr>
<td>28.4.27</td>
<td>2,600</td>
<td>&quot;</td>
</tr>
<tr>
<td>5.5.27</td>
<td>2,680</td>
<td>&quot;</td>
</tr>
<tr>
<td>12.5.27</td>
<td>2,620</td>
<td>2,620</td>
</tr>
<tr>
<td>21.5.27</td>
<td>2,680</td>
<td>2,600</td>
</tr>
<tr>
<td>30.5.27</td>
<td>2,620</td>
<td>2,650</td>
</tr>
<tr>
<td>5.6.27</td>
<td>2,650</td>
<td>&quot;</td>
</tr>
<tr>
<td>29.6.27</td>
<td>2,790</td>
<td>&quot;</td>
</tr>
<tr>
<td>15.7.27</td>
<td>2,650</td>
<td>&quot;</td>
</tr>
<tr>
<td>1.8.27</td>
<td>2,600</td>
<td>&quot;</td>
</tr>
<tr>
<td>29.8.27</td>
<td>2,650</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Therefore only Rabbit 150A showed slight transitory symptoms which ultimately passed away completely. These symptoms may have been due to the inoculation of a small dose of virus.
ENZOOTIC ENCEPHALO-MYELITIS

In order to determine whether the 3 rabbits, 150A, 152A, and 210A, had developed any degree of immunity as a result of inoculation with the filtrate, 167 days after the first injection they received intracerebrally a virulent emulsion of brain at the same time as a normal rabbit, 25A, which served as a control. Rabbit 150A died on the 49th day, Rabbit 152A on the 40th day, and Rabbit 210A on the 40th day, and the control rabbit on the 48th day after inoculation of the test dose. Typical lesions were found in all cases on microscopic examination of sections of brain and spinal cord.

Conclusions. The results in our few experiments support the conclusion of Zwick, Seifried, and Witte (1927) that the virus of Borna disease can pass, though with great difficulty, through bacteriological filters which retain ordinary bacteria, and that the concentration of virus in the filtrate is much reduced.

Zwick succeeded in obtaining an active filtrate after filtering a virulent emulsion of brain through a Zsigmondy Bachmann collodion membrane, of which the size of the pore was estimated to be 0.75 µ.

Effect of dilution. Few titration experiments have been made. Zwick records that a virulent emulsion of brain is still capable of producing the disease in a dilution of 1:10,000.

III. CENTRIFUGALIZATION.

Experiment 1.

A homogeneous emulsion of virulent brain was made, and after the larger particles had been allowed to deposit, the supernatant fluid was pipetted and centrifugalized for 5 minutes at 5,400 revolutions per minute. The supernatant fluid after centrifugalization was carefully pipetted off and inoculated intracerebrally into three rabbits weighing between 1,300 and 1,500 gms. These three rabbits fell ill, showed typical symptoms and died, 39, 48, and 90 days respectively after the inoculation. The characteristic lesions of Borna disease were found in the central nervous system of all three.

(a) Rabbit 322B. Weight 1,540 gms.
(b) Rabbit 321B. Weight 1,300 gms.
(c) Rabbit 320B. Weight 1,500 gms.

The inoculation was made on 15.3.27. The protocol of the three rabbits is given below:

Rabbit 322B.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,540</td>
<td></td>
</tr>
<tr>
<td>23.3.27</td>
<td>1,680</td>
<td>Normal</td>
</tr>
<tr>
<td>25.3.27</td>
<td>1,700</td>
<td></td>
</tr>
<tr>
<td>31.3.27</td>
<td>1,700</td>
<td></td>
</tr>
<tr>
<td>10.4.27</td>
<td>1,620</td>
<td></td>
</tr>
<tr>
<td>15.4.27</td>
<td>1,500</td>
<td></td>
</tr>
<tr>
<td>19.4.27</td>
<td>1,420</td>
<td>Paresis of hind quarters.</td>
</tr>
<tr>
<td>28.4.27</td>
<td>1,220</td>
<td>Typical symptoms of the disease.</td>
</tr>
<tr>
<td>2.5.27</td>
<td>—</td>
<td>Found dead 48 days after inoculation.</td>
</tr>
</tbody>
</table>

Culture of the brain. Negative.

Microscopic examination. Intense and characteristic lesions in the central nervous system.
BORNA DISEASE AND

Rabbit 321B.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,300</td>
<td></td>
</tr>
<tr>
<td>23.3.27</td>
<td>1,280</td>
<td>Normal.</td>
</tr>
<tr>
<td>25.3.27</td>
<td>1,380</td>
<td></td>
</tr>
<tr>
<td>31.3.27</td>
<td>1,420</td>
<td></td>
</tr>
<tr>
<td>10.4.27</td>
<td>1,480</td>
<td></td>
</tr>
<tr>
<td>15.4.27</td>
<td>1,600</td>
<td></td>
</tr>
<tr>
<td>19.4.27</td>
<td>1,640</td>
<td></td>
</tr>
<tr>
<td>28.4.27</td>
<td>2,000</td>
<td></td>
</tr>
<tr>
<td>10.5.27</td>
<td>2,100</td>
<td></td>
</tr>
<tr>
<td>21.5.27</td>
<td>2,320</td>
<td></td>
</tr>
<tr>
<td>30.5.27</td>
<td>2,080</td>
<td>Paresis of hindquarters.</td>
</tr>
<tr>
<td>5.6.27</td>
<td>1,800</td>
<td>Typical symptoms of disease.</td>
</tr>
</tbody>
</table>
| 11.6.27| 1,580          | Coma. Killed 90 days after infection.

Cultures of the brain. Negative.
Microscopic examination. Intense and characteristic lesions in the central nervous system.

Rabbit 320B.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,500</td>
<td></td>
</tr>
<tr>
<td>23.3.27</td>
<td>1,600</td>
<td>Normal.</td>
</tr>
<tr>
<td>26.3.27</td>
<td>1,620</td>
<td></td>
</tr>
<tr>
<td>31.3.27</td>
<td>1,440</td>
<td></td>
</tr>
<tr>
<td>10.4.27</td>
<td>1,320</td>
<td></td>
</tr>
<tr>
<td>15.4.27</td>
<td>1,320</td>
<td>Paresis?</td>
</tr>
<tr>
<td>19.4.27</td>
<td>1,140</td>
<td>Typical symptoms of the disease.</td>
</tr>
<tr>
<td>23.4.27</td>
<td>1,020</td>
<td>Died 39 days after infection.</td>
</tr>
</tbody>
</table>

Cultures from the brain. Negative.
Microscopic examination. Mild, but characteristic lesions in the central nervous system.

Experiment 2.

A virulent emulsion of brain was allowed to deposit, and the supernatant fluid centrifuged as in the last experiment (5,400 revs. per minute) for 15 minutes. Two rabbits were inoculated intracerebrally with the supernatant fluid. Both these rabbits developed the disease typically with paralysis, and died 28 and 45 days respectively after the inoculation, showing lesions of a characteristic nature in the central nervous system.

(a) Rabbit 324B. Weight 1,150 gms.
(b) Rabbit 326B. Weight 1,150 gms.
The inoculation was made on 15.3.27.
The protocol of these two rabbits is recorded below.

Rabbit 324B.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,150</td>
<td></td>
</tr>
<tr>
<td>20.3.27</td>
<td>1,360</td>
<td>Normal.</td>
</tr>
<tr>
<td>25.3.27</td>
<td>1,400</td>
<td></td>
</tr>
<tr>
<td>31.3.27</td>
<td>1,330</td>
<td></td>
</tr>
<tr>
<td>11.4.27</td>
<td>1,440</td>
<td></td>
</tr>
<tr>
<td>15.4.27</td>
<td>1,160</td>
<td>Commencement of symptoms.</td>
</tr>
<tr>
<td>19.4.27</td>
<td>1,160</td>
<td>Paralysis of hindquarters.</td>
</tr>
<tr>
<td>28.4.27</td>
<td>1,080</td>
<td></td>
</tr>
<tr>
<td>29.4.27</td>
<td>1,080</td>
<td>Found dead 45 days after inoculation.</td>
</tr>
</tbody>
</table>

Cultures from the brain. Negative.
Microscopic examination. The brain and spinal cord showed the presence of characteristic lesions.
ENZOOTIC ENCEPHALO-MYELITIS

Rabbit 326b.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3.27</td>
<td>1,150</td>
<td></td>
</tr>
<tr>
<td>20.3.27</td>
<td>1,360</td>
<td></td>
</tr>
<tr>
<td>25.3.27</td>
<td>1,440</td>
<td></td>
</tr>
<tr>
<td>31.3.27</td>
<td>1,380</td>
<td></td>
</tr>
<tr>
<td>11.4.27</td>
<td>1,020</td>
<td>Paresis of hindquarters.</td>
</tr>
<tr>
<td>12.4.27</td>
<td></td>
<td>Found dead 28 days after inoculation.</td>
</tr>
</tbody>
</table>

Microscopic examination. The brain and spinal cord showed the presence of typical lesions.

Conclusion. Centrifugalization for even 15 minutes at 5,400 rev. per minute does not deprive the supernatant fluid of virulence. This fact, in addition to the properties of filterability and invisibility of the pathogenic agent suggests that the size of the infective element is excessively small. It is affected by centrifugalization in the same way as other filterable viruses such as those of foot-and-mouth disease, rabies, herpes, vaccinia, and poliomyelitis.

IV. RESISTANCE TO GLYCERINE.

Moussu (1926) found that a portion of brain preserved its virulence at room temperature (July–August, Alfort) for 18 days, but that its pathogenic action was lost after 32 days. According to Zwick the brain of a rabbit preserved its virulence in glycerine for from 4 to 5 months, and in our experiments glycerinated virus kept in the cold room at 4°C was still virulent after 113, 135, and in one case 161 days.

To find the best conditions for keeping the virus in the cold room the following solutions were tried. (1) Pure glycerine, (2) pure glycerine covered with a layer of sterile paraffin oil, (3) glycerine mixed with an equal part of sterile physiological saline, (4) glycerine mixed with an equal part of phosphate saline M/25, pH 7.6. The brain of Rabbit 77A (dead of Borna disease 48 days after inoculation intracerebrally with typical lesions in the C.N.S.) was taken aseptically and divided into four equal portions, and one of the portions placed in each of the four media referred to above. At the end of a certain time a fragment of each portion was taken and an emulsion of it inoculated intracerebrally into rabbits to test its virulence. A table of results is given on page 18.

The results recorded in the table on p. 18 show that the virus may remain virulent in the cold room at 4°C at least 113 days in a medium consisting of pure glycerine, glycerine diluted to 50 per cent. with physiological saline, or glycerine diluted to 50 per cent. with phosphate saline M/25, pH 7.6. In two further experiments under similar conditions the virus preserved in glycerine remained virulent for 135 days and 161 days respectively.

(1) An emulsion of the brain of Rabbit 25 (dead of Borna disease 85 days after inoculation) which had been kept in glycerine in the ice-chest for 135 days was inoculated intracerebrally into Rabbit 145A.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus kept in pure glycerine</td>
<td>20 days 113</td>
<td>232 140A</td>
<td>1,340 2,020</td>
<td>20th day 30th</td>
<td>11 days 10</td>
<td>31st day 40th</td>
</tr>
<tr>
<td>Virus kept in pure glycerine covered with paraffin oil</td>
<td>20 113</td>
<td>244 146A</td>
<td>1,200 1,780</td>
<td>33rd day 26th</td>
<td>12 20</td>
<td>45th 26th</td>
</tr>
<tr>
<td>Virus kept in glycerine mixed with equal parts of physiological saline.</td>
<td>22 113</td>
<td>261 147A</td>
<td>1,280 1,680</td>
<td>23rd day 26th</td>
<td>8 14</td>
<td>31st 40th</td>
</tr>
<tr>
<td>Virus kept in glycerine and phosphate saline. M/25, pH. 7.6</td>
<td>22 113</td>
<td>219 143A</td>
<td>1,820 1,780</td>
<td>16th day 20th</td>
<td>5 6</td>
<td>21st 32nd</td>
</tr>
</tbody>
</table>
ENZOOTIC ENCEPHALO-MYELITIS

Rabbit 145A. Weight 2,500 gms.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.6.27</td>
<td>2,500</td>
<td>Normal</td>
</tr>
<tr>
<td>22.6.27</td>
<td>2,500</td>
<td>&quot;</td>
</tr>
<tr>
<td>29.6.27</td>
<td>2,500</td>
<td>&quot;</td>
</tr>
<tr>
<td>7.7.27</td>
<td>2,500</td>
<td>First symptoms of the disease</td>
</tr>
<tr>
<td>14.7.27</td>
<td>1,900</td>
<td>Typical symptoms of the disease in an advanced stage</td>
</tr>
<tr>
<td>19.7.27</td>
<td>1,550</td>
<td>Died 47 days after inoculation</td>
</tr>
</tbody>
</table>

Cultures from brain. Negative. Microscopic examination. Brain and cord showed presence of intense lesions.

(2) Virus (brain of Rabbit 100 dead of enzootic encephalo-myelitis in the 87th day after inoculation) kept in glycerine 161 days in the best, was inoculated intracerebrally into Rabbit 142A on the 3.6.27.

Rabbit 142A. Weight 2,440 gms.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.6.27</td>
<td>2,500</td>
<td>Normal</td>
</tr>
<tr>
<td>22.6.27</td>
<td>2,320</td>
<td>&quot;</td>
</tr>
<tr>
<td>29.6.27</td>
<td>2,440</td>
<td>&quot;</td>
</tr>
<tr>
<td>7.7.27</td>
<td>2,040</td>
<td>Typical symptoms commencing</td>
</tr>
<tr>
<td>14.7.27</td>
<td>1,850</td>
<td>&quot;</td>
</tr>
<tr>
<td>16.7.27</td>
<td>1,600</td>
<td>Died 43 days after inoculation</td>
</tr>
</tbody>
</table>

Cultures from brain. Negative. Microscopically. Characteristic and intense lesions were present in the central nervous system.

We have observed that certain of the rabbits infected intracerebrally with virus kept in glycerine succumbed to the disease at an earlier date than rabbits inoculated with an emulsion of fresh virulent brain as is indicated by the following experiment.

(3) Rabbit 275 was inoculated intracerebrally with a virus kept in pure glycerine for 48 days on 10.2.27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight in gms.</th>
<th>Clinical observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.2.27</td>
<td>2,100</td>
<td></td>
</tr>
<tr>
<td>10.2.27</td>
<td>2,150</td>
<td>No abnormal symptoms observed</td>
</tr>
<tr>
<td>23.2.27</td>
<td>2,040</td>
<td>&quot;</td>
</tr>
<tr>
<td>27.2.27</td>
<td>1,940</td>
<td>&quot;</td>
</tr>
<tr>
<td>5.3.27</td>
<td>1,700</td>
<td>&quot;</td>
</tr>
<tr>
<td>7.3.27</td>
<td>1,520</td>
<td>Commencing paresis of the hind quarters</td>
</tr>
<tr>
<td>12.3.27</td>
<td>1,200</td>
<td>Paresis of hindquarters more advanced</td>
</tr>
<tr>
<td>13.3.27</td>
<td>1,250</td>
<td>Animal remained in corner of the cage hunched up, depressed. The paresis was still present.</td>
</tr>
<tr>
<td>14.3.27</td>
<td>1,220</td>
<td>Intense salivation. Paralysis of the hind quarters.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Died 32 days after the inoculation.</td>
</tr>
</tbody>
</table>

The microscopic examination of sections of brain and cord of Rabbit 142A revealed the presence of intense and characteristic lesions.

The following table, which shows the chain of the series of passages of the virus from rabbit to rabbit, of which Rabbit 275 forms a connecting link, demonstrates the fact that although it weighed more than 2,000 gms. it died at an earlier date than the other rabbits of the same series.
BORNA DISEASE AND

<table>
<thead>
<tr>
<th>No. of Rabbit</th>
<th>Inoculated intracerebrally with</th>
<th>Internal between inoculation and death</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Fresh virus.</td>
<td>37 days.</td>
</tr>
<tr>
<td>77A</td>
<td>Fresh virus from Rabbit 100.</td>
<td>48</td>
</tr>
<tr>
<td>275</td>
<td>Virus from Rabbit 77A kept in glycerine for 48 days.</td>
<td>32</td>
</tr>
<tr>
<td>211</td>
<td>Fresh virus from Rabbit 275.</td>
<td>42</td>
</tr>
<tr>
<td>212</td>
<td>&quot;</td>
<td>44</td>
</tr>
</tbody>
</table>

A similar observation has been made a number of times in the course of our experiments, and this is recorded as a typical example. Levaditi, Harvier, and Nicolau record similar findings for the virus of herpes, and this has been confirmed more recently by Perdrau.

V. SENSITIVENESS TO HEAT AND DESICCATION.

(a) Heat. Zwick and his collaborators found that cerebral emulsions heated for 5, 10, 15, 20, and 25 minutes respectively at 50°C preserved their virulence for the rabbit. In some instances a similar emulsion heated for 30 minutes at 50°C became avirulent. Heat for 30 minutes at 57°C or 10 minutes at 70°C in the water-bath destroyed the virulence of the emulsion.

(b) Desiccation. Zwick found that a virulent emulsion of brain dried for 6 to 10 hours at 30°C proved to be avirulent when inoculated after such desiccation.

VI. ACTION OF ULTRA-VIOLET LIGHT.¹

We proceeded in the following manner. A homogeneous emulsion of virulent brain was centrifuged for 5 minutes. The supernatant fluid was carefully pipetted into a small quartz flask and exposed for 5 minutes to the rays from a mercury arc. Two mercury vapor lamps (K.B.B. type, 25 amperes, 210 volts D.C.) were employed, 8 inches distant. The flasks were slowly rotated during the exposure so that a fresh thin film of fluid was constantly exposed to the light. The flasks dipped periodically into cold water in a trough to prevent overheating during the experiment. The irradiated fluid was inoculated into the brain of a rabbit. At the same time a portion of the non-irradiated emulsion was inoculated intracerebrally into rabbits, which served as controls. The protocols of these experiments are recorded on page 21.

The rabbit inoculated intracerebrally with the virus which had been subjected to the rays of the mercury arc, did not show symptoms during three months, while the controls died after 25 and 34 days respectively, showing that the virus subjected to the action of ultra-violet rays (radiations of wave-lengths 5,720–2,320 A.U.) was killed in a maximum of 5 minutes.

Rabbit 163A was reinoculated, along with a control rabbit No. 25A, with fresh passage virus 86 days later and died on the 34th day.

¹ We are indebted to Dr. Eidinow of the Department of Applied Physiology (National Institute for Medical Research) for his collaboration in these experiments.
ENZOOTIC ENCEPHALO-MYELITIS

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38th day. The control rabbit succumbed to the injection on the 48th day.

<table>
<thead>
<tr>
<th>Irradiated emulsion.</th>
<th>Controls—non-irradiated emulsion.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit 163A.</td>
<td>Rabbit 165A.</td>
</tr>
<tr>
<td>Weight, 2,120 gms.</td>
<td>Weight, 2,010 gms.</td>
</tr>
<tr>
<td>22.6.27. 1,900 gms.</td>
<td>22.6.27. 1,900 gms. Typical symptoms.</td>
</tr>
<tr>
<td>29.6.27. 2,000 gms.</td>
<td>29.6.27. 1,400 gms. Effected.</td>
</tr>
<tr>
<td>7.7.27. 2,100 gms.</td>
<td>7.7.27. 1,490 gms. Typical symptoms.</td>
</tr>
<tr>
<td>14.7.27. 2,800 gms.</td>
<td>14.7.27. 1,000 gms. Rabbit dying, killed 34 days after injection.</td>
</tr>
<tr>
<td>15.8.27. 2,800 gms.</td>
<td>Microscopic examination. Intense characteristic lesions in the central nervous system.</td>
</tr>
<tr>
<td>3.9.27. 2,800 gms.</td>
<td></td>
</tr>
</tbody>
</table>

VII. THE ACTION OF HEXAMETHYLENETETRAMINE (UROTROPINE).

The experiments of Moussu (1926) show that when equal quantities of a virulent emulsion of brain and a 10 per cent. solution of urotropine are mixed and kept at room temperature for 12 hours, the virus can still be demonstrated in the mixture after this time.

VIII. ACTION OF CHLOROFORM AND ETHER.

(a) Action of Chloroform. A thick emulsion of the brain of a rabbit dead of experimental Borna disease (Rabbit 182A, dead on the 40th day after cerebral infection) was mixed with five volumes of chloroform, and the mixture kept for 18 hours at room temperature. The fluid part of the mixture was removed by evaporation in a vacuum over sulphuric acid. The dried brain residue was powdered in a mortar and suspended in physiological saline. This suspension was then inoculated intracranially into two rabbits (234A and 235A). Rabbit 219A, which was inoculated with an emulsion of the same brain not mixed with chloroform, served as control.

Five days later both Rabbits 234A and 235A received a further inoculation with the virus which had undergone similar treatment with chloroform.

<table>
<thead>
<tr>
<th>Virus treated with chloroform.</th>
<th>Control rabbits—virus not treated with chloroform.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8.27. Inoculated.</td>
<td>5.8.27. Inoculated.</td>
</tr>
<tr>
<td>10.8.27. Second inoculation given.</td>
<td>15.8.27. Second inoculation given.</td>
</tr>
<tr>
<td>15.8.27. 2,300 gms. Normal.</td>
<td>15.8.27. 2,400 gms. Normal.</td>
</tr>
<tr>
<td>24.8.27. 2,350 gms.</td>
<td>24.8.27. 2,500 gms.</td>
</tr>
<tr>
<td>29.8.27. 2,400 gms.</td>
<td>29.8.27. 2,550 gms.</td>
</tr>
<tr>
<td>31.8.27. 2,500 gms.</td>
<td>31.8.27. Reinoculated with fresh virulent virus.</td>
</tr>
<tr>
<td>31.8.27. 2,700 gms.</td>
<td>9.9.27. 2,900 gms. Normal.</td>
</tr>
<tr>
<td>32.8.27. 2,800 gms.</td>
<td>15.9.27. 2,700 gms.</td>
</tr>
<tr>
<td>28.9.27. 2,670 gms.</td>
<td>28.9.27. 2,670 gms.</td>
</tr>
<tr>
<td>5.8.27. Inoculated.</td>
<td>15.8.27. 2,100 gms. Normal.</td>
</tr>
<tr>
<td>24.8.27. 1,789 gms. Commenence of disease.</td>
<td>29.8.27. 1,580 gms. Typical disease.</td>
</tr>
<tr>
<td>2.9.27. Found dead 31 days after infection.</td>
<td>Passage of brain to fresh rabbit is positive.</td>
</tr>
<tr>
<td>Microscopic examination of sections of brain and cord revealed intense and characteristic lesions.</td>
<td></td>
</tr>
</tbody>
</table>
These experiments show that the virus is inactivated by contact with chloroform for 18 hours at room temperature.

(b) Action of Ether. The technique was similar to that employed in the above experiment with chloroform. Rabbit 281A, which received an intracerebral inoculation of ether-treated brain emulsion, was kept under observation for 5 months. It never showed any symptoms of Borna disease and gained 780 gms. in weight. The control rabbit, 219A, inoculated with non-treated brain died in 31 days of typical infection.

NOTE.—The experiments on the effect of chloroform and ether on the virus are only preliminary. Obviously there are certain details in the technique used which will have to be controlled, and improved methods are now being employed.

IX. ACTION OF FORMALIN.

About one gramme of the brain of Rabbit 355A (which died 27 days after intracerebral inoculation with the passage virus of Borna disease) was emulsified in 15 c.c.m.s. of a solution of formalin in physiological saline (2:1,000). The emulsion was rendered as homogenous as possible, and was then placed at the temperature of the laboratory for 18 hours. Subsequently rabbits were inoculated intracerebrally with the formalized emulsion. An emulsion of the brain of Rabbit 355A in a similar dilution not treated with formalin was inoculated as a control into the brain of a rabbit.

Protocols. The inoculations were made 10.9.27.

<table>
<thead>
<tr>
<th>Virus treated with formalin inoculated intracerebrally.</th>
<th>Control—virus not treated with formalin.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rabbit 44A. 2,710 gms.</strong></td>
<td><strong>Rabbit 45A. 2,900 gms.</strong></td>
</tr>
<tr>
<td>15.8.27. 2,600 gms. Normal.</td>
<td>15.8.27. 1,800 gms. Normal.</td>
</tr>
<tr>
<td>23.9.27. 2,700</td>
<td></td>
</tr>
<tr>
<td>30.9.27. 2,640</td>
<td></td>
</tr>
<tr>
<td>5.9.27. 2,750</td>
<td></td>
</tr>
<tr>
<td>11.9.27. 2,740</td>
<td></td>
</tr>
</tbody>
</table>

| **Rabbit 358A. 2,100 gms.** |
| 15.8.27. 2,050 gms. Normal. |
| 23.9.27. 2,150 ||
| 30.9.27. 1,900 ||
| 11.9.27. 1,780 gms. Disease commencing. |
| 14.9.27. Found dead 30th day. |

Conclusion. The conclusion arrived at is that formalin in a concentration of 0.2 per cent. inactivates the virus after 18 hours' contact at room temperature.

X. CULTURE.

All attempts at cultivation of the virus have remained negative up to the present.

XI. SUMMARY.

From the observations of the authors quoted and our own, appears that the virus of Borna disease possesses the properties common to those of vaccinia, herpes, rabies, and poliomyelitis which Levaditi has grouped together under the name 'ectodermoses neurotropes.' Under favourable conditions it filters through bacteria-proof filters.
although most of the virus is held back, and through a collodion ultrafilter which will allow colloidal particles of large dimensions to pass. The infectivity of the supernatant fluid cannot be removed by centrifugulation for 15 minutes at 5,400 revs. per minute. It is resistant to the action of glycerine, but sensitive to desiccation, ultra-violet light and heat. It is destroyed by ether, chloroform, and formalin. It has not been propagated outside the body.

3. TRANSMISSION OF EQUINE STRAIN TO RABBIT AND FROM RABBIT TO SHEEP, AND VICE VERSA

Moussu (1926) inoculated an emulsion of the brain of a rabbit previously infected with the virus from a horse into the anterior chamber of the eye of a horse. The animal developed symptoms 3 days after the inoculation and died in 8 days. The lesions found in the brain were very intense, infiltrative, and haemorrhagic. A rabbit inoculated with an emulsion from the brain of this horse died 4 days later. This same author failed to infect horses with virulent material from rabbits by subcutaneous inoculation or *per os*. Zwick and his collaborators (1926) inoculated a horse intracerebrally with the brain of a rabbit suffering from experimental Borna disease. The virus had been passaged in this species of animal nine times. The horse fell ill 58 days after the inoculation. For 11 days it showed the typical symptoms of encephalo-myelitis and death followed 64 days after the inoculation. The lesions of the nervous system were characteristic of Borna disease. The intranuclear corpuscles of Joest-Degen were demonstrated in the ganglion cells of the Ammon’s horn.

We have referred previously to the experiments of Beck and Frohböse (1926), Moussu (1926), Miessner (1926), and Ernst and Hahn (1927), which showed that the virus of encephalo-myelitis originating from sheep can be transmitted to rabbits by experimental inoculation. Moussu and Marchand (1924) succeeded in passing the disease from sheep to sheep. Zwick and his collaborators (1926) failed to transmit the disease to adult sheep by intracerebral inoculation with a strain derived from a horse and passed through rabbits. Using the same strain of virus they succeeded, however, in conferring the disease on young lambs. Death followed 88 days after the inoculation and typical cerebral lesions were revealed.

Direct inoculation from horse to lamb was also successful. The lamb showed characteristic symptoms and died 92 days after infection. Inoculation of the brain of this lamb to a rabbit gave a positive result, but inoculation of the cord gave a negative result. Beck and Frohböse (1926) did not succeed in infecting the horse by the intracerebral route with virus from sheep dead from the spontaneous

1 The experiments of Moussu and Marchand have been criticized since his inoculated animals succumbed very early, and also because the lesions of the brain were surprisingly acute when compared with those found in the classical disease occurring spontaneously or in animals infected with the viruses isolated by the German school. We had the intention of comparing their strain with those of Zwick and Miessner, but Moussu has informed us that his strain is not now available.
disease. On the other hand, they succeeded in infecting sheep with virus obtained from horses.

From the foregoing résumé of the literature the following conclusions may be drawn:

1. The virus originating from horses passaged through rabbits can be transmitted back to the horse.
2. The virus taken directly from the horse or subsequently passaged through rabbits is pathogenic for lambs.
3. Attempts at transmitting the disease from sheep to horses have so far been unsuccessful.

4. EXPERIMENTAL DISEASE IN THE RABBIT

I. EXPERIMENTAL TRANSMISSION OF THE DISEASE TO THE RABBIT

Moussu (1926) inoculated an emulsion of the brain of a horse dead of encephalo-myelitis into the anterior chamber of the eye of the rabbit. In one experiment three animals were inoculated by this route. One died on the 9th day; the two others survived; the rabbit which died constituted the head of the series of passages that the author continued until he obtained a ‘fixed’ virus which killed the rabbits infected by the intraocular route in 4 to 6 days. In another experiment using similar material one out of five animals inoculated intraocularly died 11 days after receiving the injection; the other four survived. In the majority of cases a marked excitability was a characteristic symptom. Moussu states, however, that certain rabbits die following an infection with a slower evolution, lasting more than a fortnight.

Zwick inoculated rabbits intracerebrally with the cerebral substance (Ammon’s horn, caudate nucleus, and cerebral cortex) taken from the brain of a horse dead of Borna disease. He observed typical symptoms of the disease after a period of about 4 weeks, and the lesions were analogous to those described previously in the horse. Passage from rabbit to rabbit was effected, the period of incubation after inoculation being about 3 weeks.

Zwick and his collaborators (1926) succeeded in infecting rabbits with the virus from 16 out of 21 cases of horses dead of Borna disease, which was verified histologically. The incubation period of the disease in such rabbits infected by the intracerebral route was from 3 to 6 weeks. Death ensued 8 to 14 days after the appearance of the characteristic symptoms.

Beck and Frohbose (1926) also infected rabbits with the virus from horses and sheep. Miessner (1926) with sheep virus, and Ernst Hahn (1927) with viruses from horses, sheep, and cattle.

II. AUTHORS’ OBSERVATIONS.

The virus of encephalo-myelitis of equine or ovine origin isolated by the German workers does not become ‘fixed’ when passaged through rabbits. The period of incubation varies from 15 to 50 days and we have also observed recurrent forms of the disease in rabbits.
ENZOOTIC ENCEPHALO-MYELITIS

We have inoculated more than 200 rabbits by the intracerebral route with the virus originating from horses or sheep. In Table I the period of incubation and the time between the onset of the disease and death is recorded for a total of 50 rabbits used for passaging a virus originally obtained from a horse and sent to us by Professor Zwick.

Table I.

<table>
<thead>
<tr>
<th>Number of rabbit</th>
<th>Weight in gms.</th>
<th>Commencement of disease.</th>
<th>Duration of the disease.</th>
<th>Death.</th>
<th>Lesions in C.N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2,160</td>
<td>21st day</td>
<td>14 days</td>
<td>35th day</td>
<td>Intense.</td>
</tr>
<tr>
<td>22</td>
<td>2,000</td>
<td>20th</td>
<td>4</td>
<td>24th</td>
<td>Positive.</td>
</tr>
<tr>
<td>24</td>
<td>1,420</td>
<td>31st</td>
<td>19</td>
<td>40th</td>
<td>Intense.</td>
</tr>
<tr>
<td>10a</td>
<td>2,500</td>
<td>27th</td>
<td>10</td>
<td>37th</td>
<td></td>
</tr>
<tr>
<td>11d</td>
<td>1,000</td>
<td>17th</td>
<td>10</td>
<td>27th</td>
<td></td>
</tr>
<tr>
<td>#93a</td>
<td>1,520</td>
<td>7th</td>
<td>1</td>
<td>8th</td>
<td>Slight.¹</td>
</tr>
<tr>
<td>68a</td>
<td>1,150</td>
<td>20th</td>
<td>5</td>
<td>25th</td>
<td>Intense.</td>
</tr>
<tr>
<td>50a</td>
<td>1,620</td>
<td>40th</td>
<td>13</td>
<td>53rd</td>
<td></td>
</tr>
<tr>
<td>77a</td>
<td>1,570</td>
<td>38th</td>
<td>10</td>
<td>48th</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>1,350</td>
<td>21st</td>
<td>7</td>
<td>28th</td>
<td></td>
</tr>
<tr>
<td>51a</td>
<td>1,350</td>
<td>20th</td>
<td>8</td>
<td>28th</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>2,000</td>
<td>43rd</td>
<td>14</td>
<td>37th</td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>820</td>
<td>26th</td>
<td>14</td>
<td>30th</td>
<td></td>
</tr>
<tr>
<td>275</td>
<td>2,100</td>
<td>26th</td>
<td>7</td>
<td>32nd</td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>1,050</td>
<td>29th</td>
<td>3</td>
<td>32nd</td>
<td></td>
</tr>
<tr>
<td>214</td>
<td>1,200</td>
<td>27th</td>
<td>4</td>
<td>31st</td>
<td></td>
</tr>
<tr>
<td>261</td>
<td>1,230</td>
<td>23rd</td>
<td>8</td>
<td>45th</td>
<td></td>
</tr>
<tr>
<td>232</td>
<td>1,340</td>
<td>29th</td>
<td>11</td>
<td>31st</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>1,820</td>
<td>16th</td>
<td>5</td>
<td>21st</td>
<td></td>
</tr>
<tr>
<td>295</td>
<td>920</td>
<td>9th</td>
<td>10</td>
<td>19th</td>
<td>Very intense.</td>
</tr>
<tr>
<td>211</td>
<td>1,580</td>
<td>31st</td>
<td>11</td>
<td>42nd</td>
<td></td>
</tr>
<tr>
<td>212</td>
<td>1,400</td>
<td>31st</td>
<td>13</td>
<td>44th</td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>1,500</td>
<td>31st</td>
<td>8</td>
<td>39th</td>
<td></td>
</tr>
<tr>
<td>222</td>
<td>1,540</td>
<td>35th</td>
<td>13</td>
<td>48th</td>
<td></td>
</tr>
<tr>
<td>224</td>
<td>1,150</td>
<td>32nd</td>
<td>13</td>
<td>45th</td>
<td></td>
</tr>
<tr>
<td>226</td>
<td>1,150</td>
<td>24th</td>
<td>4</td>
<td>29th</td>
<td>Positive.</td>
</tr>
<tr>
<td>70</td>
<td>1,750</td>
<td>20th</td>
<td>2</td>
<td>32nd</td>
<td>Intense.</td>
</tr>
<tr>
<td>56</td>
<td>1,400</td>
<td>20th</td>
<td>6</td>
<td>26th</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>1,500</td>
<td>24th</td>
<td>6</td>
<td>30th</td>
<td></td>
</tr>
<tr>
<td>251</td>
<td>1,250</td>
<td>18th</td>
<td>9</td>
<td>27th</td>
<td></td>
</tr>
<tr>
<td>237</td>
<td>1,680</td>
<td>18th</td>
<td>13</td>
<td>31st</td>
<td></td>
</tr>
<tr>
<td>255</td>
<td>1,920</td>
<td>35th</td>
<td>7</td>
<td>42nd</td>
<td></td>
</tr>
<tr>
<td>256</td>
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<td>15</td>
<td>47th</td>
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</tr>
<tr>
<td>30</td>
<td>1,200</td>
<td>38th</td>
<td>8</td>
<td>36th</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>1,350</td>
<td>26th</td>
<td>7</td>
<td>33rd</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>1,000</td>
<td>27th</td>
<td>7</td>
<td>34th</td>
<td></td>
</tr>
<tr>
<td>#291</td>
<td>950</td>
<td>No sympt.</td>
<td>—</td>
<td>15th</td>
<td>Slight.¹</td>
</tr>
<tr>
<td>85a</td>
<td>1,500</td>
<td>32nd</td>
<td>6</td>
<td>38th</td>
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</tr>
<tr>
<td>43</td>
<td>1,020</td>
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<td>—</td>
<td>12th</td>
<td>Slight.</td>
</tr>
<tr>
<td>60</td>
<td>1,300</td>
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<td>7</td>
<td>37th</td>
<td>Intense.</td>
</tr>
<tr>
<td>62</td>
<td>1,610</td>
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<td>17</td>
<td>39th</td>
<td></td>
</tr>
<tr>
<td>271</td>
<td>1,250</td>
<td>31st</td>
<td>10</td>
<td>41st</td>
<td></td>
</tr>
<tr>
<td>*90</td>
<td>820</td>
<td>No sympt.</td>
<td>7h</td>
<td>7th</td>
<td>Slight.</td>
</tr>
<tr>
<td>269</td>
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<td>2</td>
<td>21st</td>
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</tr>
<tr>
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<td>No sympt.</td>
<td>—</td>
<td>13th</td>
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</tr>
<tr>
<td>296</td>
<td>2,900</td>
<td>29th</td>
<td>7</td>
<td>33rd</td>
<td>Intense.</td>
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<tr>
<td>277</td>
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<td>11</td>
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</tr>
<tr>
<td>78s</td>
<td>1,700</td>
<td>24th</td>
<td>8</td>
<td>32nd</td>
<td></td>
</tr>
<tr>
<td>116a</td>
<td>1,700</td>
<td>18th</td>
<td>4</td>
<td>22nd</td>
<td></td>
</tr>
</tbody>
</table>

¹ When the brain of these rabbits was passaged, positive results were obtained. Rabbits inoculated with passage of virus from the brain of Rabbit No. 93A died in...
BORNA DISEASE AND

Of 50 rabbits inoculated in the brain:
23 died between 21 and 33 days after inoculation.
6 died in less than 21 days.
21 died in more than 33 days.

Only exceptionally did the rabbit die in less than 3 weeks when infected by the intracerebral route. The detailed histopathological study of each case showed that the rabbits dead 7 to 8 days after inoculation had minimal infiltrative lesions in the central nervous system. The presence of virus in the brain was proved by subsequent passage and in all cases complete autopsies were made to exclude the possibility of death from other causes.

The animals which died between 21 days and 57 days presented the characteristic lesions in the nervous system, which are described in the chapter dealing with the histopathology of the disease. The intensity of the lesions was not in direct relationship with the duration of the malady. As has also been observed by Zwick the incubation period was longer in larger animals. Generally, rabbits weighing less than 1,500 gms. were more susceptible to infection than older rabbits. Excluding the five animals in Table I marked with an asterisk, all of which died in 15 days or under, the average time which elapsed between the intracerebral inoculation and the death of the animal in our experiments was 20 days in rabbits of less than 1,500 gms. and 36 days in rabbits over this weight at the time of infection.

Table II.

<table>
<thead>
<tr>
<th>Number of rabbit</th>
<th>Weight in gms.</th>
<th>Commencement of disease.</th>
<th>Duration of disease.</th>
<th>Death.</th>
<th>Lesions in C.N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>164</td>
<td>1,220</td>
<td>21st day</td>
<td>8 days</td>
<td>29th day</td>
<td>Intense.</td>
</tr>
<tr>
<td>18</td>
<td>1,240</td>
<td>20th day</td>
<td>9 days</td>
<td>35th day</td>
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</tr>
<tr>
<td>245</td>
<td>1,380</td>
<td>25th day</td>
<td>10 days</td>
<td>38th day</td>
<td></td>
</tr>
<tr>
<td>234</td>
<td>1,420</td>
<td>7th day</td>
<td>7th</td>
<td>7th</td>
<td></td>
</tr>
<tr>
<td>243</td>
<td>1,450</td>
<td>20th day</td>
<td>9 days</td>
<td>33rd</td>
<td></td>
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<tr>
<td>235</td>
<td>1,500</td>
<td>22nd day</td>
<td>12 days</td>
<td>35th</td>
<td></td>
</tr>
<tr>
<td>262</td>
<td>2,220</td>
<td>40th day</td>
<td>8 days</td>
<td>48th</td>
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<tr>
<td>330</td>
<td>3,640</td>
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<td>12 days</td>
<td>35th</td>
<td></td>
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<td>10</td>
<td>2,140</td>
<td>18th day</td>
<td>9 days</td>
<td>27th</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>1,620</td>
<td>19th day</td>
<td>8 days</td>
<td>27th</td>
<td></td>
</tr>
<tr>
<td>274</td>
<td>1,770</td>
<td>15th day</td>
<td>10 days</td>
<td>28th</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1,900</td>
<td>23rd day</td>
<td>14 days</td>
<td>37th</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>1,800</td>
<td>20th day</td>
<td>12 days</td>
<td>32nd</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>1,600</td>
<td>20th day</td>
<td>8 days</td>
<td>28th</td>
<td></td>
</tr>
<tr>
<td>162A</td>
<td>2,180</td>
<td>21st day</td>
<td>12 days</td>
<td>33rd</td>
<td></td>
</tr>
<tr>
<td>167A</td>
<td>2,420</td>
<td>22nd day</td>
<td>7 days</td>
<td>29th</td>
<td></td>
</tr>
<tr>
<td>178</td>
<td>1,770</td>
<td>25th day</td>
<td>9 days</td>
<td>34th</td>
<td></td>
</tr>
<tr>
<td>207A</td>
<td>840</td>
<td>19th day</td>
<td>2 days</td>
<td>21st</td>
<td>Well marked</td>
</tr>
<tr>
<td>191A</td>
<td>2,100</td>
<td>26th day</td>
<td>6 days</td>
<td>32nd</td>
<td></td>
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<td>1,670</td>
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<tr>
<td>180A</td>
<td>1,890</td>
<td>27th day</td>
<td>12 days</td>
<td>39th</td>
<td></td>
</tr>
<tr>
<td>179A</td>
<td>2,220</td>
<td>26th day</td>
<td>6 days</td>
<td>32nd</td>
<td></td>
</tr>
</tbody>
</table>

Table II sets forth similar observations in the case of 22 rabbits 53 days; from No. 291, in 38 days; from No. 77s in 21 days. Intense lesions were found in the C.N.S. of these latter rabbits.
incubated with a strain of ovine origin kindly sent to us by Professor Miessner. The average time between inoculation and death was 33 days for the 15 rabbits weighing more than 1,500 gms. and 28 days for the 6 rabbits weighing less than 1,500 gms.

III. Symptomatology of the Disease in the Rabbit.

Observations have been made on over 200 rabbits. During the first 2 to 4 days following the injection the weight of the animal decreases slightly, to return later to the normal. Once the period of traumatic shock has subsided the animal puts on weight and no morbid symptoms are seen during 15 to 20 days. Subsequently it becomes slow in its movements and appears depressed; the weight decreases progressively and the first characteristic symptom develops. When the rabbit is placed on its side it makes efforts to recover its feet, beating the air with its hind legs before eventually recovering the normal position. While the depression referred to above suggests modifications in the meninges and cerebrum, the symptoms described later point to changes in the spinal cord.

The animal assumes a characteristic attitude in the cage with the head in the angle formed by two walls; it appears somnolent and the somnolence lasts till the end of the disease. The symptoms of nervous origin become intensified gradually; among these are those of amaurosis. When the animal is allowed to run towards an object it runs into it as if it had not seen it. Grinding of the teeth is observed, sometimes with increased salivation. There is paresis of the ears, which fall to the right and left of the head. The head itself is depressed. When the animal is placed at the edge of a table it hangs its head over the edge below the level of the rest of the body. Trismus may occur. The symptoms of a myelitic character become exacerbated. Taken from the cage and placed on its side, the rabbit makes vain efforts to rise. At this stage its position at rest is characteristic; it remains hunched up in a corner, the head is dropped as if it was no longer capable of supporting it (Fig. 1), and sometimes the back is humped. The muscles of the back become soft and flaccid. Attempts to resist with the hind legs when the animal is held by the skin of the back are feeble or absent. Finally paralysis of the hind quarters occurs, which spreads later to the fore-limbs. The animal ceases to feed, either from loss of appetite or difficulty in deglutition, and loses weight'. In certain cases the loss of weight may be the dominant feature of the disease. Very often rabbits at the end of the disease have lost nearly half of their original weight (Charts I and II).

We have never observed excitement in our experimental animals, but always depression.

The study of the blood has given inconstant results. In certain animals a slight hyperleucocytosis has been observed with a slight increase in polymorphonuclears. In others the leucocytosis 16,000 to 18,000 per c.mm. was accompanied by lymphocytosis. In the
Chart I.

Chart II.

DIED ON 57TH DAY.
terminal stage a marked increase in polymorphonuclears is the rule. The number of erythrocytes remains unchanged, and they show no morphological changes.

Chart III shows the parallelism between the augmentation of the number of leucocytes per c.mm. and the number of lymphocytes obtained from the leucocytic count. This modification of the number of leucocytes is not constant.

No fever exists during the course of the disease in the rabbit. This fact was noted constantly when the temperature of a series of rabbits inoculated intracerebrally was taken daily at the same hour. Death takes place in coma—the temperature being hypothermic (35° C.—34° C.; see Chart II).

IV. ROUTES BY WHICH RABBITS CAN BE INFECTED.

Intracerebral.

Intracerebral inoculation produces the disease in a constant manner, followed by death.

Intrathecal.

Beck (1925) infected by introducing virus intrathecally, and we have also succeeded in infecting rabbits by this route.

Experiment 1. Rabbit 207, weighing 780 gms. was inoculated intrathecally in the lumbar region with 0.5 c.cm. of a virulent emulsion of brain diluted 1:10 in physiological saline. Forty-three days
after the inoculation the condition of the animal was such that when taken out of the cage it walked with its legs spread out from the body. Paresis of the hind quarters was well marked, and increased gradually. The animal wasted and died 66 days after the inoculation. Examination of the brain revealed the presence of characteristic lesions, and a passage of this brain to a healthy rabbit gave a positive result.

Sciatic Nerve.

Introduction of several drops of a virulent emulsion of the virus into the sciatic nerve did not infect animals with encephalo-myelitis in the experiments of Moussa (1926), and Zwick and his collaborators (1926). We have, however, succeeded several times in producing Borna disease in rabbits inoculated by this route.

Experiment 2. The right sciatic nerve of Rabbit 208, weighing 850 gms., was exposed by incision and 2 or 3 drops of a virulent emulsion of the brain of a rabbit infected with encephalo-myelitis was injected into the substance of the nerve-trunk. The point of introduction of the needle was seared to prevent the exit of fluid into the surrounding tissues. The operation was carried out aseptically and the incision healed by first intention. The animal showed no morbid symptoms for 35 days and put on weight, reaching 1,350 gms. Subsequently it became prostrate, wasted progressively, and showed marked inco-ordination, which became accentuated later; the animal died on the 48th day. The brain was proved to be bacteriologically sterile, and no lesions were found which might serve to explain the cause of death, other than those in the central and peripheral nervous system. These were of an intense character, and were found in the brain, in the spinal cord (cervical, thoracic, dorsal, and lumbar regions), and also in the inoculated nerve.

Emulsions from the brain and also the dorso-lumbar part of the spinal cord were inoculated into the brain of fresh rabbits. These inoculations produced the disease, showing that the virus was present both in the brain and cord of rabbits inoculated into a peripheral nerve.

Rabbit 270 was also inoculated into the sciatic nerve by the same method as recorded above for Rabbit 208. This rabbit (270) showed 65 days after the inoculation, paralysis of the leg into the sciatic nerve of which virus had been inoculated. Paralysis of the hind quarters followed, with grinding of the teeth, and other typical symptoms. The animal was found dead on the 78th day. Lesions were demonstrated throughout the nervous system (brain, mesencephalon, cord in all regions, inoculated nerve, the sciatic of the opposite side not inoculated), as well as in the brachial nerves.

The detailed description of these lesions will be given later.

Rabbit 276 was inoculated into the right sciatic with the same technique as before. The leg on the side of inoculated nerve became useless after 28 days, and the animal died 8 days later with lesions in the central and peripheral nervous system.
Moussu (1926) and also Zwick (1926) have shown that it is possible to infect the rabbit by inoculation of a virulent emulsion of the brain of the horse into the anterior chamber of the eye, and we have confirmed the possibility of infection by this route.

Rabbit 206 received several drops of the supernatant fluid from a virulent emulsion of brain into the anterior chamber. The point of inoculation was carefully seared. During the period immediately following the inoculation a coagulum of fibrin could be seen in the eye, but this was absorbed later. The animal died 23 days after the inoculation, and lesions characteristic of Borna disease were found in the central nervous system; these, however, were not very acute.

The control rabbit inoculated intracerebrally with the same emulsion died in the average time with well-marked lesions in the central nervous system.

Rabbits 86s and 89s inoculated in the anterior chamber with an emulsion containing virus fell ill 29 and 36 days respectively after infection, and died on the 34th and 51st day with typical symptoms in the central nervous system. The control rabbit (intracerebral route) died 37 days after inoculation.

A fourth rabbit inoculated intraocularly with the virus survived without having shown any symptoms.

Thus, of four rabbits inoculated in the anterior chamber three became infected and died of the disease, while the fourth showed no obvious symptoms and survived. These results are in accordance with those of Zwick who had five positive results in six attempts to infect rabbits by the intraocular route.

No macroscopic modification of the cornea followed the introduction of the virus, but microscopically there was slight infiltration with lymphocytes between the corneal laminae. In one case an interstitial infiltration with mononuclear cells of the optic nerve was found.

Corneal Scarification.

Zwick (1926) has stated that infection by corneal scarification causes the disease only rarely. We have inoculated four rabbits by scarification of the cornea; none became infected, nor was there any visible keratitis. However, one of the rabbits (Rabbit 81s) which was subsequently reinoculated intracerebrally with a virulent emulsion of brain 110 days later remained well, whereas a control rabbit which had received a cerebral inoculation with the same emulsion died 8 days later after showing typical symptoms of the disease, and with the characteristic lesions. The corneal inoculation may have rendered the rabbit refractory to infection by the intracerebral route.

Conjunctival Sac.

Instillation of a virulent emulsion into the conjunctival sac of the eye produced no effect.

Nasal Mucosa.

The nasal mucosa appears to be a possible portal of entry of the virus, and Joest (1927) has suggested that natural contagion in the
horse is effected by this route. The results of attempts by Zwick (1926), Beck and Frohböse (1926) to infect rabbits by this route have, however, been inconstant.

Scarified Skin.

Zwick applied a virulent emulsion of brain to the scarified skin without result. Our results confirm those of Zwick. As, however, the experiments of Flexner and Amos (1917) with poliomyelitis, and of Levaditi and Nicolau (1922, 1928) with herpes and neurovaccinia show that previous injection of substances like physiological saline bouillon, or normal serum into the brain may increase susceptibility, we introduced physiological saline either into the brain or intrathecally in rabbits which had received an application of the virus to the depilated, shaved, and scarified skin. The animals prepared by the inoculation of saline intrathecally did not show any symptoms of the disease, while those which had been subjected to an irritation of the brain with saline subsequently contracted the disease, and died. Typical lesions were found in the central nervous system, and passage of the brain to new rabbits by the intracerebral route gave positive results. This experiment shows that when the nervous system is a state of special receptivity due to the diminution of the normal power of defence, infection can take place from the skin. The virus probably reached the brain by way of the intercostal nerves, the being protected from the action of leucocytes circulating in the blood. This interpretation is supported by the following experiments. Four rabbits were inoculated with 1.5 c.c.m. of a centrifugalized emulsion of virulent brain into the marginal vein of the ear. Two of the rabbits inoculated intravenously received simultaneously 0.8 c.c.m. of physiological saline into the brain, but neither of these animals contracted the disease, nor did the two which received only the intravenous inoculation. Two months later the immunity of these four rabbits was tested by intracerebral inoculation, and all proved susceptible to infection. The virulence of the emulsion used to infect the four rabbits intravenously was proved by the intracerebral inoculation of two controls. So that it would appear that the virus was destroyed in the blood-stream, or in the tissues before it reached the brain.

Our further attempts to infect by the intravenous route gave negative results, but Zwick (1926) succeeded exceptionally when intravenous injections were given at intervals. Positive results have also been obtained by Ernst and Hahn (1927). Usually, however, they found that repeated inoculations by the intravenous route instead of conferring the malady produced solid resistance. This will be discussed in the chapter dealing with immunity.

Subcutaneous.

Subcutaneous inoculation may, exceptionally, lead to a fatal cephalo-myelitis. Zwick and his collaborators (1926), who employed repeated injections of the virus, produced the disease with a great

1 At no time did the skin show any macroscopic changes which could be attributed to the virus.
ENZOOTIC ENCEPHALO-MYELOITIS

They obtained similar results by inoculation of the virus intraperitoneally; intramuscular injections with the virus did not confer the disease in their experiments.

Intratesticular.

According to the latter investigators the introduction of virus by the intratesticular route did not produce infection in rabbits, but in our experiments it has done so.

Rabbit 209, weighing 1,850 gms., was inoculated under anaesthesia into both testicles with a virulent emulsion of the brains of four rabbits dead of experimental Borna. The dilution of the emulsion of brain substance in physiological saline was 1 : 20. During a period of 3 days the animal showed no morbid symptoms and its weight increased to 2,540 gms.; then, without other symptoms, wasting commenced; 19 days later the weight had fallen to 1,860 gms. (680 gms. loss), and inco-ordination with slight paresis of the hind quarters was noticed. Paresis became accentuated and other symptoms characteristic of the disease became manifest. On the 71st day after the inoculation, 20 days after the first loss of weight was recorded and 19 days after the first clinical symptom, the animal died, weighing only 1,480 gms. The lesions found in the central nervous system were characteristic and were especially intense in the lumbar region. The topography of the lesions in the cord indicated that the virus had spread from the point of inoculation to the brain through the cord anteropetally. The intranuclear inclusions of Joest-Degen were found. The passage of the brain and cord of Rabbit 209 to fresh animals gave positive results, indicating the presence of virus in both. The control rabbit inoculated intracerebrally with the emulsion of brain had served to infect Rabbit 209 died 32 days after inoculation.

Rabbit 289, weighing 1,720 gms., was inoculated into the right testicle with a virulent emulsion of brain. During 60 days it put on weight, reaching 2,320 gms. This weight was maintained for 14 days, then wasting began. On the 90th day after inoculation paresis of the hind quarters was observed. The animal died 105 days after inoculation.

Rabbit 273 inoculated into the right testicle at the same time showed no symptoms and survived, while the control rabbit inoculated intracerebrally died after 37 days with the typical symptoms and lesions characteristic of the disease.

Intratracheal.

We have made two unsuccessful attempts to infect rabbits by intratracheal inoculation. In the first attempt two rabbits received each 0·3 c.c.m. of a thick emulsion of brain containing virus into the trachea, which had been exposed by incision; both these rabbits survived without having shown any symptoms, while the control which had received an intracerebral inoculation with the same virus succumbed to the infection. In the second experiment four young rabbits, between 670 and 860 gms., were inoculated. Each received 0·5 c.c.m. of a thick emulsion of the brain of a rabbit, dead of Borna disease,
diluted 1:5. Kept under observation more than six months they maintained their normal state of health, more than doubling the weight. The controls of this experiment inoculated intracerebrally died of a typical encephalo-myelitis on the 42nd and 44th day after injection.

Per Os.

Attempts at infecting rabbits per os are of special interest for the interpretation of natural infection in horses, cattle, and sheep. Zwiuk and his collaborators (1926) succeeded in infecting rabbits by mixing virulent brain with the food. He refers to this as infection by the intestinal route, but as the virus was administered by the mouth with the food, the pre-existence of small traumatic lesions in the mouth might permit the implantation of the virus. As infection by the nasal mucosa has been shown to be possible, one cannot exclude the possibility that infection took place by the buccal mucous membrane especially when one considers the existence of nervous tissue immediately below the mucous membrane covering the tongue (Manoue and Viala, 1926). Supposing the virus to have been implanted in nervous tissue, it is quite easy to conceive how it might ultimately reach the brain.

Attempts to infect rabbits by cohabitation have been unsuccessful.

5. AUTHORS EXPERIMENTS ON THE TRANSMISSION OF THE DISEASE TO MONKEYS (MACACUS RHESUS) AND SYMPTOMS OCCURRING IN THESE ANIMALS

Monkey M. 1. A fine specimen of Macacus rhesus weighing 3,800 gms. was kept under observation during 16 days prior to inoculation. The animal's temperature varied very little.

On 15.3.27 it was inoculated intracerebrally under anaesthesia with 1·5 c.c.m.s. of a virulent emulsion of the brain of a rabbit dilute 1:5. Two control rabbits were inoculated intracerebrally at the same time as the monkey; they developed typical symptoms on the 29th and 31st day, and died of Borna disease on the 42nd and 44th day.

Between 15.3.27 and 11.5.27, a period of 57 days, the monkey showed no symptoms and the temperature remained normal.

15.3.27. Weight 3,800 gms. Temperature 38-9° C. Received inoculation with brain emulsion from Rabbit 275.

11.5.27. Fifty-seven days after inoculation the monkey appeared depressed. Temperature 38-4° C. Slight diarrhoea.

12.5.27. Same condition.

14.5.27. Temperature 38-5° C. No diarrhoea. Less lively than usual, appeared to prefer to remain with the back to the light (photophobia?).

15.5.27. Condition unchanged.

16.5.27. Photophobia well marked. The monkey hid its head under the side of the cage and would not move when disturbed. It allowed itself to be caught easily and defended itself when approached almost exclusively with the left hand and foot. Slight paresis of the right arm was detected. The pupils were equal and reacted normally to light.

18.5.27. Weight 3,280 gms. Temperature 38-2° C. Animal feeding normally remained in a corner of the cage with the head dropped like that of a [One corner of the cage]
rabbit ill from Borna disease. It allowed itself to be caught easily, offer-
ing but little resistance and with the left arm only. It was found possible
to introduce the thermometer into the rectum without holding the legs,
which fell practically inert. When the monkey was put on the ground it
moved much more slowly than normally, dragging the right leg, which
showed parasia. Parasia was less evident in the left leg. The animal could
grip the cage with the left hand, the only limb which preserved its normal
function. The right leg and arm did not grip or gripped only badly. The
animal attempted to climb, but fell exhausted by the effort. It was
roused with difficulty. There appeared to be no trouble in preserving
equilibrium. The animal appeared to be able to see. The diarrhoea was
replaced by constipation.

27. Temperature 38.5° C. Same symptoms as on the preceding day. On
opening the cage the monkey did not move or try to get out. When
taken out and left to run it fell on the right side, of which the paralysis
was more accentuated. When the monkey was held by the skin of the
neck, the legs, which showed a marked flaccidity, fell inert without any
resistance (Fig. 2). If a finger was presented, the monkey gripped it with
the left hand only, the right hand showed paralysis of the flexors of the
digits.

27. Temperature 38.5° C. Same condition.

27. Temperature 38.1° C. Weight 3,280 gms. Animal still feeding. When
taken out of the cage it was found to tire quickly, and after a feeble
effort at escaping it remained on the ground immobile for several
minutes.

27. Temperature 38.5° C. Motor disturbances were accentuated. Monkey
remained hunched up in a corner of the cage (Fig. 3).

27. Temperature 37.5° C. Same condition.

27. Temperature 37.3° C. Complete paralysis of the legs. When making
movements, it supported itself especially with the left arm and dragged
the paralysed legs. For the most part it preferred to remain hunched up
in the corner of the cage.

27. Found procumbent. Weight 3,180 gms.

27. Temperature 36.5° C. Animal procumbent, the respirations were irregu-
lar and infrequent. Incontinence of urine, no grinding of the teeth, no
oculor symptoms, no hypersalivation, both legs paralysed. As the animal
was dying it was killed by means of chloroform at 6 o'clock in the evening
73 days after the inoculation, 16 days after the first symptoms were
observed.

Topsy of Monkey 1.

The dura mater, pia mater, and the brain substance appeared normal.
The cord was slightly hyperaemic, but no haemorrhagic areas were
found.

The buccal epithelium and tongue showed desquamation of the epi-
thelium and redness.

The parotid gland. Normal in aspect.

Liver. Normal.

Kidney, liver, and gall bladder. Normal.

Liver. Congested in both the cortical and medullary zones.

Spleen. Normal.

Peritoneal cavity, bladder, and intestines. Normal.

Samples made from the brain, spleen, and blood proved to be bac-
terologically sterile.

A study of the blood, made during the course of the infection, indicated no
decided changes; the leucocytic formula remained normal, except for the slight
increase in the number of polymorphonuclears in the last stages of the disease.
Passages into rabbits made with emulsions from various parts of the central nervous system of this monkey gave positive results (see p. 48).

An emulsion of the brain of M. 1 was likewise inoculated into two monkeys of the same species by the intracerebral route (Monkey M. and M. 3) on the 28.5.27.

Monkey M. 3 (Macacus rhesus). Weighed 3,150 gms.

During a period of 71 days after the inoculation, the animal remained free from all morbid symptoms, and the temperature remained normal. On the 72nd day the monkey was found prostrate, although the day before there was no obvious illness. It was paralysed in the legs and arms, and it could not assume an upright position. Slight ptosis of the right eyelid was observed. The temperature was subnormal. The following day the respirations were irregular, gasping, and moist râles were heard. As the animal was in convulsions it was killed. The findings on autopsy were the same as for Monkey M. 1. Rabbits inoculated with emulsions of various parts of the nervous system died of Borna disease with characteristic lesions.

Monkey M. 2 (Macacus rhesus). The evolution of the disease in this monkey was entirely different.

28.5.27. Animal inoculated. Weight 3,200 gms. Temperature 38.5° C. During a period of 33 days after the inoculation the blood, the body weight, and the temperature curve showed no marked changes, and there was no evidence of any symptoms.

30.6.27. Temperature 38.2° C. Weight 3,100 gms. When a stick was given to the animal it could not seize it with the right hand, and if irritated it defended itself with the left hand. The monkey was able to run and climb normally.

1.7.27 and 2.7.27. No change in condition.

3.7.27. In addition to the paresis of the right arm the animal showed lasting ptosis and appeared less agile.

From the 3.7.27 to the 11.7.27. the condition remained unchanged.

14.7.27. Forty-seven days after the inoculation. Weight 2,790 gms. When the monkey was in the cage the animal ran and climbed with difficulty. The paresis of the right arm was ameliorated, but there was paresis of the leg accentuated on the left side. The left arm also showed slight paralysis.

18.7.27. The left eye was closed completely, due to ptosis of the upper eyelid (Fig. 4). The face was drawn to the right side. The paresis of the arm and leg on the left side was now easily discernible. If the animal was seated it was gently pushed it fell on to the left side. It uttered a passive cry from time to time. No lesions could be seen on the cornea conjunctiva.

20.7.27. Marked excitation was observed. The animal appeared to have hallucinations. It threw itself at imaginary objects, attempted to bite, and struggled and fell as if it was in a fit. There was nearly complete paralysis of the left side and ptosis of the left eyelid.

22.7.27. The symptoms appeared to be ameliorated. The animal was calmer and the eye could be opened partially.

25.7.27. Eye nearly completely open. Animal still aggressive, uttered cries time to time, paralysis less noticeable.
ENZOOTIC ENCEPHALO-MYELITIS

7.27. Eye appeared normal, and the condition of the monkey practically normal.

8.27. Ptosis of the right eyelid. Slight paresis of the hind quarters, which, however, was not sufficient to prevent the monkey from climbing. It became fatigued easily, however, and remained in a corner of the cage crying out from time to time.

8.27. Paralysis of the right side of the face (see Fig. 5) was observed. Animal could still run and climb.

8.27. Same condition. Weight 3,170 gms.

8.27. Paralysis of face diminished. Animal irascible.

8.27. Spasmodic contractions of muscular groups of the back and shoulders were the only signs of the animal being other than normal.

8.27. Left eye deviated to the internal canthus. Tendency to remain hunched up.

8.27. Animal decidedly ill. Head hanging over on to right shoulder: internal strabismus still present and left pupil dilated. Right eye mobile. Pupil of this eye reacted to light. Nystagmus present. The animal had periods of excitement.

8.27. Monkey uttering cries with a low feeble raucous voice. Mouth opened after showing dragging to the right side. Internal strabismus on the left side, pupil on this side also dilated. Nystagmus exacerbated. Paralysis of muscles of left shoulder, head falling over on to the right shoulder.

8.27 and 21.8.27. Condition unchanged.

8.27. Until 27.8.27 animal appeared normal. On this date the animal had complete aphonia (probably paralysis of the recurrent nerve). The head hung over the right side and the monkey showed signs of cerebral disorder. Strabismus was less, face was drawn to the right side, and there was frequent spasmodic contraction of the facial muscles ('tic). The pupils were unequal in size. The animal carried out movements of mastication without the teeth coming in apposition, for this reason it fed with difficulty. Deglutition was not carried out easily, and the animal appeared to have difficulty in orientation. Taken out of the cage it could not co-ordinate its movements in the direction desired.

8.27. Weight 2,900 gms. Animal still made movements of mastication continually, and twitching of the muscles of the mouth was present. There was internal strabismus, as well as aphonia and weakness of the muscles of the neck on the left side. The monkey remained hunched up, or had periods of excitement which were increased by noises, movements, &c.

8.27. Condition unchanged.

9.27. Weight 2,820 gms. Spastic contractions of divers muscular groups were produced by noises. The tongue was drawn to the right side. The animal fed no more owing to the impossibility of swallowing. 'Tic' of the mouth persisted, also the strabismus and the inequality of the pupils.

9.27. The left eye appeared practically normal and the pupils equal in size. The animal, however, still trembled at times and bit at imaginary objects; frequent ‘tic’ was observed with aphonia. The animal, however, fed well.

9.27. The condition of the animal had not changed.

9.27. The cry appeared more normal, ocular disturbances were absent, although ‘tic’ and champing of the jaws was still observed. The head was hanged over the right shoulder, and the animal showed increased salivation.

9.27. The condition of the animal was unchanged; to a certain degree the syndrome in this monkey might be compared to that exhibited by a man affected with post-encephalitic Parkinsonism.

9.27. Same condition. Weight 3,000 gms.

9.27. Up to this date the monkey remained in a similar state, this being the 130th day since the inoculation. At this time the animal was inoculated with the virus of polio-myelitis. The result is given in the chapter dealing with immunity.
These three monkeys showed three different forms of the disease. In all three cases the incubation period was very long, but the duration of the morbid symptoms varied. Monkey 3 had an acute attack which lasted only 2 days. In Monkey 1 the evolution was slower, the time between the onset of symptoms and death being 16 days. Monkey 2 had recurrent attacks during a period of 97 days (see subsequent history, p. 80).

The virus after passage through monkeys had not lost its virulence for the rabbit or guinea-pig.

In the chapter dealing with histopathology the lesions in the nervous system will be described in full. We may, however, anticipate here by the statement that we found lesions of an intense character in the brain, and of a more discrete character in the cord. Lesions were also found in the spinal ganglia, posterior nerve-roots, and peripheral nerves. Both clinically and pathologically the disease may be described as an encephalo-myelitis complicated with a ganglion radiculitis and peripheral neuritis. The virus when introduced into the brain evidently spreads not only to the cord, but also to the peripheral nerves, for not only have we found lesions in these, but we have also been able to demonstrate the presence of virus.

DISCUSSION.

The pathogenicity of the virus for the monkey, and the clinical features presented in this animal, raise the question of the relation between enzootic encephalo-myelitis of domestic animals and polio-myelitis in man. There are marked resemblances in the clinical aspects as well as in the alterations in the cord and spinal ganglia of these two diseases. The former virus is, however, pathogenic for the rabbit, while the latter is generally considered not to be so. The incubation period of experimental encephalo-myelitis in the monkey is longer than in the disease produced by the virus of polio-myelitis.

Another question is whether the virus of encephalo-myelitis is pathogenic for man, and if such is the case, whether some human disease of the nervous system at present of unknown origin may possibly be due to it. It is a common observation in clinical medicine that exposure to cold may determine an attack of facial neuritis or sciatica. Conceivably, such attacks might be the expression of unrecognized infection by some virus, akin to enzootic encephalo-myelitis, which in the first place caused only slight, if any, general symptoms of disease and then proceeded down into the peripheral nerves, where it remained latent until the added factor of cold determined the local incidence of paralysis or pain. Similar views may be argued with regard to herpes zoster, recurrent herpes, or the so-called peripheral forms of epidemic encephalitis. An analogous action of cold was recorded by Pasteur, when certain rabbits that were resistant to the inoculation of an attenuated rabies virus, at once showed paralytic symptoms after exposure to severe cold.

The curious epidemic at Lille, studied by David and Dekass
ENZOOTIC ENCEPHALO-MYELITIS

(1926), has suggested that a certain form of sciatica, at least, is an infectious disease; and there are many human cases on record of myelitis associated with peripheral neuritis, apparently of a contagious type, in some of which, as in the instance of the child described by Péhu and Dechaume (1927), there were in the peripheral nerves inflammatory lesions closely resembling those described by us in monkeys and rabbits infected with Borna disease.

We give below a résumé of Péhu and Dechaume's case in some detail because the symptoms resemble in certain respects those which our Monkey M. 2 showed after inoculation with the virus of Borna disease. The child, 20 months old, was in perfect health up to the day when it showed some lassitude three months before going to hospital. The temperature did not exceed 36.5°C. Two months later it could no longer walk and had lost power in the arms. At the time of entering the hospital it showed flaccidity and paralysis in the lower extremities without Babinski's sign. There was also slight paresis of one arm. The following day the child collapsed although not losing consciousness. There were no cerebral symptoms, nor vomiting, neither did somnolence exist, but the pulse was rapid and irregular. The condition lasted 11 days and the child died suddenly without convulsions. The case was diagnosed as a peripheral form of epidemic encephalitis, referred to as pseudo-myelitic.

On autopsy there were no macroscopic changes. On microscopic examination of sections, discrete lesions, for the most part exudative, were found in the cord, especially in the lumbar region. Perivascular infiltrations occurred in the brain. No neuronophagia was recorded, and the spinal ganglia were not examined. The lesions found in sections of the median nerves, sciatic nerve, and posterior tibial nerves were comparable to those described by us in the sciatic and brachial nerves of rabbits and monkeys inoculated intracerebrally with the virus of Borna disease (see pages 61 and 67).

Péhu and Dechaume suggested that the presence of lesions in the peripheral nerves might be coexistent with the presence of virus. In the case of Borna disease we have proved that the presence of virus is coexistent with the existence of lesions in the peripheral nerves (see page 45).

6. PATHOGENICITY OF THE VIRUS OF ENZOOTIC ENCEPHALO-MYELITIS FOR THE GUINEA-PIG, RAT, MOUSE, AND FOWL

I. GUINEA-PIG.

The introduction of virus intracerebrally into guinea-pigs may produce the disease. The incubation period varies in individual cases and death is inconstant. Zwick and his collaborators (1926) were the first to transmit the disease to guinea-pigs, and to make passages in series from brain to brain. Some guinea-pigs proved to be resistant to infection. According to their experiments death followed infection in
from 3 weeks to 13 months. They also succeeded in infecting rabbits with the virus passaged through guinea-pigs.

In Table III we give the period of incubation and the duration of the disease in a batch of guinea-pigs which in our experiments proved to be susceptible to the virus inoculated intracranially.

### Table III

<table>
<thead>
<tr>
<th>No. of guinea-pig</th>
<th>Weight in gms.</th>
<th>Commencement of disease</th>
<th>Death.</th>
<th>Lesions in brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>85e</td>
<td>470</td>
<td>73rd day</td>
<td>83rd day</td>
<td>Intense.</td>
</tr>
<tr>
<td>84e</td>
<td>400</td>
<td>107th day</td>
<td>132nd day</td>
<td>&quot;</td>
</tr>
<tr>
<td>94e</td>
<td>500</td>
<td>23rd</td>
<td>114th day</td>
<td>&quot;</td>
</tr>
<tr>
<td>95e</td>
<td>500</td>
<td>58th</td>
<td>66th</td>
<td>&quot;</td>
</tr>
<tr>
<td>93e</td>
<td>480</td>
<td>40th</td>
<td>58th</td>
<td>&quot;</td>
</tr>
<tr>
<td>98e</td>
<td>450</td>
<td>57th</td>
<td>64th</td>
<td>&quot;</td>
</tr>
<tr>
<td>99e</td>
<td>580</td>
<td>18th</td>
<td>19th</td>
<td>&quot;</td>
</tr>
<tr>
<td>100e</td>
<td>560</td>
<td>58th</td>
<td>182nd day</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

We have also made experiments to determine the relative susceptibility of guinea-pigs to infection. Forty-five guinea-pigs of about the same size (400–600 gms.) were divided into three lots of 15.

Lot A were injected with an emulsion of the brain of a rabbit dead of Borna disease diluted 1:10.

Lot B were injected with the same emulsion diluted 1:100.

Lot C were inoculated with the emulsion diluted 1:1,000.

The results were as follows:

In Lot A all the guinea-pigs died after showing typical symptoms of the disease, 52, 58, 74, 133, 189, 139, 141, 141, 143, 148, 153, 156, and 176 days after the inoculation. Lesions characteristic of enzootic encephalo-myelitis were found in sections of the brain and spinal cord of these animals; moreover, the corpuscles of Jose Degen were demonstrated.

### Table IV

**Lot A.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>1A</td>
<td>52nd day</td>
<td>Intense lesions in central nervous system</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>58th</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>3A</td>
<td>74th</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>4A</td>
<td>133rd</td>
<td>Very intense lesion in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>5A</td>
<td>134th</td>
<td>Discrete lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>6A</td>
<td>139th</td>
<td>Intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>7A</td>
<td>141st</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>8A</td>
<td>141st</td>
<td>Mild lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>9A</td>
<td>143rd</td>
<td>Intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>10A</td>
<td>148th</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>11A</td>
<td>150th</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>12A</td>
<td>153rd</td>
<td>Very intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>13A</td>
<td>156th</td>
<td>Intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>14A</td>
<td>176th</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>15A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ENZOOTIC ENCEPHALO-MYELITIS

In Lot B two of the guinea-pigs succumbed to an intercurrent infection. The thirteen others died 58, 105, 130, 130, 135, 137, 138, 140, 140, 147, 149, and 153 days after infection.

**TABLE V.**

<table>
<thead>
<tr>
<th>Dilution of emulsion of brain inoculated</th>
<th>No. of guinea-pig</th>
<th>Animal died</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:100</td>
<td>1b</td>
<td>58th day</td>
<td>Intense lesions in central nervous system.</td>
</tr>
<tr>
<td></td>
<td>2b</td>
<td>105th day</td>
<td>Slight lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>3b</td>
<td>130th day</td>
<td>Very intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>4b</td>
<td>130th day</td>
<td>Intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>5b</td>
<td>135th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>6b</td>
<td>137th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>7b</td>
<td>138th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>8b</td>
<td>140th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>9b</td>
<td>140th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>10b</td>
<td>140th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>11b</td>
<td>147th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>12b</td>
<td>149th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>13b</td>
<td>153rd day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>14b</td>
<td>5th day</td>
<td>Accidental death.</td>
</tr>
<tr>
<td></td>
<td>15b</td>
<td>12th day</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

In Lot C five of the animals died of an intercurrent infection, the ten remaining died 70, 90, 96, 101, 140, 141, 141, 147, 149, and 150 days after intracerebral inoculation.

**TABLE VI.**

<table>
<thead>
<tr>
<th>Dilution of emulsion of brain inoculated</th>
<th>No. of guinea-pig</th>
<th>Animal died</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1,000</td>
<td>1c</td>
<td>70th day</td>
<td>Discrete lesions in central nervous system.</td>
</tr>
<tr>
<td></td>
<td>2c</td>
<td>90th day</td>
<td>Intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>3c</td>
<td>96th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>4c</td>
<td>101st day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>5c</td>
<td>140th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>6c</td>
<td>141st day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>7c</td>
<td>141st day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>8c</td>
<td>147th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>9c</td>
<td>149th day</td>
<td>Very intense lesions in C.N.S.</td>
</tr>
<tr>
<td></td>
<td>10c</td>
<td>160th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>11c</td>
<td>5th day</td>
<td>Accidental death.</td>
</tr>
<tr>
<td></td>
<td>12c</td>
<td>8th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>13c</td>
<td>8th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>14c</td>
<td>8th day</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>15c</td>
<td>11th day</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

As in the case of the guinea-pigs of Lot A, the symptoms in guinea-pigs of Lot B and C were characteristic, and sections made from the
central nervous system showed the typical changes produced by the virus of Borna disease in other animals.

These results, while demonstrating the variation in individual susceptibility, also point to the fact that resistance to infection in the guinea-pig is not so marked as it appeared to be from the results obtained by Zwick, since of thirty-eight guinea-pigs inoculated in our experiments thirty-eight succumbed to the disease (the seven guinea-pigs dead from other causes are not included).

The virus passed through guinea-pigs still preserved its pathogenicity for the rabbit.

The disease in the guinea-pig is similar to that of the rabbit. After a variable period the animal appears depressed, there is marked somnolence, and abstention from food. Characteristic nervous symptoms follow, these indicating affection of the cord being especially well marked. The syndrome is as described in the rabbit. The hind legs become paralysed (Fig. 6) and the fore legs are involved later (Fig. 7). The loss of weight is less marked than in the case of the rabbit.

From the four following experiments the susceptibility of the guinea-pig after the virus is inoculated would appear to be diminished by a simultaneous inoculation of the same material intramuscularly.

An emulsion of virulent brain originating from a rabbit dead of experimental Borna disease was inoculated into the brain of eight guinea-pigs, and at the same time 1 c.c.m. of the same emulsion was inoculated into the quadriceps group of muscles of four of them. The results of these four experiments are tabulated below:

<table>
<thead>
<tr>
<th>Table VII.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>I. Inoculated into the brain</td>
</tr>
<tr>
<td>Inoculated into the brain and muscle</td>
</tr>
<tr>
<td>II. Inoculated into the brain</td>
</tr>
<tr>
<td>Inoculated into the brain and muscle</td>
</tr>
<tr>
<td>III. Inoculated into the brain</td>
</tr>
<tr>
<td>Inoculated into the brain and muscle</td>
</tr>
<tr>
<td>IV. Inoculated into the brain</td>
</tr>
<tr>
<td>Inoculated into the brain and muscle</td>
</tr>
</tbody>
</table>

This observation is comparable to that of Ernst and Hahn (1927) who found that when rabbits inoculated intracerebrally with virulent emulsion received, either at the same time or subsequently injections of virus into the veins, they did not develop a fatal encephalitis.
Attempts at infecting the guinea-pig by intradermal inoculation of an emulsion of virulent brain into the metatarsal pad (following the technique used by Waldmann and Pape (1921) in foot-and-mouth disease, and by Gildemeister and Herzberg (1925) in experimental herpes) did not succeed. The guinea-pigs inoculated varied in weight from 100 gms. to 750 gms., and were kept under observation for over seven months, but no symptoms were seen at any time during this period.

II. Rat.

Zwick, Seifried, and Witte (1926) infected rats with the virus of Borna disease by intracerebral inoculation. Death supervened 40, 53, and 62 days respectively, after infection. Some rats showed no symptoms and survived. The virus passaged through the rat had not lost its pathogenicity for the rabbit.

In our experiments large rats appeared to be more susceptible to the disease than young animals. Four rats (three old and one young) were inoculated intracerebrally with an emulsion of the brain of rat No. 1 which died 67 days after infection. (Typical lesions of Borna disease were found in sections of the brain of rat No. 1.) The three large rats died 22, 37, and 74 days respectively after inoculation. They all developed typical symptoms, and sections of the brain showed the presence of characteristic lesions microscopically. The young rats kept under observation for six and a half months remained perfectly normal.

The control rabbit inoculated with the same emulsion of brain from Rat 1 died on the 27th day of a typical infection.

Subsequently four rats (two large and two small) were inoculated with an emulsion of one of the brains of one of the large rats mentioned above (that dead on the 37th day). The two older rats died on the 40th and 82nd day after infection, while the two younger animals survived 124 days, succumbing later to an intercurrent infection. In all, we have inoculated twenty-eight rats; of these only the older rats contracted the disease.

The symptoms in the rat are similar to those in the guinea-pig. They commence with motor disturbances, inco-ordination, and difficulty in maintaining equilibrium. Paralysis, coma, cachexia, and death follow later.

Up to the time of writing we have succeeded in making at least four passages in this species. The rats in the series died 67, 37, 82, and 47 days respectively, after inoculation, showing that the course of the disease in the rat is as variable as in the guinea-pig. It would appear that virus passaged through rats when inoculated intracerebrally into rabbits produced the disease after a shorter incubation period than when the virus was passaged in series through rabbits.

In sections of the brain of our experimental rats the corpuscles of Joest-Degen were found. Zwick (1926), however, failed to find them in the brain of rats inoculated with the virus of Borna disease.
III. Mouse.

We have been able to infect mice by the intracerebral route, but this species of rodent is apparently less susceptible to the infection. As in the rat, age appears to have an important bearing on the susceptibility of the mouse to the disease. Mice weighing more than 20 gms. generally contracted the disease and died, while smaller mice survived without showing symptoms. In our experiments mice died on the 37th, 52nd, 81st, and 126th day respectively, after inoculation.

These mice wasted considerably, walked with tortoise-like movements, and showed other motor disturbances. Typical lesions were demonstrated in the brains of the mice and the intranuclear 'inclusions' of Joest-Degen were present in the Ammon's horn.

IV. Fowl.

Zwick, Seifried, and Witte (1926) found the fowl to be susceptible to intracerebral inoculation. In one case the incubation period was 37 days, and death followed 15 days later. Passage from fowl to rabbit gave a positive result.

7. ANIMALS WHICH HAVE BEEN FOUND TO BE RESISTANT TO INFECTION WITH THE VIRUS OF ENZOOTIC ECEPHALO-MYELITIS

I. Dog.

According to Zwick, Seifried, and Witte (1926) the dog appears to be resistant to infection with the virus of Borna disease. This fact obviates up to a certain point confusion with the virus of rabies. A greater number of experiments require to be done, however, before the dog can be definitely classed among the animals resistant to infection.

II. Pigeon.

These same authors demonstrated that the pigeon is resistant to intracerebral infection with the virus.

III. Ferret.

We inoculated six ferrets, three young and three adults, by the intracerebral route and kept them under observation for seven months, but no morbid symptoms developed.

8. DISTRIBUTION OF THE VIRUS OF BORNA DISEASE IN THE ANIMAL BODY

I. Passage of Virus through the Placenta.

Ernst and Hahn (1927) showed that the virus is capable of passing the placenta of the mare and infecting the foetus during intrauterine life. In two cases the virus was demonstrated by inoculation
of rabbits intracerebrally with the brain of foals born of mothers ill with enzootic encephalo-myelitis. Further, they demonstrated lesions characteristic of Borna in the sections of the brain of both the foals and the mothers in these cases.

II. DISTRIBUTION OF THE VIRUS IN VARIOUS ORGANS AND TISSUES

Zwick, Seifried, and Witte (1926) tested four samples of blood from infected rabbits, three samples of blood, two of spleen, two of kidney, and two of liver, from horses ill from Borna disease, but failed to find the virus. Ernst and Hahn (1927), on the other hand, proved the blood to contain the virus during some stages of the illness of a rabbit suffering from Borna disease.

Similar apparently contradictory results have been obtained in experimental infections produced by other filterable viruses where the virus may sometimes be found in the blood, e.g. rabies, vaccinia, herpes, and foot-and-mouth disease.

Ernst and Hahn (1927) found the vitreous body of the eye infective after a rabbit had been inoculated intracerebrally. The virus has also been demonstrated by Zwick and his collaborators (1926) in the submaxillary salivary gland in inoculated rabbits.

III. PRESENCE OF VIRUS IN THE PERIPHERAL NERVES OF RABBITS INOCULATED INTRACEREBRALLY.

The present writers have demonstrated the virus in the peripheral nerves of rabbits infected by the intracerebral route in which infiltrating lesions in the nerve occurred.

**Experiment 1.** A portion of both sciatic nerves taken from 1 cm. below their emergence from the greater sciatic foramen to the popliteal region was removed aseptically from Rabbit 130A, which died 50 days after intracerebral inoculation. An emulsion of these two portions of sciatic nerve was made in physiological saline and inoculated into the brain of Rabbits 213A and 214A.

**Rabbit 213A.**

Weight 2,000 gms.

14.7.27. Intracerebral inoculation with emulsion of sciatic nerve.
1.8.27. Animal normal. 2,000 gms.
7.8.27. Animal normal.
15.8.27. Commencement of paresis of the hind quarters. 2,000 gms.
24.8.27. Typical symptoms of the disease. 1,600 gms.
29.8.27. Animal very ill. 1,350 gms.
29.8.27. Died the 46th day after the inoculation.

**Autopsy.** All organs macroscopically normal. Cultures from the brain negative.

**Sections.** Intense lesions of a characteristic type in the central nervous system. The corpuscles of Joest-Degen were also demonstrated.
BORNA DISEASE AND

Rabbit 214A.

Weight 2,100 gms.

14.7.27. Intracerebral inoculation with an emulsion of the sciatic nerve.
1.8.27. Normal. 2,250 gms.
7.8.27. Animal normal.
15.8.27. Typical symptoms of the disease. Head depressed; placed on its side the animal showed the characteristic myelitic syndrome. 1,800 gms.
20.8.27. Animal very ill. 1,600 gms.
22.8.27. Found dead 39 days after the inoculation.

Autopsy. No lesions in organs. Cultures from the brain negative.

Sections. Intense lesions characteristic of Borna disease were demonstrated throughout the central nervous system.

Passage. The brain of this rabbit was passaged to Rabbit 291A.

Rabbit 291A.

24.8.27. Intracerebral inoculation with an emulsion of the brain of Rabbit 214A.
15.9.27. Typical symptoms of the disease. 1,590 gms.
21.9.27. Found dead the 28th day. 1,150 gms.

Characteristic intense lesions were found in the central nervous system, and the corpuscles of Joest-Degen were demonstrated.

The two rabbits died after showing typical symptoms of the disease. Lesions of a characteristic type were demonstrated throughout their central nervous system, and moreover, the virus was demonstrated in their brain.

Experiment 2. In a second experiment the virus was sought for in the brachial nerve of a rabbit which had succumbed 31 days after inoculation into the brain. The nerves were removed aseptically and emulsified in sterile mortars. The emulsions were then inoculated intracerebrally into rabbits. The results of the inoculation are given below.

(1) Brachial Nerve.

Rabbit 36A. Weight 2,870 gms.
15.9.27. Inoculated intracerebrally with an emulsion of the brachial nerve of Rabbit 252A.
22.9.27. No symptoms. 2,850 gms.
28.9.27. Animal normal. 2,700 gms.
6.10.27. Animal normal. 2,620 gms.
10.10.27. Commencement of paresis. 2,350 gms.
12.10.27. Typical symptoms of the disease. 2,150 gms.
14.10.27. Animal died 36th day after inoculation. 1,800 gms.

Autopsy. All organs appeared normal.

Cultures of the brain. Negative.

Microscopic examination of sections of the brain and other parts of the central nervous system showed the presence of typical lesions.

(2) Sciatic Nerve.

Rabbit 35A. Weight 1,200 gms.
15.9.27. Inoculated intracerebrally with an emulsion of the sciatic nerve of Rabbit 252A.
22.9.27. Animal normal. 1,050 gms.
28.9.27. Animal normal. 1,020 gms.
6.10.27. Animal normal. 1,100 gms.
ENZOOTIC ENCEPHALO-MYELITIS

10.10.27. Commencement of paresis. 1,150 gms.
12.10.27. Typical symptoms of the disease. 1,050 gms.
14.10.27. Typical symptoms of the disease. 960 gms.
15.10.27. Found dead 37 days after infection.

Autopsy. All organs normal.

Cultures of the brain. Negative.

Typical lesions were found on microscopical examination of sections from the central nervous system.

This second experiment shows that when the virus is introduced into the animal organism by the intracerebral route, it may subsequently be found in the brachial as well as the sciatic nerves.

In a third experiment Rabbits 27A and 30A were inoculated intracerebrally with an emulsion of the sciatic nerve taken as in experiment 1.

Rabbit 27A.

Rabbit 27A. Weight 1,690 gms.

6.9.27. Date of inoculation.
15.9.27. Animal normal. 1,750 gms.
22.9.27. Animal normal. 1,800 gms.
28.9.27. Animal normal. 1,870 gms.
6.10.27. Animal normal. 1,880 gms.
10.10.27. Commencement of the disease. 1,850 gms.
12.10.27. Typical symptoms of the disease. 1,700 gms.
15.10.27. Animal in agonal stage of death. 1,450 gms. Found dead later, 39th day after infection.

Autopsy. No macroscopic lesions.

Rabbit 30A.

Rabbit 30A. Weight 1,750 gms.

6.9.27. Date of inoculation.
15.9.27. Animal normal. 1,750 gms.
22.9.27. Animal normal. 1,820 gms.
28.9.27. Animal normal. 1,800 gms.
6.10.27. Animal normal. 2,000 gms.
10.10.27. Slight paresis of hind quarters. 2,050 gms.
12.10.27. Slight paresis of hind quarters. 2,000 gms.
21.10.27. Typical symptoms of the disease present. 1,900 gms.

All these eight rabbits inoculated intracerebrally with emulsions of either the brachial nerve or sciatic nerve of rabbits dead of experimental enzootic encephalitis contracted the disease and died. The presence of lesions, and of the corpuscles of Joest-Degen in the central nervous system of these eight rabbits, as well as the positive passage made with the brain of one of them, indicates that the virus of Borna disease generalizes into the peripheral nervous system centrifugally. The lesions occurring in the peripheral nerves are described later (see p. 61).
IV. DISTRIBUTION OF THE VIRUS IN VARIOUS ORGANS AND TISSUES OF THE MONKEY.

We have studied the distribution of the virus in the animal organism of Monkey M. 1 (Macacus rhesus) (see p. 34) which died of Borna disease 73 days after infection.

Experiment 1. Two rabbits were inoculated intracerebrally with emulsions of the cerebrum, medulla oblongata, spinal cord (dorsal), parotid, spleen, testicle, blood, and adrenal glands. The results are set forth in Table VIII.

**Table VIII.**

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of rabbit</th>
<th>Weight in gms.</th>
<th>First appearance of disease</th>
<th>Death</th>
<th>Lesions</th>
<th>Parasymp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum</td>
<td>115A</td>
<td>2,100</td>
<td>37th day</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>126A</td>
<td>2,200</td>
<td>30th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medulla oblongata</td>
<td>117A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>123A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal cord</td>
<td>109A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>124A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parotid gland</td>
<td>121A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>122A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>125A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>127A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicle</td>
<td>131A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>132A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>119A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>133A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Blood (defibrinated)</td>
<td>120A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>122A</td>
<td>1,640</td>
<td>20th day</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The virus was demonstrated in the brain, medulla oblongata, spinal cord, parotid, and adrenal glands; it was not demonstrated in the spleen, testicle, or blood.

The observations were repeated with the organs of Monkey M (see p. 36), and the results are given in Table IX.
In this monkey the virus was found in the brain and spinal cord only, and could not be demonstrated in the ovary, spleen, bone marrow, liver, kidney, the blood, the lung, the mesenteric glands, adrenal glands, or parotid gland.

9. ELIMINATION OF THE VIRUS FROM THE ANIMAL ORGANISM

As is the case with the viruses of herpes, rabies, &c., the pathogenic agent of enzootic encephalo-myelitis is eliminated by the saliva and nasal secretions. The results of the German school (Zwick, Seifried, and Witte, 1926; Ernst and Hahn, 1926) are in agreement with regard to this point. The urine of animals ill from the disease has always proved avirulent.
10. HISTOPATHOLOGY OF BORNA DISEASE

I. Horse.

Siedamgrotzky and Schlegel (1896) described the disease as 'serous leptomeningitis'. Both Johe (1896) and Ostertag (1900 seq.) failed to find lesions in the brain or its coverings, and considered the disease to be an intoxication of the central nervous system by bacterial toxins. Dexler (1900) refers to Borna disease as a disseminated encephalo-myelitis with leucocytic infiltration around blood vessels and in the nerve substance. Oppenheim (1907) considered it to be an acute localized meningo-encephalitis of a non-purulent nature, the meninges being more especially affected. Joest and Degen (1909), who made a more detailed study of the histopathology of Borna disease of the horse, regarded it as an acute meningo-encephalitis, non-purulent in character, with perivascular infiltration by lymphocytes in the cord and brain. Cellular 'inclusions' were as found in the ganglion cells of the Ammon's horn, in the hippocampus and sometimes also in other regions of the brain. These 'inclusions' they described were within the nucleus, round in shape, and arranged sometimes in pairs. They stain red with Mann's or Lentz's stain, and have often an unstained halo around them. Their dimensions vary from the limit of visibility to the size of a nucleolus. Heydt (1919) was able to confirm the findings of Joest and Degen in every detail. Moussu and Marchand (1924) (see also thesis of Moussu, 1926) did not find the intranuclear corpuses described by Joest and Degen.

The cases investigated by Moussu and Marchand were of an acute haemorrhagic type and polymorphonuclear leucocytes were found in the lesions in large numbers. They described an agglomeration of infiltrating mononuclear cells surrounding the nerve-cells in the brain producing in certain cases deformation of these cells by compression of the cell membrane. These authors did not record actual neuronophagia.

Zwick, Seifried, and Witte (1924), Beek and Frohböse (1926), and Ernst and Hahn (1927) confirmed the work of Joest and Degen (1909). Zwick and his collaborators emphasize the fact that the German workers have never found degenerative lesions of the neurons or neuronophagia in any region of the nervous system where they were searched for.

II. Cattle.

The similarity of the disease in cattle and horses, as indicated by a study of the lesions in the central nervous system with the presence of virus in these lesions, has been mentioned by Ernst and Hahn (1927). They demonstrated the corpuses of Joest-Degen in the brain of cattle dead of the disease. A detailed description of the lesion has also been given in the thesis of Moussu (1926). He states that the process in the central nervous system in enzootic encephalomyelitis of cattle consists of a diffuse polio-encephalitis with a dominance of lesions in the cerebral cortex, of which the deeper layers...
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are more particularly affected. There are similar lesions in the basal ganglia of the cerebral hemisphere, in the peduncle, in the medulla oblongata, and even in the spinal cord; but these lesions are always less marked than those in the cerebral cortex. This encephalitis is accompanied sometimes by perivascular lesions and diffuse capillary hemorrhages. It is easy to understand that these lesions may lead to rapid death when affecting the medulla oblongata.

III. SHEEP.

Priemer (1925), Beck (1925), Moussu (1926), Miessner (1926), and Ernst and Hahn (1927) made a study of the lesions in the central nervous system in sheep dead of the disease. They came to the conclusion that the tissue changes were analogous to those produced by the virus of the equine type. Beck was the first to demonstrate the intranuclear corpuscles of Joest-Degen in the nerve-cells of the brain of sheep dying from the spontaneous disease.

No observer has described any departure from the normal in the histology of other organs either in sheep, horses, or cattle.

IV. EXPERIMENTAL BORNA DISEASE IN HORSES AND SHEEP.

The lesions described in the nervous system of horses and sheep infected experimentally are identical with those found in the spontaneous disease.

V. RABBITS INFECTED EXPERIMENTALLY.

The first description of lesions in the central nervous system in rabbits infected with the virus of encephalo-myelitis was given by Moussu and Marchand (1924). These authors were apparently working with a virus which differed from those isolated by workers in Germany; and, while this difference may possibly be accounted for by an increased virulence, their description of the lesions has remained unique and unconfirmed up to the present. It is as follows:

In rabbits which die soon after the inoculation, the alterations are those of an acute meningo-encephalitis with a predominance of lesions in the pia mater. The meninges are infiltrated with immature cells of which a large number contain eosinophile granules. These same cells can be found around intracerebral vessels (cortical or subcortical). There are alterations of the choroid plexus, and epithelium of the ventricles; there is infiltration of the subependymal zone and the pyramidal zones are much altered. The lesions of the cerebellum are identical with those of the brain, while the alterations in the vessels of this region are also as intense as in the meninges.

No bacteria were found, nor intranuclear inclusions. In animals which died at a later stage the lesions were less intense. The lesions predominate in the anterior region of the brain and are those of a subacute encephalo-myelitis. The inflammatory lesions of the meninges are only observed in the septum and in the spaces between the convolutions. The same may be said of perivascular infiltrations.
Embryonic cells ("cellules embryonnaires") are present, containing eosinophile granules. There is an inflammation of the ependyma with subependymal lesions.

In the olfactory lobe one finds small inflammatory areas. The cellular lesions are well marked, but less intense than in the preceding form. In the cerebellum one finds several areas of periarteritis situated in the white matter. No bacteria or inclusions can be found.¹

Zwick, Seifried, and Witte (1926), like Beck and Frohböse, Ernst and Hahn (1927), and Miessner (1927), who have been interested in passing, in the histopathology of the central nervous system of rabbits infected experimentally, devote only a few lines to the question. In general, the summary of their findings may be given as follows: Macroscopically, apart from the brain and cord, which appeared to be hyperaemic, all the organs preserved their normal aspect. A microscopic study of the lesions in the brain revealed slight meningitis with mononuclear cells; more or less infiltration in the cerebral cortex and the Ammon's horn with lymphocytes; perivascular infiltrations, especially in the small and middle-size vessels and the presence of intranuclear corpuscles in the large ganglion cells of the Ammon's horn. Zwick and his collaborators, as well as other investigators who have studied the disease in Germany, have never observed degenerative processes in the nerve-cells nor recorded neuronophagia. Zwick found perivascular infiltrations in the spinal cord in cases which had paresis or paralysis.

The summary given above records briefly the observations made by other workers whose attention has been directed particularly to the brain, while the participation of the spinal cord in the pathological process has been referred to only exceptionally. There is published work on lesions of the nerve-roots, spinal ganglia, and peripheral nerves.

VI. Authors' Observations.

A. Rabbit.

(1) Macroscopic and Microscopic Findings in Diverse Organs.

Our observations have been made on animals infected either with the strain of virus originating from horses (Zwick) or that originating from sheep (Miessner). On post-mortem examination in the major cases, a congestion of the meninges which may sometimes be intense is found. In other cases the aspect of the nervous system may be normal.

Sometimes the stomach presents the lenticular haemorrhages described by Ernst and Hahn (1927) in the lenticular formations of rabbits dead of Borna disease. We have found these lenticular formations (see Fig. 9) in the majority of cases in which rabbits had a prolonged paralytic phase and a long agonal stage. Under the microscope they appeared to be hemorrhages following upon an autodigestion of the stomach mucosa. They did not appear to have any definite structure, nor are they specific for experimental enzootic encephalomyelitis, since we have found them also in herpetic encephalitis and other morbid conditions.

¹ Ernst and Hahn (1927) described haemorrhagic lenticular formations in the mucous membrane of the stomach of rabbits dead of Borna disease. We have found these lenticular formations (see Fig. 9) in the majority of cases in which rabbits had a prolonged paralytic phase and a long agonal stage. Under the microscope they appeared to be hemorrhages following upon an autodigestion of the stomach mucosa. They did not appear to have any definite structure, nor are they specific for experimental enzootic encephalomyelitis, since we have found them also in herpetic encephalitis and other morbid conditions.
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ferred to above, but we have found that these are not a specific reaction to the virus (Fig. 9). In the larger number of cases examined in detail (more than thirty rabbits) the kidneys showed a marked congestion. This hyperaemia was not limited to the cortical zone, but affected the medullary zone to the same extent. Sections from these kidneys showed that there were small multiple haemorrhages in the region of the glomerulus, as well as in the collecting and convoluted tubules. These extravasations formed sometimes actual haemorrhagic areas. The epithelium lining the renal tubules was normal, there were no infiltrative processes peri- or intratubular. The condition may be described as renal congestion; not a true nephritis.

On microscopic examination the parotid showed occasionally small areas of infiltration composed of lymphocytic elements surrounding certain of the striated canaliculi; at the same time the cytoplasm of certain of the cells of the acini had become oxyphilic when stained by Mann, while the nucleus appeared oedematous and took up an abnormal eccentric position in the cells. Although many sections of the parotid of rabbits were examined, these lesions were found only occasionally. Without presuming that they were produced by the action of the virus, it should be mentioned that they coincided with the presence of virus in this organ. In rabies and distemper, oxyphilic corpuscles, intra- or extra-cellular, staining red by Mann's method, have been described as concomitant with the presence of virus in the parotid. No actual corpuscles have been found by us in the parotid of animals infected with the virus of enzootic encephalomyelitis.

In the medullary zone of the adrenal small accumulations of lymphocytic elements were occasionally seen. The lung, the liver, the spleen, the testicle, and the ovary appear macro- and microscopically normal.

Characteristic lesions of an intense nature are found only in the nervous system. We may class these in two categories, (1) infiltrative and (2) degenerative. Both types of lesions may be met with in the brain, mesencephalon, cerebellum, spinal cord, and spinal ganglia, in the nerve-roots and peripheral nerves (sciatic and brachial nerves examined) only infiltrative lesions have been found.

(2) Lesions in the Central Nervous System.

(a) The Brain. The pia mater is infiltrated with mononuclear leucocytes, varying in individual cases. In some areas only a trace of this infiltrative process may be seen, while in others three or four layers of infiltrative cells occur. They are especially marked in the region of the meningeal vessels as well as in the spaces between the convolutions, and may form actual meningeal plaques. The infiltrating elements are lymphocytes, plasma cells, and large mononuclears. Blood vessels of the pia mater are often surrounded by 'cuffs' constituted by mononuclear leucocytes (Fig. 10). The most intense lesions of the meninges are generally found at the base of the brain. In sections
cut at right angles to the surface of the brain vessels passing from the meninges into the cortical substance surrounded by lymphocytic 'cuffing' have often the aspect of septa (Fig. 11). In the cortex diffuse infiltration of lymphocytes accompanied by proliferation and mobilization of the neuroglial cells occurs. Especially in the hippocampus (the so-called 'elective zone' in herpetic encephalitis of the rabbit, the neuron degenerates, the nucleus swells, the chromatin becomes rarefied, collects towards the periphery of the nucleus, and in place appear small oxyphilic globules which may be at the limit of visibility or may reach the size of a nucleolus. Some of the neuroglial cells of this region appear to undergo the same degenerative process. It is similar to, although less intense than that described by Levaditi, Harvier, and Nicolau (1922) in experimental herpetic encephalitis in the rabbit, leading to the formation of encephalitic neuroglial corpuscles, and is not of a specific nature. The oxyphilic degeneration of the nucleus may lead to the formation of larger corpuscles surrounded by a halo, morphologically identical with those described as specific in Borna disease by Joest and Degen (1909). The nuclei may react in the same way to other causes. Even the halo is wanting in the figures given by Levaditi, Harvier, and Nicolau. We regard this phenomenon as possibly the result of the action of a karyotropic virus, the degenerated karyoplasm fusing round the pathogenic agent. These nuclear lesions may be found in all regions of the brain, although the German writers have described their presence only in the Coruus Ammonis, in which, indeed, they are more constantly found. They may be single or in pairs surrounded or not by a characteristic halo. When sections are stained with Mann's stain or toluidin blue-eosin, the intranuclear corpuscles are stained rose-red, while the nucleolus is more of a violet tint. They occur about the pyramidal cells of the cerebral cortex and even in the neuroglial cells (large granulo-adipose cells of the hippocampus).

In the Ammon's horn newly formed capillaries are sometimes seen. The vessels of this region appear dilated, gorged with blood, and the adventitia infiltrated with several layers of mononuclear leucocytes. Plasma cells are abundant in the process of perivasal infiltration. We have never found such a large number in rabies of the dog or rabbit, in poliomyelitis of monkeys, in human encephalitis, in chronic herpetic encephalitis of rabbits, or in the vascular lesions produced by the presence of the so-called 'Encephalitozoon cuniculi'. Such lymphocytes may also be found in large numbers in these perivascular infiltrations, but large mononuclears are also present to a lesser extent. The presence of polymorphonuclear leucocytes is exceptional.

Sometimes in the thickness of the 'cuffing' are degenerated lymphocytes or plasma cells. The nucleus of these degenerated cells become intensely oxyphilic. It is reduced in size and condensed 'blocks' without any definite structure. When Mann's stain is used the protoplasm, in the case of the degenerated plasma cells, stains rose; the unchanged elements stain blue. Here and there in the
Pathological spontaneous animals. We pies, kits ver interior of generated, fining it ele devices. First; generation, n I. m

rn bred membrane, out expression. 1. We y, y tween vin's iule isss of etly oocytes. 1

They have occasionally plasma cells, the nucleus of which had undergone generation, which in its appearance recalled this intracellular cyst; the karyoplasm was condensed into several small round intensely chromatophilic granules, apparently attached at equal distances to the nuclear membrane. The presence of a protoplasmic membrane around this formation removed from our minds the supposition that we were dealing with a 'microsporidian cyst', and showed distinctly that it was a degenerated infiltrating cell (Pl. I, Fig. 3). The staining reactions indicated that the cystic formations were the generated nuclei of the plasma cells which had penetrated into the interior of the large cells of the Ammon's horn. We have found such cysts in approximately 5 per cent. of cases examined. They have never been met with in the brain of normal rabbits, or in the brain of rabbits which have succumbed to infection with the viruses of herpes, encephalitis, or vaccinia.

We have described these formations in detail, since the elucidation of their nature required extended observations. Many control animals were examined to ensure that we were not dealing with a spontaneous 'microsporidian' disease of the rabbit.

Pathological changes other than the above are found more con-
stantly in the Ammon’s horn, especially in cases showing intense infiltrative lesions. For instance, a number of the nerve-cells may show degenerative changes characterized by the following appearances. The nucleus appears oedematous, the chromatin is fragmented while the protoplasm shows more or less advanced tigrolysis. The protoplasm also contains vacuoles and the cellular membrane is denticulated. In some cells, also, of the Ammon’s horn, the nuclei and sometimes too the protoplasm, becomes oxyphilic. The fusion of cells which are found in the upper part of the row of large ganglion cells show intense nuclear degeneration; the karyoplasm is condensed in a ‘block’ and stains red with Mann’s stain. When a preparation so stained was decolorized gradually, and examined after each stage of the process until these degenerated nuclei became a parase colour, it was found that they had no definite structure, but consisted of a round mass of homogeneous condensed chromatin much smaller in size than the unchanged nuclei. This type of nuclear degeneration has also been met with in the mesencephalon (Pl. III Fig. 4).

We have already referred above to the fact that oxyphilic corpuscles which may or may not be surrounded by halos are found in the nucleus of some of the cells of the Ammon’s horn. They can be distinguished from the nucleolus by their different staining reactions (Pl. I, Fig. 2). These intranuclear corpuscles—the specific ‘inclusions’ of the German workers—may be single or in twos or threes varying in size. Sometimes they may be at the limits of visibility or they may be as large as 2 µ or 3 µ. These corpuscles occur elsewhere. They may be found in the cytoplasm of the cell and possibly expelled intra vitam, but as it is possible for the nucleolus a cell to be dislodged by the microtome knife, the same factor might carry the intranuclear corpuscle into the cytoplasm.

Our opinion is that the intranuclear ‘inclusion’ in Borna disease possibly a reaction of the karyoplasm against the pathological agent which penetrates the interior of the nucleus. Possibly the chromatin masses around the infective virus elements. This is suggested by the staining reactions, since the condensed mass of chromatin which forms the corpuscle undergoes degeneration from the centre towards the periphery (the centre appears oxyphilic and the periphery basophil in certain corpuscles).

Around the lateral ventricles well-marked infiltrations are found these being in some cases very intense. The choroid plexus is infiltrated. The epithelium of the ventricle and of the ependyma unchanged.

Occasionally in the parenchyma in the region of the ventricles even in the cerebral cortex itself in the superficial areas, karyokinetic figures may be seen. Probably this karyokinesis is in mobile cells of the vascular endothelium which have penetrated into the nerve substance. In certain preparations we have seen 2, 3, or even 4 karyokinetic figures. The mitosis sometimes undergoes oxyphil degeneration.
ENZOOTIC ENCEPHALO-MYELITIS

There are lesions affecting the area above and below the ependyma also the surrounding zone: a mobilization of the neuroglial elements takes place, while at the same time lymphocytic elements spread in the immediate proximity of the neuron. Up to a certain time, this phenomenon is comparable with the 'satellitism' described by Etzkoff in senility. Certain of the nerve-cells are surrounded by 'satellite' cells and cells of infiltration, which occasionally penetrate the interior of the cell. Six, eight, and even ten times may be seen 'besieged' in one microscopic field. The cells come in immediate contact with the nerve-cell push in the cellular membrane and form 'cups' in the periphery of the cytoplasm, giving a perivascular cuffs a denticulated border. This phenomenon is more commonly met with in sections from rabbits dying within the first 20 days after inoculation. The intensity of 'satellitism', is in inverse proportion to the meningal and perivascular lesions. When the meningitis is perivascular cuffing are at a minimum 'satellitism' may represent only departure from the normal discovered in the brain.

On the examination of a large number of preparations we are led to believe that 'satellitism' is a stage which may either disappear during the evolution of the disease, resulting in a quasi-normal process or become intensified and be followed by neuronophagia. We have found neuronophagia present in sections of brain showing described 'satellitism' (Fig. 17). Our conception of the various stages of the struggle against the virus is as follows: When the nervous system is invaded by the virus the neuroglial elements and mononuclear lymphocytes are attracted to the parasitized neuron. If the neuron succeeds in freeing itself from the virus, the local reaction ceases at this stage, and the resorption of the satellite elements begins; but if the neuron dies in the struggle against the virus after ongoing intense degeneration, it is invaded by the satellite cells; the process has now reached a stage of neuronophagia. When the struggle between the neuron and the virus terminates without neuronophagia taking place the neuron survives until the meningal lesions, perivascular infiltrations, meningeal and perivascular cuffs become incompatible with life. In case the death takes place at a later stage, i.e. in from 25 to 50 days, these conclusions as to the evolution of the morbid process in the brain, formed from observations on the character and position of the lesions in a large number of rabbits dying early or late after inoculation, have received further support from the study of the histogenesis and the alterations in the central nervous system of six rabbits killed at regular intervals after inoculation, i.e. on the 5th, 10th, 15th, 20th, 25th, and 27th day, and of one which died on the 31st day. In these animals also, the microscopical examination of sections of various parts of the nervous system showed that the first modification in the central nervous system is the mobilization and proliferation of the neuroglial cells around the neuron—'satellitism', this being more pronounced in the pons and the medulla oblongata. In the process of 'satellitism' one finds not only neuroglial cells but also mononuclear
cells taking part. Later, infiltration of the Cornu Ammonis, the meninges, and the vascular tissues takes place. In the subjects of our experiments the latter process began to appear towards the 15th or 20th day after the inoculation.

The presence of polymorphonuclear cells is quite exceptional at what stage of the infection or from what site one examined sections of the central nervous system; the infiltrative lesions are constituted from the beginning by mononuclear cells.

There are notable differences between the development of the encephalitis of Borna disease in the rabbit and chronic herpes encephalitis produced experimentally in the same species. In the brain infected by herpes an acute stage is observed in which polymorphonuclear leucocytes take part, and are found in large numbers in the perivascular ‘cuffing’ as well as in the nodular lesions at the base of the brain in the region of the hippocampus. If the animal survives this acute stage and recovers what have been termed by Levedatti and Nicolai (1922) ‘lésions d’immunité’ may be found. These are small nodular or diffuse areas of parenchymatous infiltration situated in the hippocampus (the ‘zone élective’) and are produced by mononuclear cells that have replaced the polymorphonuclear leucocytes with which the inflammatory process commenced. In those rabbits which just fail to resist the disease and die in 20-30 days, the lesions are more intense, but, as in the case of the ‘lésions d’immunité’, the infiltration consists of mononuclear cells which have taken the place of the polymorphonuclear leucocytes that predominated in the acute stage of the inflammatory process. In the case of the infection of the brain of the rabbit with the virus of Borna disease, polymorphonuclear leucocytes do not play a part in the early inflammatory process; during this early stage, one observes only ‘satellitism’ of the neuron, while the infiltrative lesions are produced by mononuclear cells alone from the beginning of the process until the final stage.

(b) The Mid-brain and Medulla Oblongata. In the mesencephalon and the medulla oblongata similar infiltrative and degenerative lesions occur. ‘Cuffing’ of the vessels is frequently observed. Certain of the nerve-cells appear to be in a state of advanced tigrosis (Nissl’s granules have disappeared). Degeneration both of the nucleus and the cytoplasm is frequently found. Certain of the cells appear to have their protoplasm split up, the nucleus being peripherally swollen, and completely degenerated (Pl. I, fig. 1). Occasionally typical neuronophagia is encountered. In these regions also we have demonstrated the presence of the intranuclear corpuscles of Joest-Degen. The cells which contain them generally preserve otherwise their normal aspect; the nuclear membrane is intact, while the protoplasm is structurally unchanged and stains normally. The converse is also true; we have never found the corpuscles of Joest-Degen in cells in advanced stages of degeneration or disintegration. Negri bodies in rabies are also only found in nerve-cells which are otherwise normal.

In the mesencephalon, as mentioned above, one meets more frequently occasional polymorphonuclear leucocytes. Here and there, but only in very small numbers, were found the cells of the inflammatory process, which consist of small lymphocytic and leukocytic layers, in one case and by an infiltration in another the disease is due to the degenerative process. Several days later, these cells were found in the medulla oblongata.

In some cases, polymorphonuclear leucocytes were found in the medulla oblongata, which was remarkable since this was not the site of the cerebral lesion. In other cases, the medulla oblongata was entirely free from any inflammatory process. There was no characteristic degeneration of the nerve-cells at this level, but they were not normal, and this finding of Dr. Nicolai's is of importance.

In some cases, the medulla oblongata was infiltrated with lymphocytes and plasma cells, but the latter were not numerous. The inflammatory process in the medulla oblongata was most frequent, and the nerve-cells were often replaced by infiltrating cells, and the lymphocytes and plasma cells were not uncommon.
frequently with 'satellitism' of the neuron, which in certain cases goes so far as to constitute true neuronophagia (Fig. 18). In the meninges we may find small islands of mononuclear cells in the parenchyma without any relation to the vessels. In certain cases we found neuroglial cells (granulo-adipose cells) showing nuclear oxychromasia and occasionally small oxyphilic corpuscles within the nucleus, similar to the so-called 'encephalitis neurocorpuscles' of herpes described by Levaditi, Harvier, and Nicolau (1922).

(c) Cerebellum. In the case described the lesions in the cerebellum were much more intense than the average, since usually the alterations consisted only of slight meningitis and perivascular infiltration accompanied by occasional satellitism of the cells of Purkinje. In the septum there was a marked infiltration with mononuclear cells. Massive perivascular 'cuffing' was present, especially in the white substance between the convolutions. In the vessels themselves, which were gorged with blood, an excessive number of mononuclear cells were found. There was an intense infiltration in the granular layer, which in some cases was completely destroyed and replaced by areas formed exclusively of mononuclear cells. Here and there in the islands of lymphocytes 'basket' cells with a pale-staining degenerated protoplasm were found, their border appearing irregular. Several of the cells of Purkinje appeared to be hyperchromatic, and their nucleus was in some cases eccentric and stained by acid stains. In others the nucleus was not separated from the rest of the protoplasm since the remains of the nuclear membrane appeared to have disappeared. Other Purkinje cells were degenerated and appeared as cell shadows. In other parts the karyoplasm of certain of these cells was condensed around the nucleolus. Satellitism of the basket cells and the cells of Purkinje was noticeable, but true neuronophagia was not seen. The lesions were confined to certain areas; other parts of the cerebellum were perfectly normal.

In a case where the inoculation of the virus was made by the intratesticular route the lesions in the cord were especially well marked, and in the cerebellum the satellitism of the cells of Purkinje was occasionally so advanced as to constitute almost a true neuronophagia.

(d) The Spinal Cord. Generally it may be stated that the intensity of the lesions found in the brain or in the spinal cord corresponded with the intensity of the symptoms observed during life. In the rabbits we have examined pathological changes were always present in the cord whether symptoms of affection of this part of the central nervous system were present or not, but these were much more intense when the symptoms produced by affection of that region dominated the cerebral symptoms.

As in the case of the brain, the meninges of the cord are not as a rule greatly affected. Only in isolated cases was a severe meningitis found.

The anterior and posterior septa may be more or less infiltrated with mononuclear cells. Perivascular 'cuffing' is seen both in the
grey and white matter of the cord. In the anterior and posterior horns infiltrations with mononuclear cells may be seen.

The most intense infiltrations are found in the posterior horn while in the anterior horn degenerative lesions of the neuron are more common. The process of degeneration in the nerve-cells is the same as that in other regions of the central nervous system: tigroid degeneration occurs, nuclear oxychromasia exists, while the whole cell shows marked hyperchromasia. Vacuolization of the cytoplasm, a degenerative process, may also be seen (Pl. II, Fig. 1), while in rare cases when the lesions as a whole have been exceptionally intense, occasional neuronophagia was recorded (Fig. 19). The phenomenon of "satellite" appears to be more commonly met with in the cord than in the brain itself. The intranuclear corpuscles of Joest-Degen found in the cord have generally been in the nerve-cells of the anterior horn (Pl. I, Fig. 5; Pl. III, Fig. 2).

Here and there small islands of lymphocytes may be found infiltrating both the white and the grey substance, these islands being unconnected with vessels. Frequently it has been observed that the zone of Lissauer is the site of a well-marked mononuclear infiltration.

The lesions found in the spinal cord are comparable with those found in poliomyelitis. The neuronophagia so characteristic in the case of monkeys infected with the virus of the latter disease (to which rabbits are generally considered not to be susceptible) is also present in the cord of rabbits infected with the virus of Borna disease, but to a less degree.

(b) The Spinal Ganglia. The most intense lesions in the peripheral nervous system have been found constantly in the spinal ganglia. The process of infiltration in the posterior nerve-roots becomes more intense as they enter the ganglion, and between the nerve-fibres which pass through the substance of the ganglion a well-marked mononuclear infiltration is seen. In the rest of the ganglion the lesions as a rule are very intense.

The alterations in the ganglion and the various elements taking part in the infiltrative and degenerative processes at this site are always the same, no matter from what individual case or from what region
of the cord (cervical, thoracic, or lumbar) the ganglion is taken
(Figs. 21, 22). The changes are as great and the lesions of the same
importance when the ganglion originates from a case showing alterations
in the cord which are scarcely discernible as from a case where
such changes are very pronounced.

The capsule of the ganglion shows neither infiltration nor degeneration.
In the interior of the ganglion mononuclear interstitial infiltration
is abundant. The small intraganglionic vessels show perivascularn
‘effusing’. The infiltrating mononuclear elements are found dis-
seminated between the nerve-cells or massed together forming actual
nodules comparable with those described by Van Gehuchten and Nelis
(1900) in rabies (Fig. 23). The mononuclear cells may be grouped
together in small islands between the nerve fasciculi which traverse
the ganglion. The ganglion cells themselves appear to be ‘choked’ by
the infiltrative process in some microscopic fields. In certain parts
these cells undergo profound changes: the nucleus becomes oxyphilic,
the protoplasm loses its granular nature, assuming a homogeneous
appearance, and becomes slightly oxyphilic when stained with
toluidin blue and eosin. We have found that the changes in the cells
of the ganglion are more marked than in any other region of the
nervous system; and the intranuclear corpuscles are larger and in
greater number here than in any other site. In some microscopic
fields the nucleus of every cell may contain one or two corpuscles of
Joest-Degen surrounded by a halo.

The most important and frequent type of lesion in the ganglion,
however, is neuronophagia. Lymphocytes, plasma cells, and large
mononuclears penetrate the peripheral zone of the neuron. One often
finds a clear zone in the protoplasm around these infiltrating cells
suggestive of the action of a proteolytic ferment liberated by the
invading cells. Later the mass of detritus of the neuron is removed
by the macrophages aided by occasional polymorphonuclearleu-
cytes. The number of infiltrating cells increases, the whole body
of the nerve-cell being invaded, and finally, in place of the neuron,
one finds nothing but a nodule formed by mononuclear cells (Figs.
23, 24, 25, and 26). The most intense lesions of both an infiltrative
and degenerative character are found in the peripheral zone of the
ganglion; this point will be discussed again later.

(c) The Peripheral Nerves. In the peripheral nerves infiltrative
lesions are also found. A detailed study has been made of lesions found
in the sciatic and brachial nerves. The technique employed in carry-
ing out this research was as follows:

All the rabbits of which the sciatic and brachial nerves were sec-
tioned for histological examination had been inoculated intracere-
brally with the virus of Borna disease. We removed the terminal
part of the cord (sacral) with the roots of the sciatic nerve and their
various ganglia together with a portion of the peripheral parts of the
nerves. This whole was fixed in Duboscq-Brasil-Bouin fluid. Longi-
tudinal sections were made after the manner figured (Fig. No. 27).

We have found lesions in all cases examined, these being more
intense towards the origin of the nerve and becoming less intense towards its termination. The alterations consist of interstitial or peripheral vascular infiltrations with mononuclear cells. The nerve-sheaths are a rule, unaffected. In certain cases the infiltrations appear to dissect the nerve filaments (Fig. 28). The whole process constitutes a descending neuritis produced by the virus propagating centrifugally.¹

Recently G. Marinesco and S. Draganescu (1927) published their observations on the pathogenic process in herpes zoster. A complete clinical report is given of cases in which the localization of the lesions in the nervous system suggested to the authors that the infection commenced by an ascending neuritis followed by a ganglio-radiculitis and myelitis. Wohlwill (1924), Levaditi (1926), Pette (1924), Faures (1924), and others advanced similar hypotheses as to the centripetal propagation of the infection. In support of their theory as to virus ascending from the peripheral nerves, Marinesco and Draganescu refer to the lesions in the corresponding ganglia: 'In the ganglion the most intense lesions were in the peripheral zone... this topography of the inflammation explains the spread of the infection by the peripheral lymph vessels, to the interior of the ganglion.'

However, from a comparison of the description of the lesions produced by the downward extension of the virus in rabbits infected with the virus of Borna disease, and those in herpes zoster, it will be seen that the histological pictures are identical. We have found lesions in the peripheral nerve not only close to the ganglion as described by Marinesco in zoster, but also in the terminal filament farthest removed from the ganglion, showing that the virus in experiments diffused by centrifugal propagation. These facts allow us to assume that the topography of the lesions is not a criterion by which to judge the portal of entry of the virus with a sufficient degree of accuracy. Comparable lesions can be produced in the central nervous system both by infection intracerebrally or inoculation of the virus into the sciatic, i.e. no matter whether the infection is ascending or descending.

This example of lesions being produced in the peripheral nervous system after introduction of the virus into the central nervous system (brain), suggests the possibility of infection being central in origin in the case also of herpes zoster. A similar pathogenic process is excluded in recurrent herpes, peripheral forms of epidemic encephalitis, and perhaps also in certain cases of sciatica.

(4) Summary and Discussion.

The inoculation of the virus of enzootic encephalo-myelitis into cerebrally into rabbits produces changes in the nervous system, which are those of a meningo-encephalo-myelitis, a ganglio-radiculitis, and a peripheral interstitial neuritis.

The lesions in the central nervous system as well as in the spin

¹ We have been able to demonstrate the presence of virus in the peripheral nerve by inoculation of their emulsions into the brain of rabbits (see p. 45).
ganglia are both infiltrative and degenerative. The menigitis and the perivascular and parenchymatous infiltrations are produced by mononuclear cells.

Pathological ‘satellitism’ of the neuron is most pronounced in the mesencephalon, medulla oblongata, and spinal ganglia, but may be found also in other regions of the cord and brain. It may in some cases be so advanced as to constitute true neuronophagia. The latter phenomenon is most common in the paravertebral ganglia.

The intranuclear corpuscles of Joest-Degen, considered by the present writers to be evidence of an attempt at defence by the nerve-cell, and referred to as specific ‘inclusions’ by other workers, may be found in the various regions of the brain, cord, and spinal ganglia. They are almost constantly present in the large ganglion cells of the Corpus Ammonis and the nerve-cells in the spinal ganglia. In our opinion the cell which reacts against the presence of the virus by the formation of intranuclear corpuscles has formed a barrier to the extension of the destructive action of the virus in ‘blocking’ the infective elements within a condensation of its chromatin. It is feasible to conceive that this process removes the virus and renders it innocuous: for this reason the cell maintains its integrity. In those cases where the cell becomes degenerated or neuronophagia takes place, one may suppose that the nucleus has been incapable of surrounding the infective particles by condensation of its chromatin and thus limiting the extension of the activity of the virus. This failure to form intranuclear corpuscles may be due to the quality of the virus (virulence), the quantity of the virus, or the deficiency in the normal resisting power of the neuron, the result being that the virus multiplies and ultimately destroys the cell. One must recall that the figure described on p. 56 supports this view as to the method of production of the corpuscles of Joest-Degen.

The infiltrations in the nerves are interstitial in character and are produced by the invading mononuclear cells arranging themselves in chains between the nerve filaments. Perivascular ‘cuffing’ also occurs.

A peripheral interstitial neuritis occurs in Borna disease after the introduction of the virus into the brain, and the authors, without excluding the possibility of ascending infections, have suggested that herpes zoster, precedent herpes, the peripheral forms of epidemic encephalitis, and perhaps also certain forms of sciatica may be the secondary manifestations of a disease, the original focus of which is in the central nervous system.

The hypothesis has already been advanced (see p. 38) that in cases of infections with these viruses central infection of the brain takes place; but the central nervous system, being able to resist the action of the virus more efficaciously, shows no manifest disturbances, while the peripheral nerves, poor in methods of defence, do not rid themselves of the infecting elements which proliferate and produce lesions.

1 The work of Head and Campbell (1900) on the pathology of herpes zoster also suggests that zona is a secondary peripheral manifestation of a disease originating in the central nervous system.
B. The Guinea-pig.

Macroscopic Examination. The brain and spinal cord appeared congested. No other organ showed pathological changes except the stomach, in which occasionally the non-specific lenticular haemorrhagic areas, similar to those described in the rabbit, were found.

Microscopic Examination. Lesions were found in the central and peripheral nervous system and in the kidney. The alterations in the stomach wall, when they existed, were comparable with those found in the rabbit, namely, autodigestion of the mucosa with small localized haemorrhage. As in the case of the rabbit, the kidneys showed marked congestion, but no actual nephritis was recorded.

Central Nervous System. The lesions found in the brain were similar to those in the rabbit, except that their intensity was less. The intranuclear corpuscles of Joest-Degen were usually found in the Cornu Ammonis and elsewhere (Pl. I, Fig. 4). Infiltrative and degenerative lesions characteristic of the disease were found (Pl. III, Fig. 3).

The lesions in the mesencephalon, cerebellum, and spinal cord were as in the rabbit. The intranuclear corpuscles of Joest-Degen were frequently present in the anterior horn of the spinal cord.

Peripheral Nervous System. Infiltrative processes with mononuclear elements were found in the nerve roots, but they were not marked as in the rabbit. The lesions in the spinal ganglia were similar in nature to those described in the rabbit, but were not acute. The lesions in the peripheral nerves were more intense in the portion nearest the ganglia.

C. The Rat and Mouse.

The organs, except the nervous system and the kidney, were macroscopically and microscopically normal. In the brain and spinal cord the lesions found were similar to those in the rabbit and the guinea-pig. The intranuclear corpuscles of Joest-Degen were found in the brain of rats and mice. Infiltrative lesions of a discrete nature were also found in the sciatic nerve of the rat.

D. The Monkey (Macacus rhesus).

Our description of the lesions found in the monkey is made for a study of sections from different parts of the nervous system of Monkey M. 1 (Macacus rhesus). The protocol of the experiment in which this animal was infected is given on p. 34. Similar lesions were found in Monkey M. 3, although the clinical picture in the case of the latter monkey was different from that of M. 1.

The macroscopic and microscopic examination of the spleen, liver, pancreas, lung, myocardium, testicle, ovary, parotid gland, and the mesenteric and inguinal lymph glands did not reveal any pathological changes in these organs; the kidneys were hyperaemic. In the adrenal gland there was slight infiltration with lymphocytes; the medullary zone, the lymphocytes being disseminated in the parenchyma.
parenchyma or grouped together in small islands. The brain appeared to be normal by naked-eye examination.

(1) Lesions in the Central Nervous System.

(a) The Brain. Frontal Lobe. Meningitis of a mild character was present in some areas, becoming intensified near those vessels in which slight perivascular infiltrations were observed. The pathological process in the meninges might be described as an ‘irritation’ rather than a true meningitis, while the perivascular ‘cuffing’ consisted of three or four layers of cells only in the walls of the vessels in contact with the brain; in the rest of the vessels the process of infiltration hardly existed. Certain of the small vessels penetrating the cerebral parenchyma from the meninges were surrounded by characteristic ‘cuffing’. There was infiltration with mononuclear cells in the septum. The lesions in the meninges, the ‘cuffing’ (Fig. 30), and the infiltration of the septum, were produced by lymphocytes, plasma cells, and macrophages exclusively. In the parenchyma, and especially in the white matter, extensive ‘cuffing’ of the vessels could be seen, consisting of ten to twenty layers of infiltrative cells. Some of the pyramidal cells appeared to be degenerated. Intense satellitism of the neuron was present in some instances, and in certain of the cells oxyphilic corpuscles surrounded by a halo of the type described by Joest and Degen were found (Pl. III, Fig. 1). The karyoplasm was rarified in the greater number of the cells containing ‘inclusions’, suggesting that the degenerated chromat in was condensed in the corpuscles. In the deeper part of the brain, both satellitism and the intranuclear corpuscles of Joest-Degen were less frequent than in the peripheral zone.

Parietal Lobe. The meninges were infiltrated with mononuclear cells (Fig. 29) which formed plaques in certain regions. There was discrete infiltration of the septum. In the brain substance, perivascular infiltrations, consisting of many layers of cells, were found forming small nodules: that these were perivascular was evidenced by the presence of a small vessel in the centre of the nodule (Fig. 31). No neuronophagia was recorded in this region of the brain, although the acute ‘satellitism’ of the neuron sometimes suggested the phenomenon. Occasional nerve-cells in a state of degeneration had eccentric nuclei and their protoplasm was undergoing tigrolysis. No actual parenchymatous infiltration could be seen, but rare mononuclear cells were dispersed in the parenchyma. A large number of nerve-cells in this region contained large oxyphilic corpuscles surrounded by halos within the nuclei.

Occipital Lobe. Meningitis was rarely observed in this region of the brain. Certain of the meningeal vessels had several layers of mononuclear cells on their walls in contact with the brain. In the septum, infiltration was not well marked, although several venules were surrounded by ‘cuffing’. Rich perivascular infiltrations were found in the parenchyma, more especially near the large pyramidal cells. Pathological ‘satellitism’ of the neuron by neuroglial elements and
occasional lymphocytes was not uncommon in the occipital lobe: these satellite cells sometimes produced marked depressions in the protoplasm of the host cell. The intranuclear corpuscles were of very much smaller dimensions and were observed less frequently than in the parietal lobe.

**Hippocampus.** The lesions in the meninges were similar to those found in the occipital lobe. The perivascular ‘cuffing’ in the parenchyma was poor in elements. There was a slight infiltration with mononuclear cells between the large and small pyramidal cells. The number of ‘inclusions’ was greater in this region than in the occipital lobe.

**Cornu Ammonis.** In this region of the brain the ‘cuffing’ around the vessels was so extensive as to suggest a nodule or pseudo-gumma: the presence of a small vessel in the centre of the nodule was discerned with difficulty. In addition, there were small groups of mononuclear cells bearing no relation to vessels. In certain cases ‘satellitism’ was so advanced that it could almost be described as neuronophagic. The large ganglion cells preserved their normal structure and nearly all contained within their nucleus well-marked corpuscles of the Joest-Degen type.

**Basal Ganglia.** Perivascular ‘cuffing’ was observed. In some of the degenerated nerve-cells the nucleus could not be differentiated from the rest of the protoplasm. Pathological ‘satellitism’ of the nerve cells was intense. Intranuclear corpuscles were found on occasion not only in the nucleus but even in the cytoplasm. Small islets of mononuclear cells were also found.

**Pons.** The process of perivascular infiltration was abundantly present in this region. Many of the nerve-cells were in an advanced stage of degeneration while the nuclei were swollen and unrecognizable as such. The protoplasm of the cells was in a state of tigrolysis and its contour was broken in many places by splitting. The cells containing intranuclear corpuscles were otherwise morphologically normal. No areas of infiltration were seen.

(b) **Cerebellum.** There was slight infiltration of the meninges at the septum with mononuclear cells: the parenchyma was not infiltrated. The layer of the small granular cells had a normal aspect. Certain cells of Purkinje were degenerated. In some of the latter cells oxyphilic corpuscles with a characteristic halo were recorded.

(c) **Medulla Oblongata.** Meningeal changes were slight. Vessels were normal without ‘cuffing’ and the parenchyma was not infiltrated. Occasional neurons showed evidence of commencing degeneration and in certain of these large oxyphilic corpuscles were present.

(d) **Spinal Cord. Cervical Region.** The meninges were normal and no ‘cuffing’ was present in them or in the grey or white matter. Slight diffuse infiltration was observed. In the anterior horns of degenerated neurons had intranuclear corpuscles (Joest-Degen type). In certain regions satellitism was a marked feature. The zone of cells of Lissauer was infiltrated with lymphocytes. The posterior
nerve-roots showed interstitial infiltration and sometimes even 'cuffing' in the vessels. The corresponding spinal ganglia showed intense interstitial infiltrations: only rare polymorphonuclear leucocytes were found. The protoplasm of certain of the cells in the ganglia had undergone tigrolysis, and in some cases also had become oxyphilic; the nucleus of these affected cells was eccentric. Neuronophagia was frequently seen and nodules of mononuclear cells comparable with those found in rabies were not uncommon. Large intranuclear corpuscles were found in nerve-cells of the ganglia which otherwise preserved their morphological integrity (Fig. 35).

Thoracic Region. No meningitis was present, nor was there any evidence of 'cuffing'. There was slight infiltration of the posterior horns and a concomitant degeneration of certain nerve-cells of the anterior horns. In the latter region also rare intranuclear corpuscles were found (Pl. II, Fig. 2). The posterior nerve-roots showed interstitial infiltration and 'cuffing' around the vessels.

Lumbar Region. No perivascular infiltrations and no meningitis could be seen. There was a slight diffuse parenchymatous infiltration with lymphocytes. Satellitism of the neuron was present, both in the anterior and the posterior horns. There were well-marked lesions in the lateral horns: the protoplasm was markedly oxyphilic and the nuclei of the cells were eccentric. Sometimes the satellite cells penetrated the protoplasm of the degenerated neurons and constituted almost true neuronophagia. Certain small nerve-cells of the anterior horn showed neuronophagia. Intranuclear corpuscles were rare. Interstitial infiltrations and 'cuffings' were seen in the posterior nerve-roots (Fig. 34).

In the spinal ganglia the lesions were of a similar nature to those encountered in the upper regions of the cord, but were more intense (Fig. 36). Neuronophagia was a constant feature. The nuclei of cells containing intranuclear corpuscles of the Joest-Degen type showed a rarefication of the karyoplasm, but no other morphological changes were present in such cells (Pl. III, Fig. 5).

Sacro-caudal Region. In the large nerve fasciculi rich infiltration with mononuclear cells could be seen, together with marked 'cuffing' around the vessels (Fig. 38).

In the posterior roots of the nerves the interstitial infiltration and 'cuffing' round the vessels was rich.

The spinal ganglia showed similar lesions to those recorded in the lumbar region. The capsules of such ganglia were unaffected, and the lesions were marked in the periphery of the ganglion. In the nerves, after their exit from the ganglion, lesions similar to those seen in the nerve-roots were found. Massive infiltration with mononuclear cells was seen between the fasciculi of the nerves (epineurium), in the connective tissue around the nerve (perineurium), and even in the endoneurium. 'Cuffing' of the vessels was also seen.

(e) Sciatic Nerve. A study of transverse sections from the sciatic nerve after its exit from the greater sciatic foramen showed that there was a diffuse infiltration between the nerve fasciculi; this was
generally more pronounced in the interior of the fasciculi. In the thickness of the nerve an intense perivascular infiltration was seen (Figs. 38 and 39). Often the connective tissue of the sheath was unchanged, while intense infiltrative lesions were seen in the thickness of the nerve.

Examination of longitudinal sections made from the sciatic nerve half-way between the greater sciatic foramen and the popliteal region showed that interstitial infiltration with mononuclears existed along with massive perivascular 'cuffing', while the nerve-sheath itself appeared perfectly normal.

(f) Brachial Nerve. The alterations seen in this nerve were analogous to those described in the sciatic nerve; even in the lower third of the fore limb intense lesions were found (Fig. 37).

The infiltrative lesions of the nerve-roots and peripheral parts of the nerves, as well as the alterations in the paravertebral ganglia, show that in the monkey, as in the rabbit infected intracerebrally with the virus, the infective agent travels from the central nervous system to the periphery along the nerves, producing the lesions described above which constitute a ganglio-radicularis and descending peripheral interstitial neuritis.

(2) Summary.

In the brain there existed a mild meningoencephalitis with perivascular infiltrations and diffuse parenchymatous infiltrations, which sometimes formed actual nodules; massive perivascular infiltration was also seen in the parenchyma; there was degeneration of nerve-cells and satellitism. Intranuclear corpuscles of the type described by Joest and Degen were more easily found and more numerous than the rabbit.

In the cerebellum there was found a slight meningeal reaction with perivascular 'cuffing', satellitism of the Purkinje cells in certain areas, and rare oxyphilic corpuscles (Joest-Degen type) in the medulla were recorded.

In the spinal cord no meningitis was recorded and there was a marked absence of 'cuffing' in the vessels of the coverings of the cord. In the posterior horn, a diffuse infiltration was observed, this being more marked in the lumbar region. In this region of the cord also a similar infiltration was seen in the anterior and lateral horns. In the anterior horns degeneration in the nerve-cells was recorded, and sometimes neuronophagia.

In the peripheral nervous system an infiltrative radiculitis existed.

The spinal ganglia were intensely affected, showing lesions both infiltrative and degenerative; neuronophagia was a common feature.

A peripheral neuritis (sciatic and brachial nerves) was present, consisting of interstitial and perivascular infiltration.

The topography of the lesions as a whole shows that the virus introduced into the brain produces lesions locally, spreads to the rest of the central nervous system, and finally travels down the peripheral nerves. The cells in the lesions are almost exclusively mononuclear.
11. IMMUNITY

All authors agree that in the horse an attack of enzootic encephalomyelitis contracted spontaneously does not render this animal immune to a second attack; with regard to the disease in cattle and sheep, no precise records are available as to this point. The rabbit appears to behave differently. Zwick (1926) and his collaborators observed that in one case a rabbit which had been infected experimentally and had shown symptoms typical of the disease ultimately recovered and resisted a second intracerebral inoculation. The same authors succeeded, although not constantly, in producing a solid immunity by repeated inoculation of virus either subcutaneously or intravenously. They showed likewise that the introduction of a large quantity of virus intraperitoneally may render rabbits refractory to subsequent infection. Ernst and Hahn (1926) have shown that the inoculation of virus intracerebrally into rabbits does not lead to the development of the disease, if, during the period of incubation, such animals receive repeated inoculations of virulent material into the veins.

Zwick and his collaborators (1926), in the few experiments which they record in attempts to demonstrate antibodies in the serum of immunized animals by neutralization of virus in vitro, did not obtain very conclusive results.

In passaging the strains of virus with which we have been working, viz. a strain originating from horses (Zwick) and a strain originating from sheep (Miessner), we have never found any healthy uninoculated rabbit refractory to infection.

I. ATTEMPTS AT CONFERRING IMMUNITY TO RABBITS BY INOCULATIONS OF NON-ATTENUATED VIRUS.

A. Intravenous Inoculation.

(1) Four rabbits were inoculated intravenously with 1.5 c.c.m.s. of a virulent centrifugalized emulsion, which, when inoculated into the brain of a rabbit, produced the disease and death at the end of 39 days. The rabbits inoculated intravenously did not show any symptoms and gained weight. Between 91 and 206 days later they were inoculated intracerebrally with fresh passage virus. Table X. below shows that a single intravenous inoculation of virulent emulsion does not produce immunity in rabbits.

(2) Another rabbit, No. 69, weight 1,380 gms., received three inoculations of virulent material into the veins.

<table>
<thead>
<tr>
<th>Date</th>
<th>Details of Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.5.27</td>
<td>First intravenous inoculation of 3 c.c.m.s. of centrifugalized emulsion.</td>
</tr>
<tr>
<td>21.5.27</td>
<td>Animal normal. Weight 1,780 gms.</td>
</tr>
<tr>
<td>29.5.27</td>
<td>Animal normal. Weight 1,640 gms.</td>
</tr>
<tr>
<td>30.5.27</td>
<td>Second intravenous inoculation of 5 c.c.m.s. of virulent emulsion.</td>
</tr>
<tr>
<td>8.6.27</td>
<td>Animal normal. Weight 1,600 gms.</td>
</tr>
<tr>
<td>13.6.27</td>
<td>Third intravenous inoculation of 1.5 c.c.m.s. of virulent emulsion.</td>
</tr>
<tr>
<td>14.6.27</td>
<td>Animal normal. Weight 1,680 gms.</td>
</tr>
<tr>
<td>22.6.27</td>
<td>Animal normal. Weight 1,670 gms.</td>
</tr>
<tr>
<td>No. of rabbit</td>
<td>Weight</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>294</td>
<td>1,720 gms.</td>
</tr>
<tr>
<td>288</td>
<td>2,080</td>
</tr>
<tr>
<td>296</td>
<td>1,820</td>
</tr>
<tr>
<td>297</td>
<td>1,920</td>
</tr>
</tbody>
</table>
ENZOOTIC ENCEPHALO-MYELITIS

On 23.6.27 the rabbit received a test inoculation of virulent material at the same time as a control. The control died after 34 days with symptoms and lesions characteristic of the disease in the central nervous system. The rabbit prepared by intravenous inoculations behaved as recorded below.

30.6.27. Animal normal. Weight 1,750 gms.
7.7.27. Animal normal. Weight 1,950 gms.
10.7.27. Animal normal. Weight 1,680 gms.
14.7.27. Commencement of the disease. Weight 1,580 gms.
23.7.27. Typical symptoms of the disease. Weight 1,400 gms.
26.7.27. Animal died during the day. Weight 1,230 gms.

Microscopic examination of the brain and spinal cord revealed the presence of characteristic lesions. In this case three intravenous inoculations did not lead to immunity to cerebral infection.

That four intravenous injections of virulent material may, however, produce a solid immunity is shown by the experiment recorded on p. 75, Rabbit 67.

That the intracerebral inoculation of a filtrate (Mandler filter) of virulent emulsion does not lead to the development of immunity may be concluded from the experiments mentioned on p. 13.

The effect of introducing a thick emulsion of virulent brain into the trachea of rabbits was tested, but it did not produce the disease nor immunity in animals so treated. 0.5 c.c.m. of a virulent emulsion of brain was introduced into the trachea of three animals. As they did not present any morbid symptoms for 174 days they were inoculated intracerebrally with a virulent emulsion. They all died from enzootic encephalo-myelitis after 87, 27, and 40 days respectively. (See p. 33.)

Rabbits which survive corneal or intratesticular inoculations of virulent material may become refractory to the disease as tested subsequently by intracerebral inoculation.

B. Corneal Inoculation.

The experiment recorded on p. 31 (Rabbit 81s) shows that infection by the corneal route can immunize the rabbit against a subsequent inoculation by the cerebral route.

C. Intratesticular Inoculation.

Some measure of immunity may also follow intratesticular inoculation. Two rabbits (273 and 289) were inoculated into the right testicle with 1 c.c.m. of a virulent cerebral emulsion. One of these rabbits, No. 289, died after 105 days. Typical lesions were found in sections of the various parts of the central nervous system, and passage of its brain to a fresh rabbit gave a positive result.

The other rabbit, No. 273, survived, and when inoculated by the
intracerebral route 112 days later it proved to be immune. The control rabbit inoculated by the intracerebral route died on the 48th day.

II. ATTEMPTS AT CONFERRING IMMUNITY TO RABBITS BY INOCULATION OF ATTENUATED VIRUS.¹

A. Virus Inactivated by Ether.

Roux ² showed the attenuating action of ether on the virus of rabies. Later Remlinger (1919) used an ether-treated virus subcutaneously to produce an immunity in rabbits to intracerebral inoculation with fixed virus. Alvisatos (1922) and Hempt (1925) used ether-treated virus as a means of vaccinating man against rabies.

Marie and Mutermilch (1927) have shown that one can immunize rabbits against rabbits by inoculating intrathecally virus treated with ether. We have tried to immunize rabbits against the virus of encephalomyelitis, employing a similar technique, with the difference that the virus treated with ether was inoculated into the brain; the proof of the avirulence of the virus treated with ether and the technic employed has been described on p. 22.

The results obtained are given below.

Two rabbits, 220A and 217A, weighing 2,450 and 1,950 gms respectively, received two intracerebral inoculations of 0.5 c.cm. of ether-treated virus at an interval of 5 days. Twenty-one days after the last inoculation they were inoculated intracerebrally along with a control rabbit, 14A, with fresh virus. Rabbit 217A died on the 42nd day, Rabbit 220A on the 17th day, and control Rabbit 14A on the 30th day after inoculation, and typical microscopic lesions were found in the central nervous system of all three.

Thus two successive intracerebral inoculations into rabbits of virus treated with ether did not produce immunity to subsequent intracerebral inoculation.

B. Virus killed by Chloroform.

The proof that virus treated with chloroform is inactivated has already been given on p. 21. In the experiment about to be described, rabbits were twice inoculated intracerebrally with virus treated with chloroform, the interval between the inoculations being 5 days. The animals were tested 3 weeks after the second inoculation, and since the experiments were made at the same time as the experiments with ether-treated virus, the control Rabbit 14A served for both.

The rabbits prepared with virus treated with chloroform succumbed...
to a subsequent intracerebral infection after 37 and 42 days respectively. Cultures made from these brains were negative, but lesions characteristic of infection with Borna disease were found on microscopic examination of sections of brain, cord, and spinal ganglia.

C. Virus treated with Formol.

On p. 22 it was shown that virus treated with 0.2 per cent. formalin for 18 hours at room temperature failed to infect rabbits by the intracerebral route.

The vaccine was prepared the day before use by subjecting fresh virulent brain material to the action of formalin in this concentration. A batch of ten rabbits was prepared by subcutaneous inoculation of this vaccine: the results of the experiment are recorded in Table XI. The results obtained in this experiment were not very satisfactory. Of the ten rabbits prepared by inoculation of formalized virus two died accidentally, five died following upon infection with the test dose given intracerebrally, and three proved to be immune to the test dose given intracerebrally.

D. Virus Inactivated by Ultra-violet Light.

An experiment on this subject is recorded on p. 20 in which a rabbit which survived after having received an inoculation of virus exposed for 5 minutes to the action of rays emitted by the mercury arc proved to be still susceptible to infection by the intracerebral inoculation of virulent material.

III. Search for Virucidal Antibodies in the Serum of Immunized Animals.

The serum of the immune rabbit, 223A (see p. 75), was taken and mixed with equal parts of a centrifugalized virulent cerebral emulsion; at the same time two further mixtures of the same cerebral emulsion were made, one with equal parts of normal rabbit serum and the other with physiological saline. The three mixtures were kept for 2 hours at 37° C. and were subsequently inoculated intracerebrally into rabbits.

The results were: (1) the rabbit inoculated with the mixture of virus and immune serum died on the 14th day after inoculation; its brain passaged to a fresh rabbit killed in 35 days; the lesions found in sections of the brain of both of these rabbits were those of experimental Borna disease.

(2) The control rabbits inoculated with the mixture of normal rabbit serum and virus died 89 and 84 days respectively after inoculation of Borna Disease, the lesions found on microscopical examination of sections of the nervous system being typical.

(3) The rabbits inoculated with the mixture of virus and physiological saline also succumbed to the inoculation.
<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>1.9.27. 1st inoculation of 5 c.cms. of formol-treated virus</th>
<th>5.9.27. 2nd inoculation of 5 c.cms. of formol-treated virus</th>
<th>10.9.27. 3rd inoculation of 3 c.cms. of formol-treated virus</th>
<th>15.9.27. 4th inoculation of 5 c.cms. of formol-treated virus</th>
<th>20.9.27. 5th inoculation of 5 c.cms. of formol-treated virus</th>
<th>Intracerebral inoculation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>15A</td>
<td>2,260, 2,300, 2,300, 2,270, 2,200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brain</td>
<td>Died accidentally.</td>
</tr>
<tr>
<td>16A</td>
<td>1,920, 1,900, 2,100, 2,040, 1,750</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Survived.</td>
<td></td>
</tr>
<tr>
<td>17A</td>
<td>1,910, 2,000, 2,100, 2,040, 1,730</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died accidentally.</td>
<td></td>
</tr>
<tr>
<td>18A</td>
<td>1,800, 1,900, 1,900, 1,880, 2,010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Survived.</td>
<td></td>
</tr>
<tr>
<td>19A</td>
<td>2,170, 2,100, 2,080, 2,060, 1,750</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died 37th day.</td>
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<tr>
<td>20A</td>
<td>2,150, 2,100, 2,150, 2,080, 2,250</td>
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<td></td>
<td></td>
<td></td>
<td>Died 36th day, after showing typical symptoms.</td>
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<tr>
<td>21A</td>
<td>2,590, 2,450, 2,380, 2,350, 2,300</td>
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<td></td>
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<td></td>
<td>Died 36th day, after showing typical symptoms.</td>
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<tr>
<td>22A</td>
<td>1,290, 1,250, 1,260, 1,260, 1,290</td>
<td></td>
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<td></td>
<td>Died 27th day as above.</td>
<td></td>
</tr>
<tr>
<td>23A</td>
<td>1,590, 1,600, 1,750, 1,900, 1,880</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Survived.</td>
<td></td>
</tr>
<tr>
<td>24A</td>
<td>1,330, 1,350, 1,350, 1,320, 1,230</td>
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<td></td>
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<td></td>
<td>Died 28th day with characteristic symptoms.</td>
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<tr>
<td>19A</td>
<td>2,300, 2,300, 2,300, 2,300, 2,300</td>
<td></td>
<td></td>
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<td></td>
<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>25A</td>
<td></td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>26A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>27A</td>
<td></td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>28A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>29A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>30A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>31A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>32A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>33A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>34A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>35A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>36A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>37A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>38A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>39A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>40A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>41A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>42A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>43A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>44A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>45A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>46A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>47A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>48A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>49A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>50A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>51A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>52A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>53A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>56A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>57A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>58A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>59A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>60A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>61A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>62A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>63A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>64A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<td>66A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
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<tr>
<td>67A</td>
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<td>Died 28th day: typical symptoms. and lesions.</td>
<td></td>
</tr>
<tr>
<td>68A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died 28th day: typical symptoms. and lesions.</td>
<td></td>
</tr>
<tr>
<td>69A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Died 28th day: typical symptoms. and lesions.</td>
<td></td>
</tr>
</tbody>
</table>
IV. EXPERIMENTS ON CROSS IMMUNITY BETWEEN THE STRAIN OF EQUINE AND THAT OF OVINE ORIGIN.

Our experiments have been carried out with two strains of enzootic encephalo-myelitis, the one originating from the horse (Zwick's strain), and the other from the sheep (Miessner's strain).

Experiment 1. Rabbit 223a. Weight 2,280 gms. A diluted emulsion of virulent brain (Zwick strain) which had been pulped and preserved in glycerine at room temperature for several weeks was inoculated into the brain on 22.10.26. The animal lost weight slightly, developed paresis of the hind quarters about the 20th day after the infection, but subsequently recovered, and on 15.12.26, 54 days after the inoculation, it appeared perfectly normal and had a weight of 2,400 gms. On this date it was inoculated intracerebrally with fresh passage virus (Zwick strain) and survived, while the control rabbit (Rabbit 68A, weighing 1,150 gms.) inoculated by the same route fell ill on the 20th day following upon infection, and died on the 25th day. Characteristic lesions were found throughout the central nervous system on microscopic examination.

Rabbit 223a was therefore immune against infection with the strain of equine origin. On the 12.5.27 (i.e. 202 days after the first inoculation, and 148 days after the second inoculation with the equine strain, it was inoculated intracerebrally with the strain of ovine origin. At the same time a control rabbit was similarly inoculated. Rabbit 223a continued in health, and gained in weight. The control showed symptoms of Borna disease 20 days after the inoculation, and died 27 days after the injection. The usual characteristic lesions were found in sections of the central nervous system of this control rabbit, and a portion of the brain infected another rabbit.

Experiment 2. The fact that repeated inoculations of fresh virus into the veins of rabbits may immunize them against subsequent intracerebral inoculation has already been referred to (see p. 71). We prepared rabbits by vaccinating in this way with a virus of ovine origin. A fresh virulent emulsion of brain was centrifugalized for 5 minutes. The supernatant fluid was carefully pipetted off and inoculated in the marginal vein of the ear of rabbits, care being taken that the injection was carried out very slowly.

Rabbit 67. Weight 1,580 gms.

12.5.27. First injection of 2 c.c.ms. of a virulent centrifugalized emulsion into the vein.
16.5.27. Weight 1,600 gms. Second injection of 3 c.c.ms. of a virulent centrifugalized emulsion into the vein.
21.5.27. Weight 1,680 gms. Animal normal.
30.5.27. Weight 1,700 gms. Weight normal.
5.6.27. Weight 1,800 gms. Animal normal.
7.6.27. Third injection of 5 c.c.ms. of a virulent centrifugalized emulsion into the vein.

Several animals were prepared in the same way, but we give below the protocol of the rabbit which served for an experiment of cross immunity between the equine and ovine strain.
BORNA DISEASE AND

8.6.27. Weight 1,690 gms. Animal normal.

13.6.27. Weight 1,820 gms. Fourth injection of 3 c.c.m.s. of a virulent centrifugaled emulsion into the vein.

22.6.27. Weight 1,870 gms. Animal normal.

On 23.6.27 the animal, together with a control rabbit, was inoculated intracerebrally with an emulsion of fresh virus of equine origin.

**Rabbit 67. Weight 1,870 gms.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.6.27</td>
<td>1,980 gms</td>
</tr>
<tr>
<td>7.7.27</td>
<td>2,050 gms</td>
</tr>
<tr>
<td>14.7.27</td>
<td>2,200 gms</td>
</tr>
<tr>
<td>20.7.27</td>
<td>2,170 gms</td>
</tr>
<tr>
<td>25.7.27</td>
<td>2,150 gms</td>
</tr>
<tr>
<td>28.7.27</td>
<td>2,000 gms</td>
</tr>
<tr>
<td>15.8.27</td>
<td>2,100 gms</td>
</tr>
<tr>
<td>29.8.27</td>
<td>2,150 gms</td>
</tr>
</tbody>
</table>

**Control.**

**Rabbit 178B. Weight 1,770 gms.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.6.27</td>
<td>1,820 gms</td>
</tr>
<tr>
<td>7.7.27</td>
<td>1,850 gms</td>
</tr>
<tr>
<td>14.7.27</td>
<td>1,800 gms</td>
</tr>
<tr>
<td>18.7.27</td>
<td>1,650 gms</td>
</tr>
<tr>
<td>20.7.27</td>
<td>1,600 gms</td>
</tr>
<tr>
<td>25.7.27</td>
<td>1,540 gms</td>
</tr>
<tr>
<td>26.7.27</td>
<td>1,500 gms</td>
</tr>
</tbody>
</table>

Microscopical examination of sections of the central nervous system—characteristic lesions.

This experiment shows that the virus of ovine origin immunized against that of equine origin.

**Experiment 3.** A rabbit (81s) which had resisted inoculation with virulent virus of ovine origin, by scarification of the cornea, was inoculated intracerebrally 110 days later with fresh rabbit passage virus of equine origin. At the same time a control rabbit (25a) was inoculated intracerebrally with the same fresh passage virus. Rabbit 81s remained well, while the control, 25a, showed typical symptoms of Borna disease 32 days after inoculation, and died on the 48th day. In this case also the virus of ovine origin immunized Rabbit 81s against the pathogenic action of the virus of equine origin introduced into the brain.

**Conclusion.** From the above experiments the following conclusions can be drawn:

1. Rabbits which have become resistant to the virus of enzootic encephalo-myelitis of equine origin prove also to be refractory to infection by intracerebral inoculation of the virus of ovine origin: the converse is also true.

2. Rabbits which have become immunized against enzootic encephalo-myelitis keep this acquired immunity for at least 148 days. If the immunity is reinforced by repeated inoculations it may last at least 268 days.
V. Experiments on Cross Immunity Between the Virus of Enzootic Encephalo-Myelitis and Other Viruses of the Filter-Passing Group.

A. Herpes.

On the basis of the following experiment we arrived at the conclusion published as a preliminary note in the British Journal of Experimental Pathology (1927) that rabbits immunized against the virus of herpes are still susceptible to infection with the virus of Borna disease.

Four large rabbits, 111A, 183A, 185A, and 187A were immunized against herpes. On 7.6.27 their immunity was tested by the intracerebral inoculation of fresh herpetic virus; they resisted such infection, while the control rabbit, No. 148A, infected by the same route, died on the 6th day. Sixteen days later the four surviving rabbits were inoculated intracerebrally with the virus of encephalomyelitis, together with a control rabbit, No. 182A. All the rabbits showed symptoms typical of experimental Borna disease and died; the results are recorded below.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Date of death</th>
<th>Culture of brain</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>111A</td>
<td>Died on the 40th day after inoculation</td>
<td>Negative</td>
<td>Typical</td>
</tr>
<tr>
<td>183A</td>
<td>32nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>185A</td>
<td>31st</td>
<td></td>
<td></td>
</tr>
<tr>
<td>187A</td>
<td>32nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Rabbit</td>
<td>40th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>182A</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion. No cross immunity exists between herpes and enzootic encephalo-myelitis.

B. Rabies.

On 9.9.27 two rabbits proved to have been immunized against the virus of Borna disease (Rabbit 223A, see p. 75, and Rabbit 67, see p. 75), together with, as a control, Rabbit 41A, were inoculated intracerebrally with a 'street' virus of rabies. The two rabbits, 223A and 67, which were immune to Borna disease, became paralysed on the 8th day after the inoculation with rabies and died on the 10th day; cultures from the brain were negative. Negri bodies were found in sections of the Ammon's horn of both rabbits. The control rabbit became paralysed after 8 days and died on the 12th day; Negri bodies were found in sections of the Ammon's horn.

Conclusion. No cross immunity exists between rabies and enzootic encephalo-myelitis.

1 We thank Dr. Perdrau, of the National Institute for Medical Research, for kindly putting a strain of this virus at our disposal.

2 We thank Dr. Manouelian of the Pasteur Institute, Paris, for his kindness in putting this strain at our disposal.
In the experiments with this virus two strains have been used (1) a strain of low virulence kindly furnished by Professor MacIntosh, (2) a very virulent strain kindly provided by Professor Petit. This latter strain, which has been utilized by us in another series of experiments, killed monkeys by intracerebral inoculation as indicated below:

**TABLE XIII.**

<table>
<thead>
<tr>
<th>Monkey (Macacus rhesus) No.</th>
<th>Died 9th day after inoculation.</th>
<th>Died 12th day after inoculation.</th>
<th>Died 11th day after inoculation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 5</td>
<td></td>
<td>No. 6</td>
<td>No. 13</td>
</tr>
<tr>
<td>No. 15</td>
<td></td>
<td>No. 17</td>
<td></td>
</tr>
</tbody>
</table>

The lesions produced by this strain of polio-myelitis in the central nervous system of monkeys were very intense, neuronophagia was frequently found in the anterior horns of the spinal cord as well as in the paravertebral ganglia.

The virus of polio-myelitis taken from monkeys infected experimentally is generally considered as non-pathogenic for the rabbit.

In a first series of experiments we gave repeated intracerebral inoculations of polio-myelitis to young rabbits; some time afterward the rabbits so prepared were infected by the same route with the virus of enzootic encephalo-myelitis.

Table XIV gives the results of this experiment.

Thus the four rabbits having received three successive intracerebral inoculations with the virus of polio-myelitis and one rabbit which received no such inoculation, proved to be still susceptible to infection with the virus of Borna disease.

The conclusion that absolutely no cross immunity exists between the virus of polio-myelitis and that of Borna disease, however, is weakened by the following experiment on a monkey.

Monkey M. 2 (Macacus rhesus), which had apparently almost completely recovered from an intracerebral inoculation of the virus of Borna disease, after having shown the morbid symptoms recorded on p. 86, was inoculated into the brain with a passage virus of polio-myelitis (strain Petit). At the same time two controls of a comparable size and the same species were similarly inoculated. The subsequent history of the two controls was as follows:

**M. 13 (Macacus rhesus)**

5.10.27. Inoculated intracerebrally with the virus of polio-myelitis.

10.10.27. Normal.

11.10.27. Paralysed, found procumbent.

12.10.27.

1 Our thanks are due to M. le Prof. A. Petit, of the Pasteur Institute, and Prof. MacIntosh of the Bland Sutton Institute for supplying us with strains of polio-myelitis virus.

2 Krause and Meinicke (1909) and also Dahm (1909), however, have recorded a few experiments giving positive results, using virus taken directly from human cases, but the general opinion held to-day is that the virus of polio-myelitis is not pathogenic for rabbits.
### Table XIV.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>23.5.27. 1st inoculation, virus MacIntosh.</th>
<th>4.8.27. 2nd inoculation, virus MacIntosh.</th>
<th>6.9.27. 3rd inoculation, virus Petit.</th>
<th>20.9.27. Brain with Borna.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight in gms.</td>
<td>Weight in gms.</td>
<td>Weight in gms.</td>
<td>Weight in gms.</td>
<td></td>
</tr>
<tr>
<td>103A</td>
<td>660</td>
<td>1,070</td>
<td>1,250</td>
<td>1,310</td>
<td>First symptoms 22nd day after inoculation. Died the 32nd day. Lesions in C.N.S. typical.</td>
</tr>
<tr>
<td>104A</td>
<td>620</td>
<td>1,250</td>
<td>1,380</td>
<td>1,530</td>
<td>First symptoms 22nd day after inoculation. Died the 36th day. Lesions in C.N.S. typical.</td>
</tr>
<tr>
<td>105A</td>
<td>600</td>
<td>1,200</td>
<td>1,250</td>
<td>1,390</td>
<td>First symptoms 16th day after inoculation. Died 23rd day. Lesions in C.N.S. typical.</td>
</tr>
<tr>
<td>110A</td>
<td>720</td>
<td>1,200</td>
<td>1,380</td>
<td>1,460</td>
<td>First symptoms 25th day. Died 32nd day. Lesions in C.N.S. typical.</td>
</tr>
<tr>
<td>26A</td>
<td>Control</td>
<td></td>
<td></td>
<td>1,900</td>
<td>First symptoms 16th day. Died 29th day. Lesions in C.N.S. typical.</td>
</tr>
<tr>
<td>68A</td>
<td></td>
<td></td>
<td></td>
<td>2,620</td>
<td>First symptoms 20th day. Died 28th day. Lesions in C.N.S. typical.</td>
</tr>
<tr>
<td>69A</td>
<td></td>
<td></td>
<td></td>
<td>2,650</td>
<td>First symptoms 20th day. Died 28th day. Lesions in C.N.S. typical.</td>
</tr>
</tbody>
</table>
13.10.27. Paralysed, found procumbent.
14.10.27. " " "
15.10.27. " " "
16.10.27. Found dead, 11th days after inoculation.

Cultures of brain. Negative.
Sections. Lesions typical of polio-myelitis.

M. 15 (Macacus rhesus)
5.10.27. Inoculated intracerebrally with the virus of polio-myelitis.
10.10.27. Normal.
11.10.27. " " "
12.10.27. " " "
13.10.27. " " "
14.10.27. Paralysis of the left arm.
15.10.27. Found procumbent and paralysed.
16.10.27. Found dead 11th day.

Cultures of brain. Negative.
Sections. Lesions typical of polio-myelitis.

Monkey M. 2 (Macacus rhesus), which had survived the inoculation with Borna disease, when inoculated with polio-myelitis, behaved as follows:
5.10.27. Inoculated into the brain with the virus of polio-myelitis 130 days after the inoculation with the virus of Borna disease.
10.10.27. No added symptoms up to this date.
15.10.27. " " "
17.10.27. " " "
19.10.27. " " "
20.10.27. Fifteenth day, myoclonic movements.
21.10.27. Epileptiform trembling movements.
22.10.27. Slight paresis of the hind quarters.
23.10.27. Paresis more marked.
24.10.27. Total paralysis of the hind quarters and partial paralysis of the fore limbs.
25.10.27. Could make movements with the head, but the total paralysis of the arms and legs prevented it from getting up.
26.10.27. Same condition.
27.10.27. Same condition, but still feeding.
28.10.27. " " "
29.10.27. Animal in a comatose state.
30.10.27. " " "
31.10.27. Died 26 days after the inoculation with the virus of polio-myelitis and 156 days after the infection with Borna.

We have drawn attention in the course of this monograph to the resemblance between the symptomatology and histological picture in polio-myelitis in the monkey and experimental enzootic encephalomyelitis in the same species. The differences between them are the longer period of incubation and the slower evolution and lesser intensity of the lesions in the nervous system in the latter disease. Monkey M. 2 died after a lapse of time much longer than the average for animals inoculated with polio-myelitis, the strain was generally killing the species of animal in from 9 to 11 days. We had therefore, to determine whether it died from polio-myelitis or from a persisting infection of Borna disease. On an anatomo-pathological study of the lesions in the brain and spinal cord of the animal, in the fourth and 17th days after the inoculation with Borna disease, no symptoms of the disease were found. The animal died on the 15th day after the inoculation with the virus of polio-myelitis.
study of the various parts of the nervous system of this monkey the lesions of experimental enzootic encephalo-myelitis were found, including the corpuscles of Joest-Degen. The general aspect of the alterations in the lumbar region of the spinal cord indicated that these were produced by the virus of Borna disease and not by the virus of polio-myelitis. This conclusion received further support when emulsions from various parts of the nervous system were passaged to fresh animals. The results of these inoculations were as follows:

1. Rabbit 460A, weighing 2,320 gms., and Rabbit 462A, weighing 1,740 gms., were inoculated intracerebrally with an emulsion of the brain of Monkey M. 2. The former died on the 37th day and the latter on the 38th day after infection, and lesions characteristic of experimental Borna disease were found in sections of the various parts of the nervous system of both animals. A passage was made with the brain of Rabbit 462A to a fresh Rabbit 405A, weighing 1,880 gms. Rabbit 405A succumbed on the 42nd day of Borna disease.

2. Rabbit 464A, weighing 1,960 gms., Rabbit 458A, weighing 1,760 gms., and Rabbit 469A weighing 2,500 gms., were inoculated intracerebrally with an emulsion of the cervical region of the spinal cord of Monkey M. 2. Rabbit 464A died on the 34th day, 458A on the 30th day, and 459A on the 80th day after inoculation, and lesions typical of Borna disease were found in sections of the nervous system of all three. A passage was made with the brain of Rabbit 458A to a fresh rabbit, 414A, which died on the 33rd day of experimental enzootic encephalo-myelitis.

3. Rabbit 454A, weighing 1,960 gms., Rabbit 455A, weighing 1,960 gms., and Rabbit 463A, weighing 2,300 gms., were inoculated intracerebrally with an emulsion of the lumbar cord of Monkey M. 2. The first died on the 40th day, the second on the 35th day, and the third on the 34th day of experimental Borna disease. Rabbit 415A, weighing 1,880 gms., when inoculated intracerebrally with an emulsion of the brain of Rabbit 468A, died of Borna disease on the 80th day.

The results of these rabbit inoculations proved that the virus of Borna disease still existed in the brain and spinal cord of Monkey M. 2. Monkey M. 20 (Macacus rhesus) was inoculated with an emulsion of a portion of the brain and the cervical cord of Monkey M. 2. It remained healthy for a period of 60 days, when the commencement of paresis of the hind quarters was first observed. Paresis later became accentuated and reached the fore limbs. The animal died on the 68th day after inoculation, and the lesions of experimental enzootic encephalo-myelitis were found in sections of the central nervous system. Rabbits 340A and 341A were inoculated intracerebrally with an emulsion of the brain of Monkey M. 20, and both died of experimental Borna disease.

In résumé, the activity of virus of Borna disease which had become dormant in the central nervous system of Monkey M. 21, 130 days after the inoculation, as indicated by the clinical picture, was revived by the introduction of the virus of polio-myelitis by the intracerebral route. As a result of the second infection the monkey died and we
demonstrated the presence of the virus of Borna disease in its brain and spinal cord, but the virus of polio-myelitis had been destroyed.

Without wishing to attach too much importance to this single experiment, we are forced to conclude that the monkey which survived for 130 days the infection with Borna disease showed an exceptional resistance of an anomalous character to the virus of polio-myelitis. This resistance might be the result of a certain degree of immunity conferred by the first infection. On the other hand, it might be explained in another way. The experiments by Gildemeister and Herzberg (1925) indicate that guinea-pigs which showed lesions on the pads of the metatarsus as a result of infection with the virus of vaccinia develop a certain local resistance to infection with herpetic virus inoculated by the same route. The authors conclude that there is a certain degree of immunity conferred by one virus against infection with the other. In a similar way, certain rabbits, which after recovering from vaccinal keratitis, when inoculated some time later on the same cornea with the virus of herpetic encephalitis, sometimes resist the second infection.

We do not consider in these circumstances that an immunity results in the proper sense of the word, but rather a local mobilization of the elements of defence provoked by the first virus inoculated, that conferring on the tissue a certain degree of resistance of a non-specific character which does not exist normally.

We intend to repeat our experiment. In addition, monkeys are being immunized against polio-myelitis which will be inoculated later with the virus of Borna disease.

VI. Summary.

A solid immunity can occasionally be obtained against Borna disease in the rabbit by inoculating suitably attenuated virus in the brain.

Multiple intravenous injections, infection by corneal scarification or intratesticular inoculation with fresh virus, can also produce immunity.

We have not succeeded in producing immunity by inoculating virus killed by chloroform, ether, or ultra-violet light intracerebrally into rabbits.

Multiple inoculations subcutaneously of large quantities of molized virus leads to immunity in a limited number of animals.

Rabbits immunized against an equine strain of the virus of Borna disease were resistant to intracerebral infection with an ovine strain and vice versa.

No cross immunity was obtained between Borna disease and herpes or rabies. Cross immunity between Borna disease and polio-myelitis was not observed when rabbits were the subject of experiment, but in an experiment carried out on a monkey the result suggested that some resistance to the virus of polio-myelitis may be produced by previous attack of experimental Borna disease.
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18. CHEMOTHERAPY

Various medicaments, calomel, mercuric chloride, salvarsan, atoxyl, have been tried in the treatment of enzootic encephalo-myelitis. The greatest success as regards the treatment by drugs has been claimed for the administration of urotropine (hexamethylenetetramine). Moussu and Marchand (1926) obtained remarkably good results in the treatment of the disease in horses and cattle by inoculation of urotropine intravenously. According to these authors, when 15 to 20 gms. is administered on the appearance of the first symptoms, the mortality in epizootics may be lowered from 80 or 90 per cent. to 25 per cent. In Germany Ostertag (1924) has generalized the use of urotropine on a large scale in the treatment of enzootic encephalo-myelitis. H. Bohn (1927) inoculated 80 gms. per day for 4 to 5 days intravenously into affected horses with good results. Trepel (1926) advocates the use of a total of 100 gms. during the course of a few days. Grimm (1927) obtained a smaller number of recoveries than the authors cited above. All authors appear to be agreed as to the beneficial action of the drug in Borna disease of the horse.

We have carried out experiments to see whether urotropine was as efficacious in treating the disease in the rabbit as in the horse. We commenced by determining the maximum intravenous dose supported by a rabbit. This appeared to be about 0.5 gms. per kgm., a dose which can be repeated at least eight times at 4 to 5 days' interval. Subsequently the experiment recorded in Table XV was carried out.

Summary. The dose of urotropine we gave to the rabbit was equivalent, weight for weight, to 100–200 gms. for the horse and to a total amount of 600 gms., yet we were not able to demonstrate any prophylactic or curative action.
<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Inoculation of virus intracerebrally</th>
<th>23.6.27.</th>
<th>28.6.27.</th>
<th>27.7.27.</th>
<th>6.7.27.</th>
<th>11.7.27.</th>
<th>19.7.27.</th>
<th>24.7.27.</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>180A</td>
<td>Weight 1,890 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,020 gms. Urotropine 0.8 gr.</td>
<td>Weight 1,950 gms. Urotropine 0.8 gr.</td>
<td>Weight 1,900 gms. Urotropine 0.8 gr.</td>
<td>Weight 1,900 gms. Urotropine 0.8 gr.</td>
<td>Animal showing symptoms. Weight 1,880 gms.</td>
<td>Typical symptoms. Weight 1,650 gms.</td>
<td>Died of disease 39th day.</td>
<td></td>
</tr>
<tr>
<td>181A</td>
<td>Weight 2,100 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,150 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,100 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,050 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,000 gms. Urotropine 0.8 gr.</td>
<td>Typical disease. Weight 1,600 gms.</td>
<td>Typical disease. Weight 1,650 gms.</td>
<td>Died of disease 32nd day.</td>
<td></td>
</tr>
<tr>
<td>179A</td>
<td>Weight 2,220 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,200 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,250 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,300 gms. Urotropine 0.8 gr.</td>
<td>Weight 2,200 gms. Urotropine 0.8 gr.</td>
<td>Typical commencement of disease. Weight 2,100 gms. Urotropine 0.8 gr.</td>
<td>Typical disease. Weight 1,650 gms.</td>
<td>Died of disease 32nd day.</td>
<td></td>
</tr>
<tr>
<td>Control 178A</td>
<td>Virus into brain. Weight 1,770 gms.</td>
<td>No urotropine</td>
<td>Weight 1,820 gms. No urotropine</td>
<td>Weight 1,850 gms. No urotropine</td>
<td>Weight 1,850 gms. No urotropine</td>
<td>Typical symptoms Weight 1,600 gms. No urotropine</td>
<td>Typical disease. Weight 1,300 gms.</td>
<td>Died of disease 34th day.</td>
<td></td>
</tr>
</tbody>
</table>
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DESCRIPTION OF PHOTOGRAPHS AND COLOURED PLATES

Fig. 1. Photograph of Rabbit No. 275. 29 days after intracerebral infection with the virus of Born disease, and 3 days before death. The characteristic position of the head and ears is depicted.

Fig. 2. Photograph of Macacus rhesus No. 1. 64 days after inoculation. Paralysis of the hind quarters; paresis of the right arm, the hand not being able to grip any object presented to it.

Fig. 3. Photograph of Macacus rhesus No. 1. 69 days after inoculation. Characteristic 'hunched up' attitude.

Fig. 4. Photograph of Macacus rhesus No. 2. 51 days after the infection by the intracerebral route. Facial paralysis on the left side.

Fig. 5. Photograph of Macacus rhesus No. 2. 67 days after inoculation. 3rd crisis of the disease. Facial paralysis on the right side.

Fig. 6. Photograph of Guinea-pig 95k. 60 days after intracerebral inoculation, and 5 days before death. Paralysis of the hind quarters.

Fig. 7. Photograph of Guinea-pig 1a. 50 days after intracerebral inoculation, and 2 days before death. General paralysis.

Fig. 8. Photograph of Rat No. 14. 63 days after inoculation intracerebrally, and 4 days before the death of the animal. At this stage the rat placed on the flank made several useless efforts with the forelegs to recover its normal position.

Fig. 9. Photograph of the stomach wall of Rabbit 212 which died 44 days after the intracerebral inoculation, showing the haemorrhagic lenticular areas. L=greater curvature of the stomach; P=lenticular haemorrhagic areas.

Fig. 10. Microphotograph, ×160. Rabbit 209 dead 71 days after intratesticular inoculation. Perivascular 'cuff' in the cerebral cortex cut longitudinally. V=lumen of blood-vessel; C=cerebral cortex; M=mononuclear leucocyte of inflammatory process.

Fig. 11. Microphotograph, ×300. Rabbit 51a dead 28 days after intracerebral infection. Perivascular 'cuff' in the cerebral cortex cut longitudinally. V=lumen of blood-vessel; E=endothelium of blood-vessel; M=mononuclear leucocytes taking part in the infiltrative process.

Fig. 12. Microphotograph, ×165. Rabbit 11b dead 27 days after intracerebral inoculation. Section showing the aspect of the Cornu Ammonis. F=area of infiltrating lymphocytes between the nerve-cells; L=infiltrating lymphocytes; C=cells containing the corpuscles of Jost-Degen within their nuclei.

Fig. 13. Microphotograph, ×800. Rabbit 25 dead 35 days after cerebral infection. 'Cyst' in a nerve-cell of the Ammon's horn. C='cyst'; N=nucleus; N=nucleolus.

Fig. 14. Microphotograph, ×1,000. Rabbit 80a dead 28 days after inoculation intracerebrally. 'Cyst' in the protoplasm of a nerve-cell of the Ammon's horn. C='cyst'; N=nucleolus.

Fig. 15. Microphotograph, ×900. Rabbit 11b dead 27 days after cerebral infection. 'Cyst' in the protoplasm of a nerve-cell of the Cornu Ammonis.

Fig. 16. Microphotograph, ×1,000. Rabbit 51a dead 28 days after inoculation into the brain. 'Cyst' in the protoplasm of a nerve-cell in the Cornu Ammonis.

Fig. 17. Microphotograph, ×1,000. Rabbit No. 22 dead 24 days after cerebral infection. Nerve-cell in medulla oblongata showing neuronophagia. C=degenerated cytoplasm; p=polymerphonuclear penetrating neuron; m=mononuclear cells.

Fig. 18. Microphotograph, ×230 (enlarged two diameters). Rabbit 295 dead 19 days after intracerebral inoculation. Section in the region of the pons showing (d) degenerated nerve-cells, (s) 'satellitism' with commencing neuronophagia, (n) neuronophagia, (l) lymphocytes in the parenchyma.
Inoculation. Spinal ganglion in the thoracic region. P = perivascular infiltration. Section of the lumbar region including a spinal ganglion. V = vacuole in a nerve-cell; P = polymorphonuclear; L = lymphocytes.

Fig. 21. Microphotograph, \( \times 360 \). Rabbit No. 30 dead 36 days after the inoculation given intracerebrally. Spinal ganglion in the lumbar region. N = ganglion nerve; R = remains of a destroyed neuron; V = vacuole in a nerve-cell; P = polymorphonuclear; L = lymphocytes.

Fig. 22. Microphotograph, \( \times 360 \). Spinal ganglion in the lumbar region. Spinal ganglion with the frontal lobe is infiltrated with mononuclear cells. P = perivascular infiltration. Section of the cord in the lumbar region including a spinal ganglion. V = vacuolization in certain ganglion cells; I = interstitial infiltration; N = neuronophagia.

Fig. 23. Microphotograph, \( \times 1,000 \). Rabbit 164 dead 29 days after intracerebral inoculation. Spinal ganglion in the lumbar region. Neuronophagia with the formation of nodules consisting of mononuclear cells. R = remains of a destroyed neuron; V = vacuole in a nerve-cell; P = polymorphonuclear; L = lymphocytes.

Fig. 24. Microphotograph, \( \times 800 \). Rabbit No. 30 dead 36 days after intracerebral inoculation. Spinal ganglion in the lumbar region. Degeneration of a ganglion cell and commencing neuronophagia. C = degenerated cell which has become oxyphilic and is on the way to destruction; P = polymorphonuclear cells; L = lymphocytes; M = mononuclear cells participating in the process of neuronophagia.

Fig. 25. Microphotograph, \( \times 1,000 \). Rabbit 164 dead 29 days after intracerebral inoculation. Spinal ganglion in the thoracic region. N = neuronophagia; G = part of chromat in probably originating from a pyknoted polymorphonuclear; M = macrophage; C = cell-commencing neuronophagia; L = lymphocytes.

Fig. 26. Microphotograph, \( \times 800 \). Rabbit No. 30 dead 36 days after cerebral inoculation. Spinal ganglion in the lumbar region. M = mononuclear cells infiltrating. D = commencing neuronophagia; V = vessel surrounded by perivascular 'cuff'.

Fig. 27. Microphotograph, \( \times 5 \). Rabbit 140A dead 9 days after intracerebral infection with glycerinated virus of passage. Section of the terminal part of the spinal nerve with roots of the sciatic nerve and corresponding ganglion. M = spinal cord; R = nerve root; G = spinal ganglion; S = sciatic nerve. In sections cut in this way the lesions reported above can be seen as a whole.

Fig. 28. Microphotograph, \( \times 360 \). Rabbit 140A. Longitudinal section of the spinal nerve near the popliteal region. A perivascular 'cuff' is seen. V = lumen of vessel; M = infiltrating mononuclear cells; L = lymphocytes in the thickness of the nerve.

Fig. 29. Microphotograph, \( \times 150 \). Macacus rhesus No. 1. Section of the parietal lobe. C = cerebral cortex; M = mononuclear menigitis; V = blood-vessel cut longitudinally with walls infiltrated with mononuclear cells.

Fig. 30. Microphotograph, \( \times 120 \). Macacus rhesus No. 3. Perivascular cuffing of the frontal lobe of the cerebral cortex. V = lumen of vessel; P = perivascular infiltration.

Fig. 31. Microphotograph, \( \times 150 \). Macacus rhesus No. 1. Section of the parietal lobe of the cerebral cortex. Pseudo gamma (nodule) produced by mononuclear cell with a small vessel in the centre. (Very intense process of perivascular infiltration) V = blood-vessel; E = nodule formed by mononuclear cells.

Fig. 32. Microphotograph, \( \times 360 \). Macacus rhesus No. 1. Section through lumbosacral ganglia. Small area of mononuclear cells. N = neuron; L = lymphocytes.

Fig. 33. Microphotograph, \( \times 150 \). Macacus rhesus No. 1. Vascular infiltration at terminal part of the spinal cord. V = lumen of blood-vessel; M = muscular cell; P = infiltrating mononuclear cells.

Fig. 34. Microphotograph, \( \times 360 \). Macacus rhesus No. 1. Posterior root of spinal nerve. Interstitial infiltration and perivascular 'cuffing'. V = lumen of blood-vessel; M = mononuclear cell in the process of perivascular 'cuffing'; m = macrophage.

Fig. 35. Microphotograph, \( \times 700 \). Macacus rhesus No. 3. Spinal ganglion in cervical region. Corpuscles (type Joest-Degen) surrounded by a halo in the nerve-cells of the ganglion. C = intranuclear corpuscle of Joest-Degen; N = nucleus.
DESCRIPTION OF PHOTOGRAPHS, ETC.

PLATE I

Fig. 36. Microphotograph, (×36, enlarged 2½ diameters). Macacus rhesus No. 1. Section of spinal ganglion in lumbar region of spinal cord showing the profound changes which predominate the peripheral parts of the nervous system. (i)=intraneural corpuscle (Joest-Degen) with surrounding halo; n=nodule of mononuclear cells replacing destroyed neuron; d=degenerated ganglion cells; np=neuronophagia; ic=peri- cellular infiltration; t=interstitial infiltration; c= capsule of ganglion.

Fig. 37. Microphotograph, ×55. Macacus rhesus No. 3. Section through the brachial nerve. Perivascular 'cuffing' and interstitial infiltration. G=nerve sheath; V=perivascular 'cuffs'; I=interstitial infiltration.

Fig. 38. Microphotograph, ×460. Macacus rhesus No. 1. Transverse section of the sciatic nerve several centimetres from its exit to the greater sciatic foramen—perivascular 'cuff'. V=lumen of blood-vessel; E=vascular endothelium; L=lymphocytes.

Fig. 39. Microphotograph, ×55, (photograph enlarged 2½ diameters). Macacus rhesus No. 1. Transverse section of sciatic nerve after its exit from the greater sciatic foramen. V=large vessel with perivascular infiltration; v=small vessels showing same phenomenon; l=lymphocytes (interstitial infiltration).

COLOURED PLATES

PLATE I

Fig. 1. Staining, toluidin blue-eosin. Obj. 5 mm., oc. 2, ×260. Rabbit 295 dead 19 days after intracerebral infection. Cellular degeneration in the medulla oblongata. N=neuron in normal state; C=degenerated nerve-cell—tigrolysis—oxyphilia—nucleus eccentric; E=degenerated neuron; F=fragment of nerve-cell; L=lymphocytes.

Fig. 2. Staining, Mann. 1/12 oil immersion, oc. 4. Rabbit 25 dead 35 days after cerebral inoculation. Oxyphilic corpuscle surrounded by a halo in a nerve-cell in the Ammon's horn. C=corpuscle (type Joest-Degen) surrounded by a halo.

Fig. 3. Staining, Mann. 1/12 oil immersion, oc. 4. Rabbit 80× dead 28 days after the inoculation into the brain. N=nucleus of neuron repulsed by the 'cyst'; n=nucleolus; C='Cyst', this 'cyst' is the degenerated nucleus of a mononuclear cell which has succeeded in penetrating the nerve-cell.

Fig. 4. Staining, toluidin blue-eosin. 1/12 oil immersion, oc. 4. Guinea-pig 82× dead 82 days after cerebral infection. Section of medulla oblongata. N=nucleolus; C=corpuscle of Joest-Degen; H=halo around the corpuscle.

Fig. 5. Staining, Mann. 1/12 oil immersion, oc. 4. Rabbit 209 dead 71 days after the inoculation of the virus into the testicle. Neuron of the anterior horn of the spinal cord in the lumbar region. C=Intraneural corpuscle (Joest-Degen); N=nucleolus.

PLATE II

Fig. 1. Staining, toluidin blue-eosin. 1/12 oil immersion, oc. 1. Rabbit 243 dead 22 days after inoculation into the brain. Section of the cord anterior horn showing degeneration of the nerve-cells. V=small blood-vessel; P=protoplasm of degenerated nerve-cells; D=protoplasm debris; O=vacuole in the cytoplasm of a degenerated neuron; C=cytoplasm; L=lymphocytes.

Fig. 2. Staining, Mann. 1/12 oil immersion, oc. 4. Macacus rhesus No. 1. Neurons of the anterior horn of the thoracic region of the cord containing corporalces (Joest-Degen type). N=nucleus of the nerve-cell; n=nucleolus; C=corpuscle (Joest-Degen) surrounded by a halo. At the periphery of the corpuscle a blue staining area is seen.

PLATE III

Fig. 1. Staining, Mann. 1/12 oil immersion, oc. 2. Macacus rhesus No. 1. Pyramidal cells of the frontal lobe containing oxyphilic corporpales surrounded by a halo. N=nucleolus; C=corpuscle (Joest-Degen).

Fig. 2. Staining, Mann. 1/12 oil immersion, oc. 4. Rabbit 298 dead 39 days after intracerebral inoculation. Cervical region of the cord: anterior horn. Formation of intraneural corporpales in the interior of the nucleus of the neuron. N=nucleolus; C=oxyphilic corporpales; a study of the process of the
formation of the corpuscles of Joest-Degen has shown that several small oxyphilic corpuscles fuse together to form one large corpuscle surrounded by a halo.

Fig. 3. Staining, orange G. eosin, polychrome blue (Unna). 1/12 oil immersion. Guinea-pig 96x dead 19 days after intracerebral inoculation. A lymphocyte has penetrated into the cytoplasm of a nerve-cell of the Cornu Ammonis. N=nucleus; L=lymphocytes.

Fig. 4. Staining, Mann. 1/12 oil immersion. Rabbit 80A dead 28 days after intracerebral infection. Mid-brain, oxyphilic degeneration of the nuclear chromatin forming a homogeneous 'block'. C=degenerated nucleus; N=nucleolus; S=satellite cell.

Fig. 5. Staining, Mann. 1/12 oil immersion. Macacus rhesus No. 1. Spinal ganglion of the lumbar region. Corpuscles of type Joest-Degen in the nucleus of the ganglion cells. C=intranuclear corpuscle surrounded by a halo; N=nucleolus.

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ILLUSTRATIONS
Fig. 26
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See also SHOCK, SURGICAL.

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1928

Price 5s. net.
Modification de certaines propriétés d'une souche de virus d'encéphalo-myélite enzootique (maladie de Borna), 
entretenu pendant près de cinq années par passage sur lapin,

par S. Nicolau et I.-A. Galloway.

Dans la présente note nous désirons attirer l'attention sur les modifications subies par une souche de virus de la maladie de Borna, pendant les années où nous l'avons étudiée (1*). A l'origine, cette souche de virus, inoculée aux lapins par voie sous-duremérienne, conférait la maladie après une moyenne de trois semaines d'incubation, et les animaux mouraient au bout de 14 jours de maladie (2*). Nos expériences sur l'encéphalo-myélite enzootique remontent à 1926. En août 1927, nous estimions que le virus introduit dans le cerveau des lapins, amène la mort en moyenne en 28 jours chez les animaux qui pèsent moins de 1.500 gr. (25 lapins), et en 36 jours chez les lapins dépassant le poids de 1.500 gr. (25 lapins) (3*). Plus tard, dans une monographie contenant l'ensemble des expériences faites sur la maladie de Borna au « National Institute for Medical Research » (4*), nous avons donné un tableau où l'on voyait que les lapins inoculés dans le cerveau avec le virus de passage, succombaient en moyenne en 32 jours. Depuis, nous avons remarqué que cette moyenne est devenue plus longue. Un relevé fait dans nos cahiers sur plus de 400 lapins infectés par voie sous-duremérienne, nous montre que ce virus se comporte jusqu'à un certain point comme le virus herpétique, étudié par ailleurs, par l'un de nous (5*). En effet, le tableau suivant montre qu'au cours de l'année 1927, les lapins inoculés dans le cerveau succombaient en moyenne en 32 jours (50 lapins) ; en 1928, la mort survient en moyenne après 36 jours (246 lapins : 127 inoculés à l'Institut Pasteur de Paris, et 119 à l'Institut de Londres) ; en 1929, tant à Paris qu'à Londres, la virulence de la souche s'est accrue (6*) ;

(1*) Cette souche a été mise obligément à notre disposition par le Prof. Zwick en 1926. Le savant allemand l'avait isolé du système nerveux d'un cheval mort de la maladie spontanée.
(3*) Nicolau et Galloway. Borna Disease, etc. London, His Majesty's Stationery Off., 1928.
(4*) Nicolau. C. R. de la Soc. de biol., 1926, t. 95, p. 190.
(5*) Diminution de virulence de la souche ou augmentation de la résistance du lapin ? Nous penchons plutôt vers la première interprétation.
la mort des animaux inoculés a lieu, en moyenne, 3 jours plus tôt que l’année précédente, à savoir en 33 jours (68 Lapins). Par contre, en 1930, l’incubation de la maladie expérimentale devient plus longue, ainsi que la maladie elle-même, et il faut attendre en moyenne 44 jours pour voir mourir les Lapins inoculés (7*). Dans les quatre premiers mois de l’année 1931, à l’Institut Pasteur, les 11 Lapins infectés par voie cérébrale avec le virus de l’encéphalo-mylérite enzootique, sont morts en moyenne dans un délai de 45 jours.

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Ajoutons, qu’à Paris, en 1930, pour la première fois depuis que nous manipulons le virus de la maladie de Borna, nous avons été surpris de constater au cours de nos passages, la survie de deux Lapins infectés par voie intracérébrale avec du virus frais. Ces faits montrent, d’une façon générale, que la virulence d’une souche de virus de la maladie de Borna, loin de se « fixer » et abrégant l’incubation chez l’animal utilisé pour les passage — ainsi que le fait d’ordinaire le virus rabique — s’atténue la longue, à l’exemple du virus herpétilique. Aussi, comme il est paru à l’auro le virus polioémyélitique, le grume de l’encéphalo-mylérite enzootique peut avoir dans certaines années plus de virulence que dans l’année qui a précédé ou dans celle qui suit. L’atténuation de la virulence de notre souche de virus a été plus marquée à Paris qu’à Londres. Ceci peut tenir à la différence des races des Lapins utilisés pour les passages.

Au fur et à mesure que le nombre des passages augmentait, nous avons constaté un autre fait intéressant : une diminution notable dans le nombre et la dimension des corpuscules de Josse-Degen. A l’entretien de ce que nous avons observé, il y a 5 ou 4 ans, ces inclusionssie même actuellement surtout dans les cellules nerveuses de l’écorce et rarement dans la corne d’Ammon. Chez les animaux inoculés à l’Institut Pasteur, par exemple, la

(7*) Des expériences publiées récemment par Hoffman Donald (Journ. exp. Med., 1931, t. 52, p. 153) montrent que le temps nécessaire pour provoquer la mort dans la maladie expérimentale du Lapin inoculé par voie squelettique, est actuellement en moyenne de 53,7 jours (12 Lapins).
La taille de ces corpuscules est devenue de plus en plus petite et nous pouvons affirmer que, depuis près de 2 années, nous n'avons plus rencontré de beaux corps de Jœst volumineux comme ceux qui sont figurés dans notre travail publié dans les Annales de l'Institut Pasteur (8*). Aussi, il n'est pas rare de chercher parfois 10 minutes sur une préparation avant de trouver une inclusion caractéristique, quoique de dimension réduite. Cette diminution du nombre et de la taille des inclusions est moins marquée chez les animaux inoculés à Londres. Ces différences de comportement d'une même souche de virus, pour une espèce animale déterminée, mais dans des contrées différentes, rappellent les différences signalées par Levaditi entre le comportement de son virus herpético-encéphalitique, à New-York et à Paris.

Malgré les modifications relatées plus haut de la virulence de la souche de virus de Borna en notre possession, et réserve faite pour les corps de Jœst, il faut noter que l'aspect des altérations histologiques du névraxe des Lapins morts de la maladie expérimentale est actuellement le même que celui décrit par nous dans des travaux antérieurs.

(Institut Pasteur de Paris et National Institute for Medical Research, Londres.)

EFFECT OF ULTRA-VIOLET LIGHT ON THE VIABILITY OF THE VIRUS OF FOOT AND MOUTH DISEASE.

I. A. GALLOWAY, B.Sc., M.R.C.V.S.,

AND

A. EIDINOW, M.B., B.S.Lond.

From the National Institute for Medical Research, London.
ULTRAFILTRATION ET SES APPLICATIONS À L'ÉTUDE DES VIRUS

CHAPITRE EXTRAIT DE

LES ULTRAVIRUS DES MALADIES HUMAINES

SOUS LA DIRECTION DE MM. C. LEVADITI ET P. LÉPINE

LIBRAIRIE MALOINE
PARIS - 1938
INTRODUCTION

L’ultrafiltration peut être définie comme étant une filtration à travers des gels dont les pores, ou les interstices, sont capables de déterminer une rétention totale ou partielle des espèces colloïdales présentes dans un système dispersé.

Les méthodes d’ultrafiltration peuvent être employées pour le fractionnement, et l’étude de la composition d’un système dispersé, pour préciser les données nécessaires à la mesure de la taille des particules dispersées, et enfin, pour la purification et la concentration de systèmes dispersés, tels que des suspensions de virus, de bactériophages ou d’enzymes.

L’étude de l’ultrafiltration a été étroitement reliée à celle de la dialyse et, à un degré moindre, à celle de l’osmose. Sanarelli (1891) fut le premier à introduire l’emploi de sacs en collodion dans la pratique bactériologique. Salimbeni, ayant travaillé dans le laboratoire de Sanarelli, enseigna la méthode de préparation des sacs en collodion aux travailleurs de l’Institut Pasteur. Par la suite, Metchnikoff, Roux et Salimbeni (1896) placèrent des sacs en collodion contenant des cultures de vibion cholérique dans la cavité péritonéale de cobayes et, se basant sur le fait que ces animaux succombaient avec des symptômes toxiques, supposèrent que le micro-organisme produisait une toxine. Roux, Nocard, Salimbeni, Borrel et Dujardin-Baumetz (1898) utilisèrent des sacs en collodion qu’ils introduisirent dans le péritoine de lapins, pour essayer de cultiver le micro-organisme de la pérripneumonie des bovidés.

A peu près à la même époque, Martin (1896) (67), se servit d’une bougie en porcelaine imprégnée de gélatine, ou d’acide silicique, pour séparer les colloïdes des cristalloïdes. Harden et Young (1906, etc.) (53), employant des ultrafiltres préparés de la même manière, réussirent à séparer une coenzyme de la zymase. Manea (1904) (65), sur les conseils de Borrel, utilisa des sacs en collodion
pour étudier la filtrabilité des toxines et antitoxines tétaniques et diphtériques. Enfin, en 1904, Malfitano (64), se servit également de sacs en collodion pour l'étude de solutions colloïdales, et en 1905, Lévy (63), utilisa l'ultrafiltration pour l'étude des enzymes.

I. TYPES D'ULTRAFILTRES EMPLOYÉS GÉNÉRALEMENT AU COURS DES PREMIÈRES ÉTUDES SUR LES PROPRIÉTÉS FILTRANTES DES VIRUS ET DES BACTÉRIOPHAGES

1° Membranes et filtres de Bechhold.

En 1907 et 1908, Bechhold (8, 9, 10) qui créa le terme « ultrafiltration », exposa la première étude systématique de la question. Il fut le premier à préparer une série de membranes de porosités graduées et à essayer de mesurer les dimensions de leurs pores. Il insista sur le rôle de l'adsorption et d'autres facteurs physiques dans la filtration.

Les membranes de Bechhold sont réalisées par l'imprégnation de papier filtre durci avec du collodion acétique. La graduation de la porosité est obtenue en variant la concentration de la nitro-cellulose dans la solution-stock de collodion. Le processus de gélification est effectué par l'immersion des membranes dans l'eau. Cette technique de préparation a, par la suite, comporté des perfectionnements [Elford, 1929 (22)]. L'immersion du papier filtre est faite dans l'acide acétique et précède l'imprégnation, qui a lieu dans le vide, ceci pour éviter une rétention d'air par le papier, laquelle pourrait se traduire par la formation de trous microscopiques dans la membrane finale. Le papier imprégné est passé entre des rouleaux de verre, ou de nickel recouvert d'or, avant la gélification, de manière à éviter la formation d'une couche de collodion d'épaisseur irrégulière et excessive. Du fait que les membranes de Bechhold sont faciles à préparer et qu'elles peuvent présenter une échelle de porosité allant de 1 à 5 μ, à 10 μ, on les a beaucoup employées dans les études bactériologiques, y compris celles concernant les propriétés filtrantes des virus et des bactériophages. Cependant, elles présentent de nombreux inconvénients qui rendent les études comparatives difficiles. Elles sont plutôt plus épaisses que de simples membranes en collodion, l'épaisseur augmentant lorsque la porosité décroit, et il est relativement difficile de reproduire le même diamètre de pores d'une membrane à l'autre. Elles ont également le sérieux défaut de présenter une variabilité considérable dans les dimensions de leurs pores. Elford (1931) (23) a calculé que dans n'importe quelle membrane, les pores les plus larges peuvent avoir un diamètre de 10 à 20 fois plus grand que le diamètre moyen.

Le filtre de porcelaine imprégnée de Martin a été déjà mentionné. Un autre type similaire d'ultrafiltre, très employé en pratique bactériologique, est celui de Bechhold et König, qui consiste essentiellement...
ment en une membrane de Bechhold où le papier filtre est rem- 
placé par de la porcelaine. On s’est servi de creusets, de récipients à 
evaporation, et d’autres récipients à fond non vernis, imprégnés de 
collodion acétique, la porosité variant, ainsi que dans les membranes 
de Bechhold, suivant la concentration de la solution de collodion. 
Les ultrafiltres en porcelaine imprégnée sont extrêmement épais 
Et peuvent ainsi retenir une grande partie des virus, ou d’autres 
substances, par adsorption. Comme leur calibrage est impossible, 
ces filtres sont seulement utilisables pour la filtration de grandes 
quantités de matériel qui ne nécessite pas un contrôle strict des 
membranes.

Par ailleurs, les membranes en collodion acétique doivent être du 
type « membrane imprégnée » à cause de la fragilité du gel, alors que 
les membranes en collodion alcool-éther sont suffisamment résis-
tantes pour se passer de supports. Elles peuvent être faites en forme 
de sacs, ou de disques.

2° Sacs en collodion.

Les sacs en collodion ont été fréquemment utilisés sans la re-
cherche biologique, y compris l’étude des virus par Levaditi et Ni-
colau (1923) (54) et d’autres auteurs, des toxines bactériennes et des 
bactériophages. Ils ont eu une vogue toute particulière, due à la 
facilité de leur préparation, au fait qu’ils offrent une large surface 
du tube. Les expériences de filtration et à la possibilité de les employer sans avoir recours à un 
appareil de maintien, comme cela est nécessaire dans le cas d’une 
membrane plane. Cependant, ils ne conviennent qu’à des expériences 
de filtration fractionnée, dans lesquelles un calibrage exact de la 
porosité n’est pas indispensable. Il n’est pas facile, par suite de la 
rapidité de l’évaporation des solvants et de la grande viscosité du 
collodion, de faire successivement plusieurs sacs de même porosité. 
De plus, la porosité d’un sac varie d’un endroit à l’autre, elle est 
plus grande vers la partie ouverte que vers l’extrémité fermée.

Les sacs sont faits de la façon suivante : on remplit de collodion 
un tube à essai que l’on renverse de façon à laisser une couche sur 
les parois intérieures du tube. Après l’évaporation des solvants, le 
tube est plongé dans l’eau, assez longtemps pour que le coll-
odion forme un gel. Le sac est retiré, puis lavé jusqu’à disparition 
de toute trace de solvant. Une autre méthode consiste à faire le sac 
sur la paroi externe du tube auquel on imprime un mouvement de 
rotation afin d’obtenir une couche uniforme. Pour faciliter le retrait 
du sac, il est préférable d’avoir un renflement à la partie supérieure 
de rotation ; lorsque le gel est formé, on pratique une incision circulaire 
sur ce renflement et le sac est retourné comme un doigt de gant.

On varie la porosité des sacs en collodion en modifiant le rapport 
alcool-éther du solvant, et en faisant varier le temps d’écoulement 
de la durée de l’évaporation. L’addition de réactifs divers aux solutions 
de collodion influe sur la porosité des membranes : la glycérine, 
ghi de ricin, l’acide lactique et l’acétate d’éthyle augmentent la 
porosité, alors que les alcools éthylique et amylique la diminuent.
3° Membranes planes en collodion.

En 1907, Bigelow et Gemberling (13) décrivent une méthode de fabrication des membranes en collodion alcool-éther, planes. Les membranes graduées de Zsigmondy et Bachman (1918) (88) sont faites selon un procédé semblable et furent vendues par E. de Haen, G. m. b. H. Hannover, d'où le nom de « membranes de Haen », et par la suite par la Membranfilter-Gesellschaft m. b. H. Göttingen, sous le nom de « Membranfilter » et d' « Ultrafeinfilter ». Ces dernières membranes ont été employées par plusieurs expérimentateurs pour des filtrations bactériologiques. Cependant, bien qu'elles soient utiles dans certains cas, la constance de la porosité d'une membrane d'un calibre donné n'est pas suffisante pour que l'on puisse les utiliser dans des expériences de mesure des différents virus.

II. SÉRIE DE MEMBRANES GRADUÉES D'ELFORD MEMBRANES « GRADOCOL »

Asheshov, en 1925 (2), fut le premier à utiliser des réactifs volatiles pour modifier la porosité des membranes ; il constata qu'en additionnant des quantités variables d'acétone à la solution-mère de collodion, on augmentait la porosité, alors que l'addition d'alcool amylifique diminuait cette porosité.

Par la suite, Elford en 1931 (23) entreprit une étude systématique de l'effet des différents réactifs sur la porosité des membranes en collodion. Il observa que l'acétone et l'alcool amylifique ont des actions dissolvantes contraires, à l'égard de la nitrocellulose. Alors que l'un ou l'autre de ces réactifs, combiné à l'alcool éthyle et à l'éther, est un excellent solvant, leur présence simultanée à des concentrations données détermine la coagulation de la nitrocellulose. Cette action antagoniste de ces deux composés a été utilisée pour déterminer une agrégation progressive des particules de cellulose pendant le temps d'évaporation, au cours de l'élaboration des membranes.

Elford emploie une solution standard de nitrocellulose dans l'éther et l'alcool éthyle, à laquelle il ajoute en proportions définies, de l'alcool amylifique et de l'acétone. Lorsque cette solution est versée sur des plaques de verre, de façon à obtenir l'évaporation des solvants, l'alcool amylifique, étant le moins volatile, atteint une concentration relative plus élevée et, en présence d'acétone, détermine une agrégation de la nitrocellulose, alors que l'alcool et l'éther restants maintiennent le processus de gel spontané. Les variations dans la porosité des membranes préparées avec un collodion standard, résultent des modifications définies de la teneur en eau, ou en acide acétique, en vue du résultat désiré. Les calibres intermédiaires plus fins sont obtenus en faisant varier la durée de l'évaporation, de sorte que l'on dispose d'une vaste échelle de porosités, le diamètre moyen des pores allant de 3 μ à 10 μ. Les membranes graduées d'Elford sont, jusqu'à présent, les plus satisfaisantes que l'on puisse avoir.
Les expériences poursuivies à l'aide de ces membranes à l'Institut National de Recherches Médicales de Londres par Elford et ses collaborateurs, ont été faites dans des conditions expérimentales uniformes, et ont démontré la valeur de l'ultrafiltration dans l'étude des virus. Jusqu'alors les expériences de filtration des virus et des bactériophages à l'aide de bougies et de différents types de membranes et de sacs en collodion, n'avaient fourni que des résultats particulièrement inconstants, suivis de maintes conclusions erronées. Citons comme exemple, d'une part les difficultés rencontrées dans les tentatives de filtration des virus de la vaccine, de la rage, de la maladie de Born, à l'aide de bougies, d'autre part, la détermination des chiffres aussi bas que, 2,3 à 2,5 m µ pour les dimensions du virus de la peste aviaire [Andriewsky 1915 (1)], et enfin, les conclusions de Modrow 1929 (71) qui, utilisant des membranes de Béchhold, affirmait que l'ordre de grandeur des particules du virus de la fièvre aphteuse, Vallée et Carré, type O était entre celui de la molécule d'hémoglobine et celui de l'albumine d'œuf, ou de la particule du tournesol, c'est-à-dire entre 2 et 3 m µ, étant ainsi inférieur à celui des virus Vallée et Carré type A et Waldmann et Trautwein, type C. Il est certain que dans les conclusions de ces expériences, il n'était tenu, pour ainsi dire, aucun compte, des divers facteurs que l'on sait, à l'heure actuelle, être indispensables au succès d'une filtration, soit, sur une membrane en collodion, soit sur des bougies [Galloway et Elford 1931 (46), Elford 1933 (24)].

Les membranes d'Elford ont maintenant été utilisées dans de nombreux instituts pour l'ultrafiltration des virus des plantes et des animaux, et aussi des bactériophages ; la parfaite concordance des résultats obtenus dans les différents laboratoires qui les ont expérimentées montre, entre autres, la facilité avec laquelle on peut reproduire une expérience par cette méthode. Dans certains cas, on a eu confirmation des dimensions de certains virus, par des recherches poursuivies indépendamment et simultanément dans deux laboratoires différents. [Cf. Virus de la poliomyélite : Elford, Galloway et Perdrau 1935 (38) et Theiler et Bauer 1934 (83) ; virus de l'encéphalite de Saint-Louis : Bauer, Fite et Webster (1934) (5), et Elford et Perdrau 1935 (39)]. Par conséquent, dans la discussion des résultats des expériences d'ultrafiltration des virus et des bactériophages, seuls les essais faits avec les membranes graduées d'Elford seront pris en considération. Mais avant d'entamer cette discussion, il est nécessaire de décrire la méthode de préparation des membranes et leur calibrage, l'appareil employé pour l'ultrafiltration et le mécanisme de l'ultrafiltration de systèmes dispersés, tels que les suspensions de virus.

1. PRÉPARATION DES MEMBRANES D'ELFORD

En 1931, Elford (23) donne une description complète de sa méthode de préparation et de calibrage des membranes ; d'autres détails sur le calibrage sont exposés dans un article d'Elford et

Nous essaierons de donner ici un aperçu général des aspects les plus essentiels du problème.

Il est nécessaire d'avoir, comme matériel de départ, une nitro-cellulose facilement utilisable et de qualité constante. Parmi les produits qui remplissent ces conditions on peut citer la celloïdine de Schering, « Nécol » N° 356 A/9 (un celloïdine standard de la Nobel Chemical Finishes Co. Ltd, Slough, England) et le « Parlond » (Du Pont, Amérique). Si l'on emploie la celloïdine de Schering, il faut préparer d'abord une solution stock, contenant 10 pour cent de celloïdine dans un mélange alcool (1 partie en poids), éther (1 partie) et acétone (2 parties). Les différentes étapes de la préparation des membranes sont les suivants :

a) Préparation d'une solution-mère de celloïdine à partir du « Nécol ».

Le Nécol est d'abord dilué avec un même poids d'acétone, ceci étant le Nécol-stock. On ajoute 10 cc. d'alcool amylique à 40 g. de solution stock de Nécol, ce mélange étant ensuite dilué avec son propre poids de celloïdine, d'alcool et d'éther (1 : 9 en poids) de cette solution désignée par 110/110 (1 : 9) est la solution mère de celloïdine. Dans des conditions standards (par exemple 75 cc. de celloïdine dans une cellule de 20 cm.), elle fournira une membrane dont les pores ont un diamètre moyen d'environ 0,8 µ. Pour augmenter la porosité et atteindre un diamètre de 2 à 3 µ, il est nécessaire d'ajouter 10 % d'eau à la solution mère de celloïdine. Les degrés intermédiaires peuvent être obtenus en graduant la quantité d'eau ajoutée à raison de 0,25 pour cent. Pour diminuer la porosité progressivement jusqu'à 10 m. µ, on ajoute jusqu'à 20 % d'acétone au mélange mère. Des variations de 0,5 pour cent sont communes. Les degrés intermédiaires plus fins s'obtiennent en modifiant le temps de l'évaporation.

b) Préparation des membranes.

On verse un volume connu de solution dans une cellule de verre horizontale peu profonde. On peut employer des cellules d'un diamètre de 20 à 40 cm ; elles sont faites de deux plaques de verre carrées plates et polies. L'une de ces plaques comporte un trou circulaire central de 20 à 40 cm. ; elle est scellée à l'autre par de l'albâtre d'étain, formant ainsi une cellule profonde de 1 cm. La plaque inférieure sur un triangle doit être fixée strictement horizontalement au moyen de vis. On trouve des photographies du type de cellules employées dans des publications de Bauer et Hughes (1934) (4), de Yaoi et Nakahara 1935 (85) et de Smith et Doncaster 1935 (80). Pour des cellules de 20 cm., on emploie de 50 à 75 cc.
de collodion; pour des cellules de 40 cm., la quantité de collodion est de 200 à 250 cc. On obtient ainsi des membranes dont l'épaisseur est de 0,15 à 0,20 mm. La préparation des membranes doit être effectuée à une température constante de 22°, réglée au moyen d'un régulateur électrique et de lampes chauffantes. L'entrée de la pièce (de 2,5 m. x 2,5 m. x 2 m.) consiste en une porte à glissières, munie d'une petite fenêtre, permettant de surveiller les processus d'évaporation, sans provoquer de mouvement dans l'atmosphère. La pièce utilisée par Elford à l'Institut National de Recherches Médicales de Londres ne comporte pas de dispositif spécial pour le contrôle de l'humidité, du fait qu'elle est située au centre d'un vaste bâtiment chauffé et que cette humidité se maintient à peu près stable ; mais le facteur humidité est très important du point de vue de la reproduction d'une membrane et ne doit pas être négligé. Lorsque l'évaporation des solvants est suffisante, le collodion se transforme en un gel. L'évaporation est continuée pendant un laps de temps de 1 à 3 heures, suffisant pour obtenir cette transformation spontanée en gel et la coagulation est achevée par immersion du collodion dans l'eau distillée. La membrane est ensuite lavée à l'eau distillée de façon à éliminer toute trace de solvants, cette opération dure 14 jours. Ce lavage doit être fait dans des vases stériles de façon à obtenir finalement des membranes stériles. De petites membranes circulaires sont coupées ensuite au moyen d'un emporte-pièce. Les bords du film sur une largeur de 2 cm. pour une membrane de 20 cm. et de 4 cm. pour une membrane de 40 cm., sont rejetés. En pratique, une feuille de 40 cm. peut être découpée en 30 à 40 disques, dont 5 ou 6 servent au calibrage, les autres étant ensuite utilisés pour les expériences de filtration.

2. CALIBRAGE DES MEMBRANES

Le procédé de calibrage des membranes a été décrit en détails par Elford en 1931 (23) et par Elford et Ferry en 1935 (32).

Pour définir les caractéristiques d'une membrane, il est nécessaire de calculer 1° le diamètre moyen des pores, 2° la distribution des différentes tailles des pores, 3° d'étudier son comportement dans la filtration de systèmes particuliers.

a) Diamètre moyen des pores : Le calcul du diamètre moyen des pores est basé sur le fait que l'on attribue à la membrane une structure simple et sur la validité de la loi de Poiseuille sur l'écoulement de l'eau à travers cette membrane. L'estimation du diamètre moyen des pores se fait par les mesures de l'épaisseur de la membrane, de la teneur spécifique en eau et de la vitesse d'écoulement de l'eau à travers la membrane.

b) Épaisseur de la membrane : L'épaisseur de la membrane est mesurée par un micromètre, réglé par un fil ressort (Zeiss) et gradué au 1/1000 de millimètre. On fait au moins 6 mesures successives de la membrane placée entre deux lames de verre d'épaisseur
connue, afin d'éviter la compression ou la déformation de la membrane et on prend la moyenne des résultats.

c) Teneur spécifique en eau : La teneur spécifique en eau se définit comme le volume d'eau contenu dans 1 cc. de membrane dont les pores sont remplis d'eau, ce qui est le cas d'une membrane lavée et équilibrée dans l'eau distillée. Cette teneur s'exprime selon la formule :

\[
S = \frac{W_w - W_d}{A t}
\]

dans laquelle :
- \( S \) = Teneur spécifique en eau
- \( W_w \) = Poids de la membrane lorsque les pores sont pleins d'eau
- \( W_d \) = Poids de la membrane sèche
- \( A \) = Surface transversale de la membrane
- \( t \) = Epaisseur de la membrane.

Procédé : On mesure l'épaisseur de la membrane à l'aide d'un micromètre. La surface peut être considérée comme égale à celle de l'emporte-pièce qui a servi à couper le disque, à moins que celui-ci n'ait été stérilisé, à la vapeur, ce qui entraîne une légère rétraction. Dans ce cas, la superficie doit être évaluée d'après la mesure du diamètre, cette dernière étant la moyenne d'au moins 6 lectures sur une règle graduée. Ensuite toute l'eau superficielle est absorbée par des papiers filtres de Schecher et Schull N° 575, ou Whatman N° 50. La membrane est immédiatement pesée sur un verre de montre et couvert, et ensuite mise sur le verre découvert dans un four à air chaud, à une température de 60°, jusqu'à poids constant. La température ne doit pas dépasser 70°, car une décomposition partielle de la nitrocellulose peut se produire. 6 heures sont généralement suffisantes pour cette dessication. Le verre de montre et la membrane sèche sont ensuite refroidis et pesés à nouveau. Il est également possible de calculer l'épaisseur de la membrane d'après les données obtenues, en estimant la teneur spécifique en eau selon la formule de Manegold (1930) (66) :

\[
V_w = \frac{W_d}{1.6}
\]

dans laquelle :
- \( t \) = épaisseur de la membrane.
- \( A \) = surface transversale de la membrane.
- \( V_w \) = \( W_w - W_d \) (voir ci-dessus) = volume d'eau contenu dans les pores.
- \( W_d \) = poids de la membrane sèche.

Le chiffre 1.6 représente la densité du collodion sec, et \( V_w + \frac{W_d}{1.6} \) exprime le volume total du disque saturé d'eau. L'emploi de cette formule permet un contrôle facile des données fournies par le micromètre.
d) Mesure de la vitesse d’écoulement d’eau : Elford, en 1931 (23) a défini ainsi la vitesse d’écoulement d’eau : — le volume qui passe à travers 1 cm² de la membrane, en 1 minute, à une température de 20° sous une pression de 100 cm. d’eau, l’épaisseur de la membrane étant de 0,1 mm. Cette vitesse peut être exprimée par la formule :

\[ F = \frac{Vt \times 60.000}{A \times T \times P} \]

dans laquelle

- \( P \) = pression en cm. d’eau.
- \( F \) = vitesse d’écoulement d’eau.
- \( V \) = volume d’eau en cc.
- \( T \) = temps d’écoulement en secondes.
- \( A \) = surface de la membrane en cm².
- \( t \) = épaisseur de la membrane en mm.

Un appareil permettant d’effectuer de telles mesures a été décrit par Elford en 1931 (23), Elford et Ferry en 1935 (32) et Bauer et Hughes en 1934 (4).

Dans le cas des membranes graduées d’Elford, \( F \) varie entre 35 et 0,000002, de sorte que l’appareil doit permettre la mesure de grands et petits volumes d’eau ainsi que l’application de hauteurs et de basses pressions. Pour les porosités les plus grandes, une pression de 10 cm. d’eau est suffisante ; cette pression peut, sans inconvénient, être portée à 350 cm. pour les membranes moins poreuses. La membrane doit être fixée solidement afin d’éviter les fuites, tout en évitant cependant de détériorer ses bords. La technique de cette mesure a été clairement décrite par Elford et Ferry 1935 (32).

8) Calcul du diamètre moyen des pores. — Ce diamètre peut être calculé d’après les valeurs de \( F \) (vitesse d’écoulement de l’eau) et \( S \) (teneur spécifique en eau), (voir ci-dessus) après introduction de constantes de dimensions, par l’expression simple suivante :

\[ j = \text{diamètre des pores en } \mu = 0,234 \sqrt[4]{\frac{F}{S}} \]

f) Répartition des différentes tailles de pores. — On peut avoir une idée de cette répartition par l’application de la loi de Cantor [Bechhold (1908) (10)].

La formule employée est la suivante :

\[ d = 4 \frac{r}{p} \]

dans laquelle

- \( d \) = diamètre du capillaire en cm.
- \( r \) = tension de surface eau-air en dynes-cm. à la température de mesure.
- \( p \) = pression critique en dynes-cm².

Si l’air est insufflé à travers une membrane mouillée, la simple observation visuelle de l’augmentation de la fréquence des bulles
à mesure que la pression de l'air s'élève permet une estimation grossière de la répartition des pores les plus larges. La pression à laquelle commence la formation des bulles détermine la taille maximum des pores, et le rapport de celle-ci à la taille moyenne des pores, établie d'après la vitesse d'écoulement de l'eau, est une indication, d'isoporosité ou d'hétéroporosité. Elford en 1931 (23) a montré que ce rapport descend jusqu'à 2 pour les membranes préparées selon sa méthode, alors que dans le cas des membranes en collodion acétique, il peut s'élever à 10 ou même 20.

3. STÉRILISATION DES MEMBRANES

Les membranes préparées de la manière exposée ci-dessus sont gardées dans l'eau distillée dans des bechers Pyrex à couvercle rodé et recouverts de papier à beurre. Elles peuvent être stérilisées par l'ébullition ou à la vapeur sans qu'il s'en suive une altération notable de leurs propriétés filtrantes. Il est cependant toujours indispensable de contrôler la standardisation de telles membranes après ce traitement, en déterminant à nouveau la valeur de l'écoulement d'eau (Voir ci-dessus F). La stérilisation à l'autoclave est une méthode trop brutale. En 1931, Elford (23) constate que, si les membranes, dont les pores ont un diamètre moyen de 2,0 µ à 0,5 µ ne sont que légèrement influencees par la stérilisation à l'autoclave, par contre les membranes de porosité moindre sont modifiées, et cela à mesure que l'on descend dans l'échelle des porosités ; ainsi une membrane de 62 mµ perd 10 % de sa porosité, alors qu'une membrane de 20 mµ en perd 25 %.

III. APPAREIL D'ULTRAFLTRATION

Pour le montage de la membrane, il faut un dispositif de serrage étanche, un vase contenant le système filtrant sous pression positive ou négative et un récipient pour recevoir le filtrat. Les types d'appareils indiqués par Elford sont représentés par les figures 1 : filtre à pression positive, 2 : parties détachées du filtre à pression positive et 3 : filtre à pression négative. Une description de l'appareil employé pour la filtration sous pression positive (fig. 1) est donnée par Elford dans un article de Bar- nard et Elford, en 1931 (3). Le vase de l'ultrafiltre est fait de cuivre fortement nickelé, de façon à éviter tout contact avec
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des métaux susceptibles d’avoir une action toxique ; il a une capacité d’environ 25 cc. La membrane filtrante est supportée par une plaquette métallique perforée et également nickelée. Un système d’écrou hexagonal maintient ensemble les différentes parties du filtre, ceci assurant une uniformité de distribution de la pression sur le caoutchouc qui assure la jointure effective avec la membrane. Tout danger de déchirure de la membrane est éliminé par un système spécial de fermeture.

Les différentes parties de l’ultrafiltre sont stérilisées à l’air chaud. Le filtre complet est monté dans une chambre stérile à lumière ultraviolette, ainsi que l’a indiqué Elford en 1929 (22). La filtration, avec ce type de filtre, s’opère sous une pression positive d’air ou d’azote. Ce filtre semble le plus propice aux travaux quantitatifs au cours desquels des échantillons successifs de filtrat doivent être recueillis. Grabar (1934) (51) a décrit un appareil pour le montage des membranes d’Elford dans lequel ni la suspension, ni le filtrat ne sont à aucun moment en contact avec autre chose que du verre. Ceci a son utilité dans certains cas.

Il est parfois commode, lorsque l’on veut filtrer de grandes quantités de liquide, d’employer l’appareil (fig. 3) à pression négative décrit dans le travail de Galloway et Elford (1936) (48).
Avant d'examiner les applications de l'ultrafiltration à l'étude des virus, il est nécessaire d'indiquer les facteurs qui influent sur l'ultrafiltration des systèmes dispersés en général.

IV. MÉCANISME DE L'ULTRAFILTRATION DES SYSTÈMES DISPERSÉS

Lorsqu'un système dispersé passe à travers une membrane ultra-filtrante, la phase dispersée peut se trouver moins concentrée dans le filtrat pour plusieurs raisons : 1. parce qu'elle est adsorbée par la surface de la membrane et ses pores (adsorption primaire); 2. parce qu'elle ne peut pas traverser les pores, à cause du colmatage; 3. parce qu'enfin elle est retenue mécaniquement sur le filtre (tamisage).

L'observation de la marche d'une filtration projette quelque lumière sur le problème, les divers mécanismes pouvant, en effet, varier pendant l'opération. Dans les essais faits avec des virus, on examine de petits échantillons successifs de filtrat. La concentration relative du filtrat est définie comme le rapport entre la concentration d'un petit échantillon de filtrat et celle de la suspension originale; et si l'on suit ce rapport au cours de la filtration, on peut établir une courbe. L'étude des modifications de la courbe de filtration permet d'ajuster les conditions expérimentales et d'éliminer autant que possible l'adsorption primaire et le colmatage de façon à obtenir les résultats désirés.

1. ADSORPTION PRIMAIRE

Dès 1920, Bechhold (11) a insisté sur le rôle de l'adsorption dans la rétention de la phase dispersée hors du filtrat. Un certain nombre de facteurs influencent l'adsorption primaire; examinons-les.

a) Action de la concentration. — Plus la solution filtrante est diluée, plus le volume qui doit traverser la membrane avant que sa capacité adsorbante soit satisfaite est grand, et plus long est le temps avant que la phase dispersée commence à apparaître dans le filtrat.

En 1931, Galloway et Elford (46) étudiant le virus de la fièvre aphteuse, ont précisé les détails de cette action et montré que, au delà d'une certaine concentration, l'adsorption et la concentration sont à peu de chose près inversement proportionnelles. En 1933, Elford (24) enregistre des résultats semblables dans la filtration de colorants. Les virus sont toujours utilisés en suspensions relativement diluées; si donc on recueille des volumes limités de filtrats, il est évident que la présence ou l'absence de virus dans le filtrat dépendra de la concentration initiale. Ceci a été confirmé au cours d'expériences d'Elford et Andrewes (26) sur le virus vaccinal en 1932, et d'Elford sur le B. prodigiosus en 1933 (24).
b) Pression. — L'augmentation de la pression réduit la zone d'adsorption primaire, ainsi que l'a démontré Elford en 1933 (24) avec le colorant « bleu de nuit ».

c) Epaisseur de la membrane. — Plus la membrane est épaisse, plus la capacité d'adsorption est grande et, par conséquent, plus le volume qui doit filtrer avant l'apparition de la phase dispersée dans le filtrat est important. En 1931, Galloway et Elford (46), travaillant avec le virus de la fièvre aphteuse, ont établi que le facteur d'adsorption est à peu près proportionnel à l'épaisseur de la membrane. Ils ont montré que le degré d'adsorption dans la filtration d'un virus à travers 3 membranes superposées était équivalent à l'adsorption qui a lieu lorsqu'on filtre le virus successivement à travers trois membranes simples.

d) Substances agissant sur la tension superficielle. — La présence de substances agissant sur la tension superficielle, dans un système dispersé, diminue la zone d'adsorption primaire. L'action d'un bouillon bactériologique, en tant qu'agent influençant la tension superficielle, a été observée en premier par Ward et Tang en 1929 (84) ; ces auteurs démontrèrent l'influence du bouillon sur la perméabilité des bougies filtrantes à l'égard des virus de la vaccine et de l'herpès. Le contraste, entre l'action sur la tension superficielle, du bouillon et d'une solution aqueuse tamponnée de phosphates était clairement mis en lumière par la filtration du virus de la fièvre aphteuse à travers des membranes en collodion et des filtres de Seitz [Galloway et Elford, 1931 (46)]. Cette action est probablement dû à un revêtement des parois des pores et de la surface des particules par les substances actives, déterminant une sorte de lubrification.

e) Elimination de l'adsorption primaire. — L'action de l'adsorption primaire peut, éventuellement, être annihilée par la filtration d'un volume suffisant de la phase dispersée à travers la membrane. La capacité adsorbante de l'ultrafiltre est satisfaite plus rapidement : 1° grâce à une concentration initiale plus élevée de la suspension à filtrer ; 2° par l'application d'une pression élevée ; 3° par l'emploi de membranes minces et 4° par la présence de substances modifiant la tension superficielle, telles que celles qui existent dans le bouillon, par exemple.

2. COLMATAGE

Cette question a été discutée en détails par Ferry en 1936 (44) ; cet auteur a montré, en particulier, que dans la filtration de substances à tendances lyophobiques, le phénomène est étroitement lié à l'instabilité colloïdale.

a) Particules étrangères. — Cependant, la présence de particules étrangères plus grosses que celle du système dispersé, peut déterminer le colmatage par obstruction mécanique, même dans des conditions qui ne favorisent pas le colmatage par la phase
dispersée elle-même, par suite d’instabilité colloïdale. Ainsi, Erbe en 1932 (42), a observé, au cours d’expériences sur la vitesse d’écoulement d’eau, que même la filtration d’eau distillée peut être entravée par un colmatage dû à des particules de poussière. Dans la filtration des virus, le colmatage est, avant tout, provoqué par la présence de particules étrangères plus grandes que celles du virus.

b) Concentration. — Dans les solutions protéiques, la concentration moléculaire peut être assez élevée pour empêcher la filtration par colmatage [Elford et Ferry, 1934 (31)]. Les suspensions les plus riches que l’on peut obtenir dans le cas d’un virus correspondent à une concentration moléculaire qui ne serait pas appréciable, pour une solution protéique, par le test de l’acide sulfosalicylique; en outre on a montré que la filtrabilité du virus est améliorée par l’augmentation de la concentration, surtout lorsque l’on se rapproche du point terminal de la filtration. Plus la concentration du virus est forte, plus la capacité adsorbante de la membrane est satisfaite rapidement et, par suite, plus le virus a de chance de passer dans le filtrat avant le colmatage.

c) Epaisseur de la membrane. — Le colmatage est plus fréquent lorsque l’on emploie des membranes épaisses, du fait que le rapport entre la hauteur des pores et leur diamètre est plus grand.

d) Substances agissant sur la tension superficielle. — La présence de telles substances diminue les possibilités de colmatage, ainsi qu’Elford et Ferry l’ont démontré en 1934 (31), dans leurs expériences sur les protéines.

e) Pression. — Si le système (suspension de virus) à examiner est insuffisamment débarrassé des particules plus grosses que le virus lui-même, l’augmentation de la pression favorisera le colmatage et entravera ainsi la filtration plutôt qu’elle ne la facilitera. D’autre part, si l’on emploie de basses pressions, les forces de surfaces, dans les canaux capillaires, exerceront leur action maximum.

f) Elimination du colmatage. — On peut déduire des données précédentes, que, dans la filtration des virus, les conditions suivantes permettent de diminuer le colmatage. Ce sont: 
a) l’absence de particules étrangères ; b) l’usage de membranes minces ; c) la présence de substances agissant sur la tension superficielle, et d) l’emploi de basses pressions. Lorsque l’on opère dans des conditions optima, de façon à supprimer pratiquement le colmatage et à atteindre rapidement la capacité adsorbante de la membrane, la filtration normale, c’est-à-dire le tamisage mécanique, aura lieu.

3. TAMISAGE

Dans une ultrafiltration bien réussie, le mécanisme qui agit sur la rétention de la phase dispersée est un tamisage mécanique. Lorsque l’on étudie les propriétés filtrantes d’un virus, on filtre
des volumes égaux de la suspension à travers des membranes de différentes porosités. Il est préférable de fractionner le système à étudier et de pratiquer les examens en descendant l'échelle des porosités jusqu'à ce que l'on atteigne le point terminal, c'est-à-dire la plus grande porosité capable de retenir complètement le virus.

On prétend souvent que, lorsque l'on filtre un système monodispersé à travers une membrane isoporeuse et que la filtration s'opère normalement, ou bien la phase dispersée traverse le filtre à une concentration inchangée, ou bien elle est complètement retenue. L'existence d'un tamisage, c'est-à-dire d'une rétention partielle de la phase dispersée dans l'ultrafiltration n'est pas un critère de polydispersion. Ainsi que l'a expliqué Ferry en 1936 (43), le tamisage se produit dans l'ultrafiltration des protéines [Elford et Ferry 1934 (31)] qui sont pratiquement monodispersées comme il a été montré par centrifugation [Svedberg (1930) (81), Mc Farlane (1936) (68)]. Ferry, en 1936 (43), a insisté sur le fait que le phénomène du tamisage dans l'ultrafiltration peut être prévu, d'après des données statistiques. L'hétérodispersion peut être démontrée, soit par l'apparition du tamisage sur une plus grande échelle de porosités que dans le cas d'un système monodispersé, soit par l'ultrafiltration de fractions séparées par centrifugation, ou par filtration préalable [Bechhold (1907) (8)].

Il est important de prendre ces faits en considération, lorsque l'on examine les résultats d'expériences de filtration de virus ou de bactériophages. Ainsi, Ferry en 1936 (43), a montré que des courbes établies d'après une expression simple pour la constante de tamisage calculée en rapport avec la porosité de la membrane calibrée et la taille des particules, concordent pratiquement avec les données expérimentales fournies par l'ultrafiltration du virus de la fièvre aphteuse [Galloway et Elford., 1931 (46)] ; il est donc inutile de concluir à l'existence de particules de taille supérieure à la moyenne, ou à celle d'agrégats de virus retenus par les membranes de grandes porosités, ou encore à un certain degré d'hétéroporosité du filtre utilisé. Il est intéressant de noter que des essais récents de centrifugation [Schlesinger et Galloway, 1937 (76)], ainsi que des essais combinés de centrifugation et d'ultrafiltration [Elford et Galloway, 1937 (77)], ont apporté des preuves nouvelles de la monodispersion du virus de la fièvre aphteuse. Il est probable, d'après les constatations faites, que les autres virus et les bactériophages sont des systèmes pratiquement monodispersés.

Il est évidemment impossible dans un résumé succinct sur l'ultrafiltration des virus, de discuter plus à fond le mécanisme de l'ultrafiltration d'un système dispersé, et d'examiner la question de la structure et des caractéristiques des membranes. On trouvera, du reste, un exposé plus complet des processus physiques qui régissent l'ultrafiltration, ainsi que de ses applications, dans les publications et revues récentes d'Elford [1933 (24)], Grabar [1935 (52)] et Ferry [1936 (44)].
V. L’ULTRAFILTRATION DE SYSTÈMES BACTÉRIOLOGIQUES, TELS QUE LES SUSPENSIONS DE VIRUS OU DE BACTÉRIOPHAGES

Nous n'essaierons pas ici de faire une revue des travaux antérieurs relatifs à cette question. La plus grande partie de ces essais ont été faits au moyen, soit de sacs en collodion, soit de membranes ou de filtres en collodion acétique, qui, ainsi qu'il a été montré précédemment, sont incapables de fournir des résultats précis et constants. Beaucoup d’expériences ont, de plus, été poursuivies sans tenir compte de facteurs importants, tels que l’épaisseur de la membrane, le milieu et la concentration, lesquels, ainsi qu’on l’a vu, jouent un rôle primordial dans la filtration. Ceci a conduit à une discordance complète entre les différentes recherches sur l’ultrafiltration. Les membranes employées pour des essais quantitatifs doivent, avant tout, pouvoir être reproduites, et être strictement isoporeuses. Les membranes les plus satisfaisantes, sous ces différents rapports, sont, comme nous l’avons indiqué, les membranes graduées de collodion « gradocol », d’Elford. La supériorité de ces membranes et de la technique de filtration de cet auteur, sont amplement prouvées par la confirmation complète de ses résultats par les différents laboratoires qui les ont employées, contrairement à ce que l’on a pu observer lorsque d’autres méthodes étaient utilisées.

I. FRACTIONNEMENT

L’ultrafiltration peut être appliquée au fractionnement et à l’étude de la composition des systèmes dispersés. Comme exemple de cette utilisation, citons l’élimination des particules d’un ordre de grandeur différent, dans la stérilisation de systèmes bactériologiques par l’ultrafiltration. Les avantages des membranes d’Elford sur les bougies en porcelaine ou en terre d’infusoires, telles que celles de Chamberland et de Berkefeld, résident dans l’uniformité plus grande de leurs porosités, dans leur faible pouvoir d’adsorption spécifique, et dans la suppression du traitement de nettoyage. Il a été démontré que toutes les membranes dont les pores ont un diamètre moyen inférieur à 0,75 µ, retiennent complètement le B. pro-digiosus. Tout le monde s’accorde à reconnaître la difficulté éprouvée à obtenir des filtrats actifs stériles à partir du virus de la vaccine, de la maladie de Borna et de la rage, par l’emploi de bougies filtrantes. Par contre, il est facile de préparer des filtrats très actifs et stériles à l’aide de membranes « gradocol », avec les virus de la vaccine [Elford et Andrewes, 1932 (26)], de la maladie de Borna [Elford et Gallo-way, 1933 (34)], de la rage [Galloway et Elford, 1936 (49)] et de la paralysie bulbaire infectieuse [Elford et Galloway, 1936 (36)].

Cependant, même avec des membranes en collodion, on n’obtient de bons résultats que si l’on tient compte du rôle du milieu et si l’on pratique un fractionnement progressif de la suspension de virus. Une clarification préliminaire de la suspension de tissu, par
la centrifugation et la filtration à travers des filtres standardisés de sable et de pulpe de papier, est toujours nécessaire et peut être suivie de filtrations successives à travers des membranes en collodion de porosités sélectionnées. On sait que, à l’aide de ces méthodes et avec les membranes « gradocol », Elford et Galloway, en 1933 (34), ont réussi à préparer 20 filtrats stériles du virus de la maladie de Borna, titrant de \(1 \times 10^3\) à \(1 \times 10^7\), alors que dans les expériences antérieures, faites avec des bougies Chamberland ou Berkefeld, Zwick, Seifried et Witte [1926 (89)], et Nicolau et Galloway [1928 (72)] n’avaient obtenu que des filtrats stériles très peu actifs et cela seulement 3 fois sur 46 expériences.

On trouve un autre exemple de l’emploi de l’ultrafiltration par le fractionnement de systèmes dispersés dans les expériences de Burnet [1933 (17)], qui, à l’aide de membranes de collodion « gradocol » de porosités convenablement choisies, réussit à obtenir une séparation quantitative entre bactéries, bactériophage et une substance spécifique soluble résultant de la bactériolyse.

Par ailleurs, l’application de l’ultrafiltration à la purification des virus est un autre exemple de fractionnement par cette méthode, exemple qui sera examiné plus loin.

2. DIMENSIONS DES PARTICULES DES VIRUS ET DES BACTÉRIOPHAGES

Le succès de l’utilisation de l’ultrafiltration pour la détermination de la taille des particules d’une suspension donnée, exige une compréhension parfaite des processus physiques de cette opération, lesquels ont été rapidement exposés dans le paragraphe ayant trait au mécanisme de la filtration.

Les virus humains et animaux sont présents dans les liquides organiques, ou, plus souvent, dans les organes. Avant tout, le virus sera libéré, autant que possible, par un broyage des tissus dans un mortier, avec du verre pilé (Pyrex) s’il est nécessaire. Les débris tissulaires et tous autres matériaux étrangers, doivent ensuite être éliminés par centrifugation et par une filtration préliminaire à travers des filtres standardisés de sable et de pulpe de papier [Barnard et Elford, 1931 (3)]. Pour certains virus, tels que ceux de l’influenza humaine et porcine [Elford, Andrewes et Tang, 1936 (30)], on emploie, au lieu de filtres de sable et pulpe de papier, des filtres d’amiante, mais ceux-ci se sont révélés tout à fait inutilisables pour certains virus. Ensuite, la suspension devra être filtrée successivement à travers des membranes en collodion de porosités choisies, bien avant le point terminal, de façon à retenir les particules plus grosses que celles du virus examiné et à obtenir ainsi un filtrat stérile très actif.

Ainsi donc, bien qu’une membrane de porosité 0,75 μ donne un filtrat stérile, si, par ailleurs, une membrane de 0,1 μ fournit un filtrat de même activité, on devra employer ce dernier comme filtrat stock dans les expériences ultérieures servant à déterminer le point ter-
minal de filtration. On utilise un milieu tel que le bouillon, lequel contient des substances agissant sur la tension superficielle, et les conditions de viabilité du virus doivent être contrôlées de façon à éviter une fausse interprétation des résultats, due à l'instabilité du virus. On a constaté que la plupart des virus animaux sont stables à un pH de 6,5 à 8,5 et que la variation du pH dans cette zone est sans effet sur la filtrabilité du virus à travers les membranes « gradocol ». La plupart des expériences de filtration sont faites en bouillon de pH 7,4 à 7,6. On sait que la variation du pH du milieu, entre 6,4 et 8,7, n'a pas d'action sur la filtrabilité du virus de la fièvre aphteuse à travers les membranes en collodion [Galloway et Elford, 1931 (46)]. Par contre, Busch en 1932 (19) a constaté la supériorité marquée des milieux aqueux (exemptes de bouillon) à réaction alcaline (pH 8,5) dans la filtration de ce virus à travers les ultrafils de Bechhold-König, dont le pouvoir d'adsorption est beaucoup plus grand.

a) Détermination des points terminaux de filtration.

On ne peut tirer des conclusions valables que si la filtration a été conduite dans les conditions les plus favorables. Lorsque l'on détermine le point terminal pour un virus donné, il est nécessaire d'établir une courbe de filtration d'après les données concernant le pourcentage de la concentration du virus par rapport au volume du filtrat, pour des membranes de porosité de plus en plus réduite, dans des conditions standard. L'échelle de la porosité des membranes doit être descendue graduellement, afin de préciser le point auquel les forces de surface commencent à exercer une influence anormale sur le cours de la filtration. A partir de ce point, la courbe de filtration doit être étudiée avec le plus grand soin, en réalisant les conditions qui diminuent l'action des forces de surface, telles que d'une part la présence de substances agissant sur la tension superficielle et, d'autre part, une pression optimum, jusqu'à détermination du véritable point terminal de la filtration. La précision avec laquelle on définit le point terminal dépend de l'isoporosité des membranes et de l'uniformité de la dispersion du système examiné. Au cours d'expériences faites avec les membranes « gradocol » d'Elford on a constaté que la première chute du maximum de la concentration d'un virus, ou d'un bactériophage, se produit généralement avec une membrane dont la valeur de la porosité est de 2 à 3 fois la valeur limite absolue à laquelle la filtrabilité est nulle [Elford, 1933 (24)]. Galloway et Elford, (1931) (46) ont donné un exemple de courbe de filtration du virus, d'après les résultats de leurs expériences sur le virus de la fièvre aphteuse. La forme de cette courbe est normale et il n'y a aucune évidence de colmatage. La première diminution marquée de la concentration du filtrat apparaît avec une membrane dont les pores ont un diamètre de 60 m. µ, alors que le point terminal est 25 m. µ. On trouve également dans l'article de Elford et Andrewes, 1932 (27), les courbes de filtration de plusieurs souches de bactériophage de tailles différentes, les points terminaux variant de 25 m. µ à 110 m. µ. Les courbes de filtration sont très inclinées.
la première chute de concentration du phage apparaissant à une porosité dont la valeur est environ deux fois celle du point terminal.

b) Estimation des diamètres des particules de virus et de phages d'après les points terminaux de la filtration.

Lorsque, en filtrant un virus dans les meilleures conditions possibles, on trouve que la porosité d'une membrane est telle, qu'elle retenait la totalité du virus, le point terminal de filtration est déterminé et il est possible de calculer la taille des particules (diamètre). On doit, toutefois, employer des facteurs de correction (F) [Elford, 1933 (24)]. La lumière du pore d'un ultrafiltre, n'est pas utilisable en totalité pour le passage de la particule, puisqu'un certain volume sera occupé par des couches d'eau ou des substances adsorbées sur les parois. Plus les particules du système filtré sont petites, plus le rôle de l'épaisseur de la couche protectrice adsorbée devient important.

Ainsi :

Pour des membranes de diamètre 10 - 100 m. μ, \( F = \frac{1}{3} \) à \( \frac{1}{2} \).

\[ \text{— } 100 - 500 \text{ m. μ, } F = \frac{1}{2} \text{ à } \frac{1}{3} \]

\[ \text{— } 500 - 1000 \text{ m. μ, } F = \frac{3}{4} \text{ à } 1 \]

\( F \) représentant le rapport entre le diamètre de la particule de virus et le point terminal. Ces facteurs de correction ont été déterminés d'après une comparaison entre les résultats de l'ultrafiltration de bactéries et de virus, de sols d'or et de protéines, dont la taille des particules pouvait être déterminée par d'autres méthodes. Ainsi, à la partie supérieure de l'échelle des porosités, le rapport \( Fa \) a été établi avec des bactéries, des gros virus, et des sols d'or dont les dimensions peuvent être appréciées respectivement, au microscope, par la microphotographie dans l'ultraviolet ou par la numération à l'ultra-microscope. A la partie inférieure de l'échelle des porosités, le rapport \( F \) a été calculé d'après la filtration de protéines dont la taille avait été appréciée par ultracentrifugation. Les quelques exemples suivants illustrent clairement l'application de ces facteurs de correction :

Le point terminal de filtration du virus de la rage, calculé par Galloway et Elford en 1936 (49), est de 200 m.μ ; le facteur 0,5 à 0,75 a permis d'attribuer une valeur de 100-150 m. μ au diamètre du virus ; de même le point terminal de filtration du virus du "louping-ill" estimé à 40 m.μ [Elford et Galloway, 1935 (35)], a fixé, après correction par le facteur 0,33 à 0,5, la taille de la particule de virus à 15-20 m. μ.

Dans le tableau I figurent les diamètres des particules de divers virus, bactériophages et protéines, calculés par Elford et ses collaborateurs, ainsi que par d'autres travailleurs s'étant servi de ses membranes, dans des conditions déterminées.

Récemment, Levaditi et ses collaborateurs, Paic, Krassnoff, Voet et Haber (1936) adoptant la technique d'ultrafiltration décrite ci-dessus, ont fait des essais avec les virus de l'herpès (55), de la vaccine (57), de la lymphogranulomatose inguinale (61), de la sto-
matite vésiculeuse (58), de la peste aviaire (59), de la rage (60), et de la fièvre aphteuse (58). En général, leurs résultats concordent avec ceux consignés dans le tableau I. Ces auteurs estimant, que, dans certains cas, le nombre des expériences a été trop limité pour permettre le calcul des dimensions du virus, continuent leurs recherches.

VI. DIMENSIONS DES VIRUS ET DES BACTÉRIOPHAGES, SELON DIFFÉRENTES MéTHODES

Il existe actuellement, outre l’ultrafiltration, d’autres méthodes, telles que l’ultracentrifugation et la photographie dans l’ultraviolet, qui peuvent fournir des données relatives au diamètre des particules des virus ou des bactériophages. La première de ces méthodes est maintenant applicable même aux plus petits virus et bactériophages connus, tels que le virus de la fièvre aphteuse [Elford et Galloway, 1937 (37)] et le phage S13 [Elford, 1936 (25)]. La photographie dans l’ultraviolet (Barnard) peut être employée comme moyen de mesure des diamètres des virus plus gros, tels que ceux de la vaccine, de la variole des canaris et de l’ectromélie infectieuse des souris. Dans le tableau II, sont groupés les calculs des dimensions d’un certain nombre de virus et de bactériophages, évalués selon différentes méthodes. Les résultats fournis par la centrifugation ont été obtenus par l’emploi d’une nouvelle technique décrite par Elford [1936 (25)]. Les données recueillies par l’ultrafiltration sont celles du tableau I et enfin les diamètres de gros virus, estimés à l’aide de la photographie en lumière ultraviolette, ont été mesurés par M. J.-E. Barnard et concordent parfaitement avec les résultats obtenus tant par centrifugation, que par filtration. La comparaison des données établies par la filtration et la centrifugation montre une concordance assez exacte entre les dimensions des différents virus, sauf à la partie inférieure de l’échelle. Ainsi, dans le cas des virus de la fièvre aphteuse et du phage S13, le diamètre de la particule, donné par la centrifugation est supérieur à celui calculé d’après les résultats de la filtration. On n’a pas, jusqu’à présent, pu fournir d’explication parfaite de ces divergences ; cependant, plusieurs facteurs peuvent être incriminés, dont on trouvera l’exposé dans un article de Elford et Galloway [1937 (37)]. Une des possibilités envisagées est que la valeur donnée par l’ultrafiltration est quelque peu diminuée par le facteur de correction (voir p. 1037), employé pour exprimer le point terminal de la porosité en termes du diamètre des particules, ce facteur étant trop bas pour permettre l’interprétation exacte des valeurs des dimensions, lorsque l’on arrive au point extrême de l’échelle des porosités.

VII. PURIFICATION DES VIRUS ET DES BACTÉRIOPHAGES PAR L’ULTRAFILTRATION ET LA CENTRIFUGATION

1° Méthodes de centrifugation. — La connaissance des dimensions des différents virus a fourni des méthodes nouvelles et
plus efficaces de purification des virus et des bactériophages, à savoir: des processus de lavages successifs comportant des filtrations et centrifugations différentes. Ainsi, grâce à la centrifugation fractionnée, il a été possible, pour certains des virus les plus gros, tels que celui de la vaccine [Craigie, 1932 (20), [Parker et Rivers, 1935 (73)] et la psittacose [Bedson, 1932 (12)] de rassembler les éléments virulents, et, après élimination du liquide superficiel, de refaire une suspension du sédiment dans un milieu approprié. La répétition de ce processus fournit un produit final dans lequel le virus a été fortement débarrassé des protéines étrangères, sans pour cela perdre beaucoup de son activité. La méthode a été également appliquée à l'un des plus gros bactériophages [Schlesinger, 1933 (76)]. Avec les modèles ordinaires de centrifugeuses et suivant la technique habituelle, cette méthode ne peut convenir aux virus et aux bactériophages dont le diamètre est inférieur à 100 m.µ. On sait maintenant que par centrifugation dans le cylindre fermé, recouvert de gélose, de la supercentrifugeuse de Sharples, on peut précipiter même les plus petits virus connus, entre autres celui de la fièvre aphtuse [Schlesinger, 1936 (77), [Schlesinger et Galloway, 1937 (78) (*)]. Dans le liquide superficial, les chutes de concentration du virus observées sont entièrement dues à la force centrifuge, et il est prouvé que le phénomène d'adsorption n'exerce aucune influence sur les résultats obtenus. Cette méthode de centrifugation peut être également employée pour la purification, car la sédimentation du virus suivie de l'extraction de la gélose dans un milieu de suspension frais, procure un matériel dont la virulence est égale à celle de la suspension originale et qui est partiellement débarrassé de protéines. Il est cependant préférable, tout au moins, dans le cas du virus de la fièvre aphtuse, de ne pas opérer les lavages dans la centrifuguse, au delà d'une certaine limite; on a, en effet, constaté que des suspensions de virus fortement purifiées peuvent être adsorbées par la gélose. Cependant cette méthode est utile, car la teneur en protéines d'une lymphe vésiculaire virulente (fièvre aphtuse) diluée seulement de moitié peut être réduite, sans qu'il s'en suive de perte de virus et il est possible de continuer la purification par des lavages répétés sur une membrane en colloidion, sans dilution.

2° Méthodes de purification par ultrafiltration. — Cette méthode, c'est-à-dire le lavage sur une membrane de colloidion de porosité adéquate, est largement appliquée. Elle a été employée, en particulier, pour la purification des virus de l'ectromélie infectieuse de la souris [Barnard et Elford, 1931 (3)] du sarcome de Rous n° 1 [Elford et Andrewes, 1935 (28)] de la fièvre aphtuse [Galloway et Elford, 1936 (48)] des bactériophages [Elford et Andrewes, 1932 (27)] et des enzymes [Dubos et Bauer, 1935 (21)]. Il est à remarquer, toutefois, que chaque virus et chaque bactériophage présentent, du point de vue de leur purification, un problème particulier, surtout

(*) Les résultats de ces expériences ont été brièvement relatés au 2ème Congrès International de Microbiologie de Londres en Juillet 1936.
en ce qui concerne leur stabilité dans différentes conditions de milieu et de pH. Ceci est nettement mis en lumière par les recherches de Galloway et Elford [1936 (48)] sur le virus de la fièvre aphteuse, et peut expliquer quelques difficultés rencontrées dans l’application de cette méthode à certains autres virus. La purification du virus de la fièvre aphteuse peut être prise comme exemple de l’emploi de la méthode. Galloway et Elford, 1931 (46) ont montré que les membranes « gradocol », dont les pores ont un diamètre moyen égal, ou inférieur à 25 m.μ., retiennent complètement le virus, alors que les protéines sérices ne sont arrêtées entièrement que par des membranes de porosité inférieure à 10 m.μ. Dans les expériences de purification, on a donc employé des membranes dont les pores ont un diamètre moyen de 25 m.μ., porosité maximum permettant une réten-
tion totale du virus. Pour la purification par la technique des lavages, il a semblé plus commode d’utiliser le type d’ultrafiltre employé avec des pressions négatives (fig. 3, p. 1029). La méthode de montage et de fixation de la membrane est la même que dans les ultrafiltrations à prés-
sion positive (p. 1028). Toutefois, l’ouverture de la partie supérieure des appareils à pressions négatives facilite l’observation de la marche de la filtration, ainsi que l’addition de milieu de lavage qui peut être faite sans interruption de l’aspiration. En général, on procède ainsi: on charge le réservoir du filtre (hauteur: 5 cm., diamètre: 2,75 cm.) avec 20 cc. du filtrat-stock (porosité de la membrane: 0,6 μ). En-
suite, dans le vide le plus parfait réalisable par la trompe à eau, on pratique la filtration jusqu’à ce que le volume de liquide restant au-dessus de la membrane soit réduit à environ 1 cc. que l’on ramène ensuite au volume initial, par addition du milieu de lavage, c’est-à-
dire une solution de phosphates M/45, de pH.6. Ce proces-
sus de filtration suivie de dilution est répété 4 fois ; l’opération demande parfois 2 jours. On obtient ainsi une suspension parfaite-
ment virulente et exempte de protéines (test de l’acide sulfosalicy-
lique), alors que ce test révèle la présence de protéines dans l’émulsion stock, et même dans le premier filtrat donné par une membrane de 
25 m.μ., dilué au \[\frac{1}{100}\] ; dans les dilutions au \[\frac{1}{1000}\] , on ne décelle plus de protéines. Il est donc clair que, par les lavages répétés, le virus est débarrassé d’environ 99% des protéines étrangères. Il est essentiel d’éviter la dessiccation de la membrane au cours de l’expérience, ce qui entraînerait un abaissement de la concentration du virus purifié. 
Insistons particulièrement sur le fait, que pour chaque virus, le problème de la purification se présente différemment ; ainsi, le virus de la fièvre aphteuse ne peut pas être lavé avec du bouillon, puisque l’on sait que si ce virus est purifié, il est instable dans ce milieu; de même, on ne peut le laver avec des solutions telles que celle de Ringer, ou l’eau physiologique, puisqu’il n’est pas stable dans des milieux qui ne sont pas tamponnés à un pH de 7,4-7,6. D’autre part, on a constaté que le virus de la stomatite vésiculeuse, ne peut être lavé avec une solution aqueuse tamponnée de phosphates, milieu qui détruit sa stabilité. Par contre, on peut employer le bouillon pour
ce virus. Il ne faut pas négliger ces particularités lorsque l’on essaie
de purifier les virus par lavage.

3° Concentration des virus par l’ultrafiltration. — La
concentration des virus peut être obtenue au moyen de l’ultrafiltra-
tion. On emploie les membranes les plus poreuses, assurant une
rétention complète du virus examiné ; 20 cc. de virus sont placés
entre les filtres (dont les dimensions sont indiquées plus haut) et on
opère la filtration jusqu’à obtention d’un résidu de 1 cc. ou moins.
Ensuite, si le virus arrêté par la membrane n’a pas formé d’agrégats
et n’a pas adhéré partiellement à la membrane, et par conséquent est
easile resuspendu, il est plausible de conclure que la concentra-
tion du virus dans le liquide restant doit être à peu près 20 fois celle
du liquide original. Ceci a été contrôlé par les recherches sur le virus
de la fièvre aphteuse [Galloway et Elford, 1936 (48)]. Pour d’autres
virus, tels celui de la maladie de Borna [Elford et Galloway, 1933
(34)], la concentration n’a pas exactement atteint le degré prévu.
Tout comme pour la purification, chaque virus doit être considéré
en particulier.

4° Purification et concentration des virus par des métho-
des de centrifugation et ultrafiltration combinées. — Dans
le paragraphe concernant la purification des virus par centrifugation,
sous avons relégué que Schlesinger et Galloway, (1937) (78) ont mon-
té que la centrifugation dans la super-centrifugeuse de Sharples, à
cylindre fermé recouvert de gélose, peut être utilisée pour purifier,
partiellement tout au moins, le virus de la fièvre aphteuse. D’autres
expériences de Galloway et Schlesinger, (1937) (50) (*) ont établi
que la teneur protéique de la lymphe vésiculaire virulente (diluée
de moitié) pouvait être réduite sans entrainer de perte de virus,
jusqu’à un degré permettant de continuer la purification par des
lavages répétés sur une membrane en collodion de porosité 25 m.µ
et sans dilution. Par une combinaison de trois méthodes : lavage du
virus dans la super-centrifugeuse de Sharples, puis sur une mem-
brane de collodion de 25 m.µ, et enfin concentration à l’aide d’une
bougie recouverte de collodion acétique à 9 %, on peut obtenir une
préparation purifiée dont le titre est 10^-9, c’est-à-dire dont le pouvant
infectieux limite est 100 fois celui de la lymphe originale (10^-9).
Ces auteurs ont également montré, par des expériences dans lesquelles
le virus est en suspension dans du sérum sanguin, qu’il est possible
de concentrer dans 5 cc. seulement de gélose le virus contenu dans
un grand volume de liquide (100 cc. et plus), dont la teneur protéique
est aussi élevée que celle de la lymphe vésiculaire. Par conséquent,
si l’on peut se procurer une quantité suffisante de matériel, on obtien-
dra par les méthodes combinées de purification et de concentration
utilisables à l’heure actuelle, des filtrats de virus purifiés et infectieux
t une dilution limite de 10^-9 et même supérieure.

(*) Les résultats de ces expériences ont été brièvement relatés au aème Con-
pes International de Microbiologie de Londres en Juillet 1936.
**TABLEAU I**

**CALCUL DES DIMENSIONS DES PARTICULES DES ULTRAVIRUS, DES BACTÉRIOPHAGES ET DES PROTÉINES AU MOYEN DE L’ULTRAFILTRATION**

<table>
<thead>
<tr>
<th>Virus, bacteriophage ou protéine</th>
<th>Point terminal de filtration en mμ</th>
<th>Facteur de correction</th>
<th>Taille des particules d’après différents auteurs en mμ</th>
<th>Diamètre moyen des particules en mμ</th>
<th>Auteurs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psittacose</td>
<td>400</td>
<td>0,5-0,75</td>
<td>200-300</td>
<td>250</td>
<td>Levinthal (1935) (62)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>250</td>
<td>0,5-0,75</td>
<td>125-175</td>
<td>150</td>
<td>Elford et Andrewes (1932) (26)</td>
</tr>
<tr>
<td>Variole des canaris</td>
<td>250</td>
<td>0,5-0,75</td>
<td>125-175</td>
<td>150</td>
<td>Burnet (1933) (16)</td>
</tr>
<tr>
<td>Lymphogranulomatose inguinale (Bubon climatique)</td>
<td>(1) 250, (2) 240</td>
<td>0,5-0,75</td>
<td>125-175</td>
<td>150</td>
<td>(1) Broom et Findlay (1936) (40), (2) Miyagawa, Mitamura, Yasi, Ishii et Okanishi (1935) (70)</td>
</tr>
<tr>
<td>Rage (souche fixe de l’Institut Pasteur)</td>
<td>(1) 200, (2) 200</td>
<td>0,5-0,75</td>
<td>100-150</td>
<td>125</td>
<td>(1) Galloway et Elford (1936) (49), (2) Yaoi, Kanazawa et Sato (1936) (87)</td>
</tr>
<tr>
<td>Rage (souche fixe Fukuoka)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maladie de Aujeszky (Pseudorage)</td>
<td>A 200, B (« Mad itch » Shope)</td>
<td>0,5-0,75</td>
<td>100-150</td>
<td>125</td>
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<tr>
<td>Souche A (Aujeszky)</td>
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<td></td>
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<td>Souche B (« Mad itch » Shope)</td>
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<td>Herpès</td>
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<td>100-150</td>
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<tr>
<td>Ectromélie infectieuse</td>
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<td></td>
<td></td>
<td>125</td>
<td>Barnard et Elford (1931) (3)</td>
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**TABLEAU I (suite)**
<table>
<thead>
<tr>
<th>VIRUS, BACTÉRIOPHAGE OU PROTÉINE</th>
<th>POINT TERMINAL DE FILTRATION EN m µ</th>
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<th>DIAMÈTRE MOYEN DES PARTICULES EN m µ</th>
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<td>160</td>
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<td>Influenza (porcin) ...............</td>
<td>160</td>
<td>0,5-0,75</td>
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<td>100</td>
<td>Elford, Andrewes et Tang (1936) (30)</td>
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<td>Maladie de Newcastle .............</td>
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<td>100</td>
<td>Burnet et Ferry (1934) (18)</td>
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<td>Sarcome de Rous N° 1 .............</td>
<td>(1) 200</td>
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<td>(2) 140</td>
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<td>85</td>
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<td>Elford et Andrewes (1932) (27)</td>
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<tr>
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<td>35</td>
<td>Levaditi, Paic, Voet et Krassnoff (1936) (56)</td>
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<td></td>
<td>(2) 90</td>
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<td>30-45</td>
<td>35</td>
<td>Tang, Elford et Galloway (1937) (82)</td>
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<td>Bactériophages (D 54, S 41) ......</td>
<td>90</td>
<td>0,33-0,5</td>
<td>30-45</td>
<td>35</td>
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<tr>
<td>« Rift Valley » Fever ............</td>
<td>70</td>
<td>0,33-0,5</td>
<td>23-35</td>
<td>30</td>
<td>Broom et Findlay (1933) (14)</td>
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### TABLEAU I (suite)

<table>
<thead>
<tr>
<th>VIRUS, BACTÉRIOPHAGE OU PROTÉINE</th>
<th>POINT TERMINAL DE FILTRATION EN m μ</th>
<th>FACTEUR DE CORRECTION</th>
<th>TAILLE DES PARTICULES D'APRÈS DIFFÉRENTS AUTEURS EN m μ</th>
<th>DIAMÈTRE MOYEN DES PARTICULES EN m μ</th>
<th>AUTEURS</th>
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<tr>
<td>Mosaique du Tabac</td>
<td>70</td>
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<td>23-35</td>
<td>30</td>
<td>Smith (1936) (79)</td>
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<td>Encéphalomyélite équine (souches américaines)</td>
<td>(1) 65</td>
<td>0,33-0,5</td>
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<td>28</td>
<td>Tang, Elford et Galloway (1937) (82) Bauer, Cox et Olizky (1935) (7)</td>
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<td>Encéphalite de St-Louis</td>
<td>(1) 66</td>
<td>0,33-0,5</td>
<td>22-33</td>
<td>28</td>
<td>Bauer, Fite et Webster (1934) (5) Elford et Perdrau (1935) (39)</td>
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<td>Bactériophages (T. III — B. typhosus)</td>
<td>60</td>
<td>0,33-0,5</td>
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<td>25</td>
<td>Yaoi et Sato (1935) (86)</td>
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<td>Bactériophages (C 36, D. 13, D. 20, D. 48)</td>
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<td>0,33-0,5</td>
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<td>Fièvre jaune</td>
<td>(1) 55</td>
<td>0,33-0,5</td>
<td>18-27</td>
<td>23</td>
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<td>Hémocyanine (Hélix)</td>
<td>55</td>
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<td>15-20</td>
<td>17</td>
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<td>Louping-ill</td>
<td>40</td>
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<td>15-20</td>
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<td>8-12</td>
<td>10</td>
<td>Elford et Andrewes (1932) (27)</td>
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<tr>
<td>Fièvre aphteuse</td>
<td>25</td>
<td>0,33-0,5</td>
<td>8-12</td>
<td>10</td>
<td>Galloway et Elford (1931) (46)</td>
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<td>VIRUS, BACTÉRIOPHAGE OU PROTÉINE</td>
<td>POINT TERMINAL DE FILTRATION EN m(\mu)</td>
<td>FACTEUR DE CORRECTION</td>
<td>TAILLE DES PARTICULES D'APRÈS DIFFÉRENTS AUTEURS EN m(\mu)</td>
<td>DIAMÈTRE MOYEN DES PARTICULES EN m(\mu)</td>
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<tr>
<td>Poliomyélite</td>
<td>(1) 25 (2) 35</td>
<td>0,33-0,5</td>
<td>8-12</td>
<td>10</td>
<td>(1) Elford, Galloway et Perdrau (1935) (38)</td>
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<td></td>
<td></td>
<td></td>
<td>(2) Theiler et Bauer (1934) (83)</td>
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<tr>
<td>Edestine</td>
<td>18</td>
<td>0,33-0,5</td>
<td>6-9</td>
<td>8</td>
<td>Elford et Ferry (1936) (33)</td>
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<tr>
<td>Pseudo-globuline sérique</td>
<td>12</td>
<td>—</td>
<td>6</td>
<td>6</td>
<td>Elford et Ferry (1934) (31)</td>
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<td>Albumine sérique</td>
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<td>5</td>
<td>5</td>
<td>Elford et Ferry (1934) (31)</td>
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<td>Oxyhémoglobine</td>
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<td>—</td>
<td>5</td>
<td>5</td>
<td>Elford (1933) (24)</td>
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<tr>
<td>Albumine d'œuf</td>
<td>6</td>
<td>—</td>
<td>4</td>
<td>4</td>
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**TABLEAU II**

DIAMÈTRE DES PARTICULES DES VIRUS ET DES BACTÉRIOPHAGES — COMPARAISON ENTRE LES DONNÉES FOURNIES PAR L'ULTRAFILTRATION (F) L'ULTRACENTRIFUGATION (C) ET LA PHOTOGRAPHIE EN LUMIÈRE ULTRAVIOLETTE (UVP)

<table>
<thead>
<tr>
<th>VIRUS OU BACTÉRIOPHAGE</th>
<th>F m(\mu)</th>
<th>C m(\mu)</th>
<th>UVP BARNARD m(\mu)</th>
<th>AUTEURS</th>
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<tr>
<td>Variole des Canaris</td>
<td>125-175</td>
<td>—</td>
<td>160-170</td>
<td>Burnet (1933) (16) F et UVP (Barnard)</td>
</tr>
<tr>
<td>Vaccine</td>
<td>125-175</td>
<td>170-180</td>
<td>150</td>
<td>Elford et Andrewes (1932) F. (26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Elford et Andrewes (1936) C. (29)</td>
</tr>
<tr>
<td>VIRUS OU BACTÉRIOPHAGE</td>
<td>F m µ</td>
<td>C m µ</td>
<td>UVP BARNARD m µ</td>
<td>AUTEURS</td>
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<tr>
<td>------------------------</td>
<td>--------</td>
<td>--------</td>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Ectromélie Infectieuse</td>
<td>100-150</td>
<td>—</td>
<td>130-140</td>
<td>Barnard (UVP) et Elford (F) (1931) (3)</td>
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<td>Sarcome de Rous N° 1</td>
<td>70-100</td>
<td>60-70</td>
<td>—</td>
<td>Elford et Andrewes (1935) F. (28) Elford et Andrewes (1936) C. (29)</td>
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<td>Stomatite vésiculeuse</td>
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<td>Fièvre Aphteuse</td>
<td>8-12</td>
<td>17-20</td>
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<td>Galloway et Elford (1931) F. (46) Elford et Galloway (1937) C. (37)</td>
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DISCUSSION ON THE ROUTES OF INFECTION AND
PATHS OF TRANSMISSION OF VIRUSES

Dr. G. M. Findlay: During the past few years a tendency has grown up, at
least in the English language, to apply the term "virus" without further
qualification, to a group of pathogenic agents that do not greatly exceed 0.1 µ in size.
This tendency, however, is not as new as it may seem, for in 1800 Jenner described
the cause of smallpox as a "perfect virus" (cf. also Ward, 1799). Included in the
group of viruses are undoubtedly agents of many diverse kinds. Certain plant
viruses are, it is claimed, autocatalytic proteins; tobacco-mosaic virus, for instance,
has been isolated in the form of fine crystalline needles that are only inactivated by
heating at 94° C. (Stanley, 1935). On the other hand, so far as animal viruses are
concerned, the evidence obtained from a number of sources is now strongly in favour
of the view that they are organisinal entities, essentially parasitic, but not
necessarily co-existent either in space or time with the hosts they parasitize. If
this view of the nature of animal viruses is correct, it follows that they have an
ecology of their own which can be studied apart from that of their hosts.

The life-history of the animal viruses may thus be divided into seven stages:

1. Transmission to new vertebrate hosts; (2) entry into new hosts; (3) spread
through the tissues of the host; (4) localization in selected cells; (5) action on
parasitized cells; (6) excretion from the host, and (in the case of certain viruses)
(7) passage through arthropod hosts.

(1) The methods of transmission to a new host may involve (a) direct contact
between skin or mucous surfaces, as in the case of the virus of lymphogranuloma
inguinale which is contracted by venereal contact; (b) "droplet" infection, the
droplets consisting of secretion from either the salivary glands, the nasopharynx or
the respiratory tract, as in mumps, measles, influenza; (c) the infected material may
be dried and may contaminate "fomites," as in variola, varicella or feline gastro-
enteritis. "Fomites" may in some cases retain their infectivity for considerable
periods, possibly for months; (d) insect vectors. In the case of plant virus diseases,
there are certain that can only be transmitted by grafting.

(2) Entry into a new host.—The portals of entry include (a) skin, (b) mucous
membranes, and (c) the placenta.

(a) Skin: certain viruses such as those of warts, molluscum contagiosum, fowl-
pox, herpes, pseudo-rabies and lymphocytic chorio-meningitis may pass through the
lightly scarified skin. Others such as rabbies and virus B are introduced by the bites
of vertebrates while a small group, yellow fever, dengue, equine encephalomyelitis,
blue tongue, sandfly fever, louping-ill, tick-borne fever, Nairobi sheep disease, fowl-
pox and equine anaemia gain entrance by means of the bites of arthropods.

(b) Mucous membranes: the portals of entry provided by the mucous membranes,
and certain viruses which enter by these portals are: Conjunctiva—trachoma,
herpes; oral mucosa—dog warts; nasopharyngeal mucosa—measles, acute coryza,
variol, varicella, poliomyelitis; respiratory mucosa—influenza, laryngo-tracheitis
of fowls; alimentary mucosa—polyedral and wilt disease of caterpillars;
poliomyelitis; genital mucosa—warts, lymphogranuloma inguinale, herpes,
exanthema pustulosum coitale of cattle.
(c) Placental transmission to the embryo may occur in certain diseases such as varicella, varicella, Rift Valley fever and rinderpest. As a rule lesions are produced in the placenta. The Islamic physicians, Rhazes and Avicenna, who flourished at the end of the ninth century, were well aware of this method of contagion in smallpox. Lanfranchi and Lenzi (1918) claim that rabies virus may pass through the placenta of the dog and rabbit, but Genevray and Dodero (1935) have recently recorded the birth of a perfectly healthy child by Cæsarian section, to a mother suffering from rabies.

It is of interest that a virus pathogenic by one portal of entry may be quite innocuous when injected by another. Swine influenza injected intramuscularly into pigs causes no illness (Orcutt and Shope, 1935).

(3) Methods of spread in the tissues.—Certain viruses, as for instance, warts, may spread directly on skin or mucous surfaces by auto-inoculation. The more usual paths of infection involve the blood-stream, as in yellow fever, the lymphatics, as in lymphogranuloma inguinale or the nerve-fibres, as in rabbits, poliomyelitis, herpes, Borna disease. In certain diseases, spread may involve the actual axones, in others the neurolymph spaces form the more probable means of dispersal. Some viruses infect cells at their portal of entry, others may travel both by the blood-stream and by nerves.

(4) Localization in selected tissues.—Some viruses produce changes in a large number of different types of tissue cells; when such cells are derived from all three embryonic layers the virus may be said to be pantropic: other viruses are highly selective in the cells they parasitize. The following simple classification of animal virus diseases according to the embryonic layers involved, is therefore proposed. It is less complicated than that recently suggested by Verge and Goret (1935):

(a) Pantropic.—All three embryonic layers attacked—(cf. Hurst, 1936).
  Type I—Herpes, pseudo-rabies, Virus B; Type II—Yellow fever, Rift Valley fever, equine encephalomyelitis, horse-sickness, louping-ill, vesicular stomatitis.
(b) Ectodermotropic.—(i) Dermotropic—warts, molluscum contagiosum; (ii) neurotropic—rabies, poliomyelitis, Borna disease.
(c) Mesodermotropic—Leukaemia of fowls, filtrable sarcomata of fowls, Shope's filtrable fibroma, virus myxomatosis, lymphogranuloma inguinale.
(d) Entodermotropic.—Encephalitis of silver foxes (?); filtrable endothelioma of fowls?

By suitable means it is possible to produce variants of certain viruses which differ from their parent strains in tissue affinities.

(5) Action on parasitized cells.—It is now recognized that the action of viruses is due to direct action on the parasitized cells. Such cells may be stimulated to active mitosis with resulting hyperplasia, or necrosis may rapidly destroy the cells. In many cases the production of a hyperplastic mass with insufficient blood supply involves subsequent necrosis, but recently Rous and Beard (1935) have found that the cells of filtrable papillomata in rabbits may undergo true neoplastic processes. Certain viruses, after parasitizing cells, may remain quiescent for weeks or months. The long incubation periods of warts, or Borna disease are well known, while mice inoculated intracerebrally with the virus of lymphogranuloma inguinale may not develop symptoms for ninety-five days after inoculation. On the other hand, mice inoculated intraperitoneally with the virus of Rift Valley fever usually die in forty-eight hours with widespread necrosis of the liver parenchyma. It must however be recognized that the intensity of virus action varies within wide limits: at times viruses may exhibit an almost symbiotic relation to their hosts' tissues, or may cause an inapparent infection.

Certain viruses have an action on the tissues that is but little understood. This consists in a reduction of the hosts' resistance to bacterial infection: the loss of
resistance may be against specific bacteria only, as in swine fever or swine influenza, or may be general, as in dog distemper or feline gastro-enteritis.

6 Methods of excretion.—An increasing number of viruses have now been isolated from the tissues of animals which have previously acquired specific immunity: vaccinia, infectious equine anaemia, laryngo-tracheitis of fowls, rabies, poliomyelitis, herpes, virus III, guinea-pig and rat salivary gland viruses, yellow fever, lymphocytic-chorio-meningitis, psittacosis and Theiler's mouse encephalitis. How long such viruses may remain in the tissues apparently in a state of commensalism is unknown—possibly they may endure for the life of their host. If, however, such viruses are to parasitize fresh hosts they must by some means be excreted from their original host. The points of exit are reached, as in the case of spread from the portals of entry, by the blood or by nerves, the spread in the latter being now centrifugal instead of centripetal. The actual portals of excretion comprise: (1) skin—variola, varicella, warts; (2) saliva—rabies, herpes, virus B, mumps; (3) secretions of respiratory tract—measles, acute coryza, poliomyelitis, influenza; (4) urine—lymphocytic chorio-meningitis; (5) faeces—bacteriophage; (6) blood—yellow fever, dengue, Nairobi sheep disease.

It will be noted that some viruses come out by the same door as they went in; others, such as variola, enter by the nose and leave by the skin. The importance of nasal infection in variola appears to have been known to the ancient Chinese since they used the nose as a route for prophylactic immunization, while Rhazes, to whom we owe the first description of smallpox, believed that the contagion was affected by a kind of leaven which fermented in the blood. The latter, trying to purify itself, rejected the peccant matter from the body through the openings of glands in the skin.

As a general rule the period of excretion and, therefore, the period of infectivity, are short. This is especially true when the virus is taken up from the blood-stream by the bites of arthropods. Herpes virus may, however, remain in the saliva of healthy persons for long periods and the same is true of the virus of rabies in the saliva of the vampire bat (Lima, 1934): Lucas and Osgood (1913) have found the virus of poliomyelitis in the nasopharynx four months after recovery from an attack, and Berry and Rivers (1935) state that a parrot was still excreting psittacosis virus, though isolated from other birds, for eighteen months. There is also evidence to suggest that mice may excrete the virus of lymphocytic chorio-meningitis in the urine for considerable periods. Prostitutes infected with lymphogranuloma inguinale were found to be contagious after more than one and a half years (Caminopetres, 1935). It is of interest that comparatively few viruses, with the exception of bacteriophage, are excreted in the faeces.

7 Passage through insect hosts.—Just as many viruses may remain infective but quiescent for long periods when attached to "fomites," so others may pass long periods in insects without producing any pathogenic effects in their arthropod hosts. If the production of pathological changes in the host is evidence of incomplete adaptation on the part of the parasite to the host's tissues, then the absence of pathogenic action would in the same way be evidence of very long standing adaptation. Many of the animal viruses transmitted by arthropods may therefore have originated as insect parasites and only later have become partially adapted to vertebrate hosts. If this view of the origin of these insect-borne viruses is correct, the viruses must, in many cases, have lost the capacity they once possessed of being hereditarily transmitted from the female to her eggs. The following animal viruses are at present known to be transmitted by insects:—

Yellow fever, dengue, equine encephalomyelitis, blue tongue, Nairobi sheep disease, louping-ill, tick-borne fever, sandfly fever, fowlpox, infectious equine anaemia. The vectors of Rift Valley fever and African horse sickness are not at present certainly known: the former is probably transmitted by certain mosquitoes of the
genus \textit{Mansonien}, but \textit{Aedes} mosquitoes do not naturally transmit horse sickness. It is possible that in the future the list may be increased by diseases such as the following: (1) Red fever of the Congo; (2) three days’ fever, such as that described by Chabrilhat (1934) as occurring in Madagascar in the absence of sandflies; and (3) three-day sickness of cattle (cf. Sen, 1931): in addition, Cowdry and Rees (1935) have brought forward evidence to suggest that \textit{Anaplasma marginale} is not a protozoon but a virus. In this case the ticks \textit{Dermacentor variabilis}, \textit{D. andersoni} and \textit{Boophilus microplus} would become virus vectors. Even with these possible additions, the list of animal viruses transmitted by insects is small and is in striking contrast to the very large proportion of plant viruses transmitted by insects.

An examination of the list of insect vectors of animal viruses shows that the two most important groups are those of the Culicine mosquitoes and the ticks. Yellow fever, dengue, equine encephalomyelitis and possibly Rift Valley fever and blue tongue of sheep are all readily transmitted by Culicine mosquitoes. It is, however, of interest to note that among the great family \textit{Culicidae}, the tribe of the \textit{Culicini} alone transmits viruses while the equally numerous tribe of the \textit{Anophelini} is entirely blameless in this respect; on the other hand, the \textit{Anophelini} comprise all the carriers of malarial protozoa, with the exception of certain species of the genus \textit{Culex} which transmit the organisms of bird malaria. Why these two tribes should exhibit this dichotomy is unknown and is the more remarkable as the \textit{Anophelini} are usually regarded as the more primitive in type. Differentiation of the \textit{Anophelini} and \textit{Culicini} probably occurred, however, as early as the mid-tertiary period. Ticks, on the other hand, transmit both protozoa and viruses.

In studying the changes undergone by viruses in insects, the following classification proposed by Huff (1931) and applicable to all organisms transmitted by arthropods is of interest:—

<table>
<thead>
<tr>
<th>Cyclical change of organisms in vector</th>
<th>Multiplication of organisms in vector</th>
<th>No multiplication of organisms in vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Cyclo-propagative, e.g. malaria by mosquitoes</td>
<td>(2) Cyclo-developmental, e.g. filaria by mosquitoes</td>
<td></td>
</tr>
<tr>
<td>(3) Propagative, e.g. typhus by lice</td>
<td>(4) Mechanical, e.g. typhoid by flies</td>
<td></td>
</tr>
</tbody>
</table>

In the case of viruses only the methods of propagative and mechanical transmission are known to occur. Propagation without cyclical change occurs in the mosquito transmission of dengue, equine encephalomyelitis and, in all probability, of yellow fever, while mechanical transmission is exemplified by the action of \textit{Stomoxys calcitrans} in transmitting the viruses of African horsesickness and infectious equine anemia and by the mosquito transmission of fowlpox. Bos (1934), however, has found that this latter virus may be carried by \textit{Anopheles maculipennis} for as long as 210 days, that is to say probably for life. The same is true of the yellow fever, dengue and equine encephalomyelitis viruses, for the vectors, once infected remain infected as long as they live. Culicine mosquitoes are unable to transmit yellow fever, dengue or equine encephalomyelitis by biting until some days after they have ingested these viruses. This period of “extrinsic incubation” is necessary to allow the virus to reach the salivary glands in concentration and can, at any rate in the case of yellow fever virus, be greatly modified by change of temperature, being retarded by low and accelerated by high temperatures. There is no evidence that yellow fever or equine encephalomyelitis viruses are transmitted from female to male mosquitoes during sexual conjugation or that they are hereditarily transmitted from the female to her eggs. The fact that the yellow fever virus is unable to penetrate into the egg is remarkable since the infection of the mosquito is apparently septicemic in character. Thus, in an isolated mosquito community, yellow fever infection gradually dies out with the death of the infected mosquitoes. The failure of yellow-fever infection to persist indefinitely in mosquitoes in the absence of susceptible vertebrate hosts raises the question whether other arthropod vectors of yellow fever may not exist either longer-lived than the culicine mosquitoes (\textit{Aedes}
Aedes cannot live more than six months under the most favourable conditions) or capable of ensuring hereditary transmission. One of the most important problems in connexion with the epidemiology of yellow fever is the reason for the persistence of infection in sparsely populated rural areas in South America and Africa. Although there is now evidence from both continents that wild monkeys may act as alternative hosts to man, it is difficult to imagine that a constant supply of non-immune monkeys can always be at hand to maintain the supply of infected mosquitoes. The search for other arthropod vectors of yellow fever therefore continues, more especially as in South America rural outbreaks of yellow fever have occurred in the total absence of the usual vector Aedes aegypti: on epidemiological grounds Soper (1935) in South America has recently incriminated, for instance, the blue mosquito Haemagogus equinus.

Dengue virus behaves in Aedes mosquitoes in very much the same way as the yellow fever virus. Here again, there is no hereditary transmission, though the virus is widely distributed in the tissues of the mosquito. A curious fact of considerable interest is that though the Western strain of equine encephalomyelitis is readily transmitted by Aedes aegypti the Eastern strain is unable to penetrate the intestinal mucosa. Tick-transmission of viruses is a somewhat more complicated process. Thus, Daubney and Hudson (1931) found that infection with Nairobi sheep-disease virus of any instar of Rhipicephalus appendiculatus results in transmission of the virus by the succeeding stage which then loses its infection at the next moult. A female tick infected as an adult thus only passes infection to the eggs. However, larvae, nymphs or adults which have successfully cleared themselves can become reinfected if their host's febrile reaction begins before the infecting meal is completed; carriage of infection for a further stage is thus ensured. With Amblyomma variegatum, a less successful vector of Nairobi sheep-disease virus, transmission from the female to her eggs does not occur (Daubney and Hudson, 1934).

Louping-ill virus was shown by MacLeod and Gordon (1932) to be transmitted by nymphs and adult female Ixodes ricinus that had engorged in their previous stage, on infected sheep, the ticks becoming infective as soon after moulting as they were capable of attachment to a host. Great variation may occur in the infectivity of different groups of ticks and in different ticks of the same group. The action of the associated virus of tick-borne fever in localizing the louping-ill virus in the brains of sheep is of great interest. Alexander and Nietz (1933) found that Rhipicephalus appendiculatus also transmitted louping-ill. The larve of the tick can pass on infection to the nymphs and similarly freshly infected nymphs can transmit infection to the adults. The virus will not, however, pass through the egg to the next generation and in this instance also infected nymphs tend to clear themselves when fed on susceptible sheep though they may reacquire infection before detachment. The exact method by which ticks clear themselves is at present uncertain, nor is it known whether viruses actually multiply in ticks.

Among the sandflies, Phlebotomus papatasii is the only species which is definitely known to act as a vector of the virus of sandfly fever. Various workers, chiefly on epidemiological grounds, have brought forward evidence that P. minutus var. africanus, P. perniciosus, P. neglectus, P. major, var. chinensis and P. sergenti may also act as carriers of the disease. The known distribution of the fever is very similar to that of P. papatasii but outbreaks of diseases with very similar characters have been reported from areas where this sandfly is not known to exist. This discrepancy may depend, as Sinton (1930) has suggested, upon (a) the presence of another vector, either another species of Phlebotomus or some other insect, (b) insufficient knowledge of the distribution of P. papatasii or (c) mistaken diagnosis, as at present the disease can only be identified by clinical symptoms.

Little, however, is yet known in regard to the transmission of sandfly fever, as there is no laboratory animal in which the disease can be studied. A few facts,
however, are certain: thus Doerr (1909) and Birt (1910) both found that the flies do not become infective till seven to ten days have elapsed after they have fed on a patient with sandfly fever. The virus is not passed through the egg, but Whittingham (1926) believes that under natural conditions infection may be transmitted from one generation to another, the larvae ingesting the dejecta or dead remains of parent flies infected with the virus. Flies infected by this means are not infective till they have had a blood meal.

Although many problems in connexion with the insect transmission of viruses still remain to be elucidated, the ease with which certain viruses live and in some cases multiply in the bodies of arthropods, is an additional argument of some weight in support of the view that viruses are organismal in character and are not chemical products derived from the tissues of their vertebrate hosts.

The means by which many viruses are transmitted in Nature is still unknown; nevertheless, in this brief summary it has been possible to sketch a life-history that is applicable to many animal viruses. As of men, so too of viruses, it can be said that they have their exits and their entrances, and each one in its time plays many parts.

**Virus Diseases of Animals Transmitted by the Bites of Insects**

<table>
<thead>
<tr>
<th>Species of vector</th>
<th>Efficiency</th>
<th>Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culicidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em> Linn.</td>
<td>++</td>
<td>Finlay (1881), Reed, Carroll and Agramonte (1901)</td>
</tr>
<tr>
<td><em>A. africanus</em> Theo.</td>
<td>+</td>
<td>Philip (1929)</td>
</tr>
<tr>
<td><em>A. albopictus</em> Skuse</td>
<td>+</td>
<td>Dinger, Schüffner, Snijders and Swelengrebel (1929)</td>
</tr>
<tr>
<td><em>A. luteocephalus</em> Nowst.</td>
<td>+</td>
<td>Bauer (1928)</td>
</tr>
<tr>
<td><em>A. simpsoni</em> Theo.</td>
<td>+</td>
<td>Philip (1929)</td>
</tr>
<tr>
<td><em>A. vittatus</em> Bigot</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. stokesi</em> Evans</td>
<td>+</td>
<td>Davis and Shannon (1929)</td>
</tr>
<tr>
<td><em>A. acapulcaris</em> Londoni</td>
<td>+</td>
<td>Davis and Shannon (1931)</td>
</tr>
<tr>
<td><em>A. fusitexta</em> Lutz</td>
<td>+</td>
<td>Davis and Shannon (1929)</td>
</tr>
<tr>
<td><em>A. tenuirohyphus</em> Wied.</td>
<td>+</td>
<td>Philip (1930)</td>
</tr>
<tr>
<td><em>Mansonia africana</em> Theo.</td>
<td>+</td>
<td>Bauer (1929)</td>
</tr>
<tr>
<td><em>Eretmapodites chrysogaster</em> Graham</td>
<td>+</td>
<td>Kerr (1932)</td>
</tr>
<tr>
<td><em>Culex thalassius</em> Theo.</td>
<td>+</td>
<td>Davis (1933)</td>
</tr>
<tr>
<td><em>Musca</em></td>
<td>+ up to 42 hours</td>
<td>Hoskins (1934)</td>
</tr>
<tr>
<td>Stomoxys calcitrans Linn.</td>
<td>+ up to 42 hours</td>
<td></td>
</tr>
<tr>
<td><strong>Hemiptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cimex lectularius</em> Linn.</td>
<td>+ up to 24 hours</td>
<td>Kumm and Probisher (1962)</td>
</tr>
<tr>
<td>Reduviidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Panstrongylus</em> (Triatoma) <em>megistus</em> Burm.</td>
<td>+</td>
<td>Davis (1933)</td>
</tr>
<tr>
<td><strong>Acari</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodidae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ornithodoros rostratus</em> (adult) <em>Arag.</em></td>
<td>+</td>
<td>De Bourrepaire Aragao (1933)</td>
</tr>
<tr>
<td><em>O. moubata</em> (adult) <em>Marr.</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><strong>Diptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culicidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em> Linn.</td>
<td>++</td>
<td>Bancroft (1906), Cleland, Bradley, and McDonald (1916)</td>
</tr>
<tr>
<td><em>A. albopictus</em> Skuse</td>
<td>+</td>
<td>Morishita (1925), Simmons, St. John, and Reynolds (1930)</td>
</tr>
<tr>
<td><strong>Diptera</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culicidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aedes aegypti</em> Linn.</td>
<td>++</td>
<td>Kelser (1933), Merrill, Laccaille, and Ten Broeck (1938)</td>
</tr>
<tr>
<td><em>A. sollicitans</em> Wlk.</td>
<td>++</td>
<td>Madsen and Knowlton (1933)</td>
</tr>
<tr>
<td><em>A. tenuirohyphus</em> Wied.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. nigromaculatus</em> Ludl.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. tornalis</em> Mg.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. crantor</em> Coq.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. vexans</em> Mg. (sylvestris Theo.)</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**2. DENGUE**

**3. Equine Encephalomyelitis**

<table>
<thead>
<tr>
<th>Species of vector</th>
<th>Efficiency</th>
<th>Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em> Linn.</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td><em>A. albopictus</em> Skuse</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. sollicitans</em> Wlk.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. tenuirohyphus</em> Wied.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. nigromaculatus</em> Ludl.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. tornalis</em> Mg.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. crantor</em> Coq.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>A. vexans</em> Mg. (sylvestris Theo.)</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
HEMIPTERA

DIPTERA

(4) Rift Valley Fever

Mansonella fuscopunctata Theo. +
M. versicolor Edw. +
M. microsaudoleti Theo. ++

(5) Blue Tongue

Aedes lineatopennis Lundl. +++

(6) Sandfly Fever

Diptera Psychodidae

Phlebotomus papatasii Scopoli +++

(7) Louping-Ill

Acari

Ixodida

Ixodes ricinus Linn. +

(8) Tick-Borne Fever

Diptera Muscidae

Stomoxys calcitrans Linn. +

(9) Nairobi Sheep Disease

Diptera Culicidae

Anopheles gambiae Linn. +

(10) African Horse Sickness

Acari

Leptodiaceae

Argas persicus Oken. +

(11) Equine Infectious Anaemia

Hemiptera Acari

Hapalodiscus Linn. +

(12) Fowl Pox

REFERENCES

Dauney, R., and Hudson, J. R. (1931.) Parasitol., 25, 175; (1932.) Lancet, 1, 611; (1933.) Parasitol., 26, 495.
De Oliveira Castro, G. M. (1930.) Ibid., 116, 316.
Mr. I. A. Galloway: The method of spread of viruses in the body and the elective affinity of viruses for tissues derived from different embryonic layers are problems which have given rise to innumerable discussion since viruses were first recognized. During recent years, in which the investigation of these infective agents has been more intensive, the interest has increased. Improved methods of study have made observations more trustworthy and the interpretation of experiments more convincing. It has come to be realized that isolated observations on the presence of the virus in certain tissues or in the circulating blood, or other body fluids, especially during the later stages of a disease, are of no assistance in arriving at a satisfactory estimate of the route by which the specific virus under study travels in the body. It is only by systematically investigating at the various stages and especially during the incubation period, and by giving, at the same time, due consideration to the portal of entry of the virus and the degree of infection (especially after experimental inoculation) that one can hope to arrive at reliable conclusions. While in the case of some viruses the evidence for elective tissue-tropism is so overwhelming as to render it unequivocal, e.g. foot and mouth disease virus for certain epithelial tissue, rubies, Borna disease and poliomyelitis viruses for nervous tissue (ectoderm affinity), in the case of others dogmatic statements as to elective tissue affinity which have been made should be received with caution. As knowledge has accumulated it has been found that some viruses, such as that of vaccinia, may possess several potential "tropisms" which can be enhanced or suppressed by experimental devices. One may also cite in this connexion the viruses of yellow fever and horse sickness which although in the spontaneous disease...
appear to possess only viscerotropic characters may, when injected intracerebrally into experimental animals (mice) or under special experimental conditions which will be referred to later, exhibit neurotropic potentialities.

In the short time at my disposal it would obviously be impossible to discuss the whole question of the mode of spread of viruses in the body. I propose, therefore, to limit my remarks to one group of viruses, i.e. those often referred to as "neurotropic" viruses. The employment of this term, which in some cases has been misapplied, has led to the erroneous conception that all those viruses which are associated with symptoms referable to lesions in the nervous system and may possess some characteristics in common, behave in exactly the same way when they gain access to the body. It has become increasingly obvious, as a result of intensive and more accurate experimental investigations, that a revision of our ideas about this group is called for and a division into sub-groups necessary. I must state my indebtedness to Dr. E. W. Hurst, with whom I have, on several occasions been able to discuss many aspects of the problem and who has afforded me the opportunity of reading not only his published papers, but also his unpublished papers relating to this subject.

One can fairly safely place the viruses of rabies, Borna disease (an enzootic encephalomyelitis affecting horses, sheep, and cattle), and poliomyelitis in a group by themselves. This is Group I Neurotropic Viruses—Strict Neurotropes. The viruses of this group are rarely found in the blood or cerebrospinal fluid, and then only in the later stages of the disease. Inoculation of moderate doses intravenously is usually without result. They all attack primarily and destroy nerve-cells. The inflammatory reactions in the nervous tissues, meningitis and perivascular "cuffing," come later into the histological picture and may be considered as secondary and reactive. It has been shown in all three diseases that if the virus is inoculated intracerebrally it will travel centrifugally in nervous tissue to reach the peripheral nervous system, and if inoculated into a peripheral site will travel centripetally in the same way to reach the central nervous system. It has also been shown experimentally, at least in the case of poliomyelitis and rabies, that the virus does not reach the cord and brain if the nerves supplying the inoculated region are resected. In the case of Borna disease, it has been possible owing to the long incubation period to obtain a very complete histological picture and story of the spread of the virus after intracerebral inoculation [Nicolau and Galloway, 1928; Nicolau, Dimancesco-Nicolau and Galloway, 1929]. The infiltrative interstitial neuritis encountered in the peripheral nerves is more intense than in rabies [Id., 1928] and poliomyelitis [Id., 1928] and infiltrative and degenerative processes as well as inclusion bodies have been demonstrated only in the nervous mechanism associated with a large number of organs and tissues.

All the evidence goes to prove that the viruses of Group I travel by some component of the nerve trunk. Hurst (1930) has submitted experimental evidence to support his belief that in the case of poliomyelitis the spread of the virus is by the axis cylinders.

The size of the virus of poliomyelitis has been shown to be about 8 to 12 mµ [Theiler and Bauer, 1934; Elford, Galloway, and Perdran, 1935]. It would appear more easy to conceive the possibility of the passage of particles of this size along the axis cylinders than viruses of the size of rabies, 120 mµ [Elford and Galloway, 1934], and Borna disease, 100 mµ [Elford and Galloway, 1933].

A recent clinical observation of interest has been made by Leake (1935), who has described cases of poliomyelitis in humans following vaccination with virus treated in different ways. In every case in which the sequence was known the level of the spinal cord first affected corresponded to the extremity in which the injection was made, paralysis beginning either in the same limb or in the contralateral limb. As the author points out, this is strong support to other evidence [Fairbrother and Hurst, 1930; Hurst, 1930] that the virus of poliomyelitis is
transmitted along nerve-fibres, since neither blood nor lymph streams would afford direct access from one extremity to the corresponding cord level.

Before leaving this group of strict neurotropes, I wish to say a few words about the confusion which appears to exist in the minds of some about the position with regard to equine encephalomyelitis. Borna disease, an equine encephalomyelitis (which may also affect cattle and sheep under natural conditions), known to occur in Europe, is produced by a virus which is quite different from that responsible for another entity which has been proved to exist among horses in the United States of America, the Argentine and Russia, and which for convenience sake may be referred to as American equine encephalomyelitis.

Although both the viruses are responsible for encephalomyelitis in the equine species, yet they are definitely not related. Miss Howitt (1935) said that

"while the two entities may differ in various characteristics, difference in incubation period, a more chronic course for the Borna type with greater virulence for rabbits, the lack of cross-immunity and the lack of similar intranuclear inclusion bodies, yet a common generic relationship seems probable."

The incubation period after intracerebral inoculation of guinea-pigs with Borna disease is about sixty days on an average, while in the case of the American type of encephalomyelitis it is about forty to ninety hours according to the strain. The size of the virus of Borna disease (the European encephalomyelitis) is about 100 m\(\mu\), while Bauer, Cox and Olitsky (1935) have recently shown that the “Eastern” and “Western” strains of the American virus have a size value of only about 20 to 30 m\(\mu\). Further, as Dr. Hurst will subsequently point out in some detail, the American virus circulates in the blood and does not travel in the body exclusively by nervous tissues. In fact, the two viruses possess nothing in common except their capability of producing an encephalomyelitis in the horse. The difference in size value is in itself sufficient to reject any conception of generic relationship. For a similar reason it appears illogical to suggest any generic relationship, as has been done by Olitsky, Cox, and Syvertson (1934) between the virus of equine encephalomyelitis (American type), size value 20 to 30 m\(\mu\), and that of vesicular stomatitis, size value 70 to 100 m\(\mu\) [Galloway and Elford, 1935].

The second group of “neurotropic” viruses has been termed by Hurst Group II Neurotropic Viruses—Pantropic Type 1. In this group may be placed such viruses as those of pseudorabies (Aujezsky’s disease, “mad itch”), herpes and Sabin’s “B” virus. These are all definitely neurotropic but also attack and produce lesions in cells derived from all embryonic layers. The lesions in the central nervous system itself are both ectodermal and mesodermal. Intravenous inoculation of moderate doses of virus is often effective in producing the disease. The virus may or may not be detected in the blood. The virus of pseudorabies, which is very typical of this group, has been studied in much detail [Hurst, 1934]. This virus, inoculated into a peripheral site in the rabbit, reaches the central nervous system by the nerve-fibres. Itching begins about the time when lesions can first be demonstrated in the corresponding spinal ganglia and segments of the spinal cord, and about two to three hours after virus can first be detected there. The infection ascends the peripheral nerves, as Hurst suggests, both interstitially and by the axis cylinders. (The size of the virus of pseudorabies has been shown to be about 120 m\(\mu\) [Elford and Galloway, 1934], and, as already emphasized with regard to the spread of the viruses of rabies and Borna disease by the nerves, it seems difficult to conceive axonic transmission of particles of this size.) The nerve-cell changes are probably responsible for the cardinal symptom of the disease, itching. Death ensues soon after the virus reaches the medulla and before visible changes have been produced there. Although Hurst has stated that the morbid changes in the lungs, viz., oedema, haemorrhage, and congestion are not necessarily related to the presence...
of virus, this has not been the speaker's experience in young Dutch or Himalayan rabbits; generally, if severe lesions were present, the lungs could be used as a rich source of virus.

The virus of pseudorabies inoculated intracerebrally spreads centrifugally by nerve paths. After intravenous inoculation the virus disappears from the blood, foci are established in the organs whence the virus reaches the central nervous system by the nerves. a fact which explains also why inoculation of the virus into a derenervated area may be followed by the development of the disease. In pseudorabies infection in the rabbit, lesions are found in the adrenal glands very similar to those encountered in herpes infection. In the pig, infection with pseudorabies by the subcutaneous route is followed by a mild febrile illness unaccompanied by itching, and nervous symptoms develop only rarely. In this animal also there is some evidence of involvement of the lymphatic system. It is of some interest that Sabin (1934) has from experimental evidence suggested that the three viruses, pseudorabies, herpes and "B" virus may to some extent be related antigenically.

In Group 3 Neurotropic Viruses—Pantropic Viruses Type 2, may be placed such viruses as those of yellow fever, louping-ill and for reasons which Dr. Hurst will give in detail the virus of equine encephalomyelitis (American type). I have added to this group the virus of African Horse Sickness. As Hurst has suggested with regard to these viruses, the similarities are sufficiently great and the points of distinction from other neurotropic viruses sufficiently marked to warrant their relegation to a special class. Yellow fever, louping-ill and equine encephalomyelitis (American type) are all transmitted by insect or arthropod vectors, in the former two diseases under natural conditions and in the last case at least experimentally and perhaps also under natural conditions. There is strong suggestive evidence that horse sickness may also be transmitted by an insect vector as yet undetermined.

These four viruses are pantropic in the sense of possessing multiple cellular affinities; this is especially so in the case of yellow fever. In louping-ill, on the other hand, these affinities are more restricted and may be limited to the blood or reticulo-endothelial system.

There is some suggestive evidence of the possibility that the viruses of this group may multiply, at least in certain susceptible hosts, in the blood or blood-vascular system [Gordon, 1934; Hurst, 1935] in which they appear at a relatively early stage of infection.

The virus of yellow fever and horsesickness, as already pointed out, resemble one another in that although they appear to be mainly viscerotropic in the hosts which they infect under natural conditions they possess neurotropic potentialities which can be enhanced relatively to their viscerotropism [Theiler (1930) (yellow fever), Nieschulz (1932) and Alexander (1933) (horse sickness)]. In the case of yellow fever these neurotropic potentialities once enhanced can again be diminished by experimental methods [Findlay and Clarke, 1935]. Findlay and Stern (1935) have shown that unmodified or so-called "viscerotropic" yellow-fever virus, instilled intranasally into Asiatic monkeys, produces ordinary yellow fever, but inoculated by the same route into mice gives rise to encephalomyelitis. Asiatic monkeys also develop encephalitis when inoculated with "viscerotropic" virus. These authors and Theiler and Hughes (1935) have found also that Asiatic monkeys inoculated intracerebrally with "viscerotropic" virus after subcutaneous injection of immune serum develop encephalitis.

After a number of passages in the brain of mice, Sellards (1931), and Lloyd and Penna (1932) showed that the virus of yellow fever loses to a large extent its capacity to produce viscerotropic lesions in man and in monkeys.

After intraperitoneal or subcutaneous inoculation of modified (neurotropic) virus, the latter circulates in the blood but only rarely does encephalitis follow unless
injury of the brain is present (Findlay, 1934). It has yet to be shown whether in those cases in which encephalitis results without brain injury the virus reaches the brain by the direct or the indirect route. If encephalitis occurs, the virus disappears from the blood and, although antibodies can be demonstrated in the serum, these have apparently no effect on the course of the encephalitis. Inoculation of neurotropic (mouse brain) yellow-fever virus intracerebrally into monkeys gives rise to encephalitis accompanied by a widespread distribution of the virus in the peripheral nervous system at the time of death. In the mouse and guinea-pig [Theiler, 1930, 1933] the neurotropic mouse strain appears to behave like a strict neurotrope.

Horse-sickness in equines is a septicaemic disease and there is a concentration of virus in the blood and highly vascularized organs such as the liver and spleen. No data are available as to the concentration of virus in the brain. Clinically, there is no record of the symptoms in horses being correlated with nervous derangement. In the mouse the virus assumes the characters of a strict neurotrope. Following the injection subcutaneously of neurotropic virus into horses, the blood is found to be highly infective but the animals do not develop encephalitis and, although the neurotropic (mouse) virus is highly infective for the mouse, when inoculated intracerebrally into horses no encephalitis follows [Alexander, 1936]. It has not yet been shown whether in this disease the neurotropism of the virus, once enhanced by mouse intracerebral passage, can be again diminished by experimental procedure.

Louping-ill is carried by an arthropod vector, and Pool, Brownlee, and Wilson (1929-30) and Gordon, Brownlee, Wilson, and MacLeod (1932) have shown that in the natural host, the sheep, the blood is infective in the early stages of the disease at the time of the first febrile reaction, but not usually once the nervous symptoms have developed. Encephalomyelitis may or may not follow the systemic invasion after infection by ticks or by experimental subcutaneous inoculation of virus, and in those cases in which encephalitis occurs it has not yet been determined how the virus reaches the nervous system. Galloway and Perdrau (1935) showed that with satisfactory experimental methods monkeys can be infected by the intranasal instillation of virus and that after such infection the virus rarely appears in the blood. Intravenous inoculation of virus was also found to be non-infective unless the brain was damaged. The virus therefore evidently reached the nervous system by the direct path.

Greig, Brownlee, Wilson, and Gordon (1931) made an attempt to infect seven sheep by the nasal route, employing a nebulizer and a crude suspension of infective brain. The technique may not have been very efficient in ensuring the entry of the virus into the posterior nares but, although only one sheep developed nervous symptoms and recovered, they all were found to be immune when subsequently tested by the cerebral route, so that some virus must have reached the central nervous system. At that time these authors stated that the experimental evidence suggested that the disease may be contracted by the nasal inhalation of virus. Whether or not sheep can be infected under natural conditions by the nasal route and without the intervention of the tick, has not yet been determined. Gordon and his co-workers are of the opinion that tick-borne fever, which ticks infected with louping-ill may also carry, plays some part in facilitating the entry of louping-ill virus into the nervous system of sheep, but the route of entry is still unknown. They also state [MacLeod and Gordon, 1932] that sheep can become infected with, and even die from, louping-ill infection by tick-bite although tick-borne fever is not present. The suggestion of MacLeod and Gordon that the presence or absence of concomitant tick-borne fever infection is a deciding factor as to whether the number of sheep which will contract encephalitis and die is high or low would of course be valid if there was found to be some correlation between high mortality and presence of tick-borne fever and vice versa. In this connexion it is of interest to consider the observations of Lennette and Hudson (1934-35) in experimental poliomyelitis. As
pointed out when discussing the strict neurotropes—Group 1, it is not possible to infect monkeys by single intravenous inoculation of small doses of poliomyelitis virus. These authors, however, inoculated their monkeys intravenously with repeated large doses of virus. In four out of five normal monkeys inoculated in this way the disease resulted, whereas five monkeys, from which both olfactory tracts had been removed five months previously, remained well after similar inoculation. The results suggest, as the authors point out, excretion of the virus on to the nasal mucosa of these intravenously inoculated monkeys and subsequent passage of the virus to the brain by the olfactory tract. This idea was supported by the observation that in at least one of six monkeys which had been inoculated intravenously virus could be demonstrated in the nasal washings. Whether experiments along these lines would always be equally demonstrative is questionable, since it is known how an old trauma may have the effect of localizing virus in the brain.

These observations are however of significance and lead one to consider whether in the case of a disease like louping-ill infection of the nervous system after systemic infection may not occur by a similar process. In this connexion it is of interest to cite the observations of Gordon, Brownlee, Wilson, and MacLeod (1932) on the subcutaneous inoculation of virus into sheep. In earlier experiments on a limited number of sheep, Greig et al. (1931) found that the subcutaneous inoculation of sheep with living virus was followed by a thermal reaction and that sheep so treated were immune on recovery to an intracerebral inoculation of virus. In the later experiments of Gordon and his colleagues 50 sheep were inoculated subcutaneously with louping-ill virus and of these 33 died before the immunity test. In 28 cases the disease to which the animals succumbed was described as "typical louping-ill." It is known that not all ticks which have fed on an infected louping-ill sheep are later infective, so that, although sheep may be heavily infested with ticks, the degree of infection with louping-ill virus may vary very considerably from case to case. This, coupled with a fair degree of resistance of the olfactory tract to virus, might account for the low percentage of frank cases of the disease. There are still certain details in connexion with louping-ill which require amplification.

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Dr. Wilson Smith: In his opening paper Dr. Findlay included influenza amongst the virus diseases; some might not agree with him on that point but, for myself, I believe that influenza is a virus disease, because for the last three years the evidence that a filtrable virus is the primary cause of at any rate one type of epidemic influenza has been steadily accumulating. My remarks are based upon the assumption that the virus of experimental influenza in the ferret and mouse is indeed the virus of the human disease. If this assumption is granted, there are certain features of the experimental disease in ferrets and mice which may have some bearing on our conceptions of the disease in man.

Firstly, the only certain way in which animals can be infected is by the direct introduction of virus into some part of the respiratory tract. Almost every other conceivable route of inoculation has been tried, but both ferrets and mice will usually tolerate massive doses of virus given in such ways without acquiring infection. There have been only a few occasions when very large doses given intravenously in mice have caused lung lesions. If the human disease is comparable, it means that influenza is essentially an air-borne droplet infection, and that the only portal of entry is the nasopharynx. It seems to me that a clear recognition of this might help in the prevention of epidemic spread, e.g. in ensuring adequate spacing of hospital cases and the use of masks by nurses whilst attending patients. The second important feature of the experimental disease is that the virus remains confined to the tissues of the respiratory tract. After the virus has gained entry into the nasopharynx and nasal sinuses of the ferret, it spreads to the lungs but does not invade the body in the true sense. There is no generalization by blood-stream or lymph-channel spread such as occurs in smallpox, yellow fever, and a host of other virus diseases. Blood, spleen, liver, glands, brain, have all been tested at the height of the disease, and some of them at different stages of the disease, but virus has only been recovered from respiratory tissues and secretions. Influenza virus may thus be regarded as of low invasive power and I cannot help thinking that there is some connexion between invasive power and the sort of immunity which follows a disease. The immunity which follows an attack of influenza in the ferret is of relatively short duration, and it is possible that it may depend more upon a local immunity of susceptible tissues than upon a general humoral immunity. The animal may be susceptible to a second attack a few months after the first and at a time when it still has circulating antibodies. It is known only too well that in man acquired immunity is not the solid life-long immunity which seems to be characteristic of many of the other virus diseases. There is a similar short immunity in other diseases of low invasive power such as herpes, staphylococcal skin infections, pneumonia, and probably the common cold. The practical outcome of this is the difficulty of immunizing by artificial means—thus in the ferret we can call forth circulating antibodies by vaccine inoculations, but we cannot yet confer a local immunity to the nasal mucosa.

The last point I wish to raise is the influence of an anaesthetic on the experimental disease. In ferrets, no recognizable lung lesions were produced until the virus had been passed a number of times from animal to animal under anaesthesia—then lung involvement became the rule when the virus was administered in this way. Although such lesions may now be caused by the lung-adapted strain without anaesthesia, an anaesthetic is still necessary for regular and extensive lung involvement. In mice, anaesthesia is even more necessary, and without it virus instilled
into the nostrils is usually innocuous. Now the whole explanation of this may be that the anaesthetic enables a massive dose to be aspirated directly into the lungs, but there remains at least the possibility that it also lowers the resistance of pulmonary cells to attack by the virus. It suggests that other factors which similarly lower cell resistance play a part in the onset of pneumonia as a complication of influenza. Certainly any factors which interfere with the normal clearing mechanism of the respiratory tract, especially damage to the cilia of tracheal and bronchial mucosa, will favour virus attack on the lungs. We are accustomed to think of bacterial infection in virus diseases being second to the virus attack, but the features of experimental influenza make one wonder if damage to the nasopharynx caused by non-specific bacterial infection or mechanical irritants may sometimes prepare the way for the virus infection and determine its extension to the lungs.

Dr. Weston Hurst: In the majority of the neurotropic virus diseases clear evidence of neural spread of the virus to the nervous system is forthcoming; thus, after inoculation into a hind limb, the lumbar cord is invariably the first part of the nervous system to become infective. This is true even of a pantropic virus such as that of pseudorabies, which though circulating in the blood, does not reach the brain or cord by its agency.

The virus of equine encephalomyelitis with which I have worked recently [1] behaves in a totally different manner. After inoculation into the muscles or skin of the leg, or even into the sciatic nerve, the lumbar cord is never first infective. The virus appears in the blood-stream, where its titre rapidly rises; but it does not penetrate directly the hemato-encephalic barrier, for after intravenous inoculation the fluid does not become virulent for several days. During its circulation in the blood, however, the virus may often be detected in the nasal washings. Taking this fact in conjunction with the observation that the anterior frontal region of the brain is almost always the first part to show virus after intramuscular or intradermal inoculation in the leg, just as it is after instillation into the nose, it seems fair to conclude that infection of the nasal secretions is the intermediate step in passage of virus from blood to brain. I suspect strongly that the viruses of yellow fever and louping-ill reach the brain by the same route.

It seems, moreover, that we must postulate a similar occurrence in those previously puzzling cases in which intravenous injection of a strictly neurotropic virus, such as that of poliomyelitis, is effective in causing the nervous disease. Lennette and Hudson [2] have recently shown that after intravenous inoculation this virus may occasionally be detected in the nasal washings; and again, while in normal monkeys repeated intravenous doses of virus will cause the nervous disease, if the olfactory bulbs be removed previously, intravenous injection is quite innocuous. These two observations, both made within the last year, indicate, I think, a formerly unsuspected mechanism of infection of the central nervous system with certain filtrable viruses.

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Professor S. P. Bedson said that in the course of his investigations he had encountered problems which were bound up with this question of the mode of transmission of virus infections. One of these concerned the readiness with which man contracted psittacosis from the infected bird and the low infectivity of the human case. Dr. Findlay had said that, apart from the bacteriophage, he had been unable to find an example of a virus excreted in the faeces. He had overlooked psittacosis, however, for in birds suffering from this disease the virus was present in the droppings. The affected bird had diarrhoea and its feathers became soiled with virulent droppings, which, when dry, were readily disseminated in the air in the form of a fine dust. It seemed most probable that this disease was passed from bird to
bird by the agency of the virulent excreta and it was also extremely likely that the same material was responsible for human infections, the virus being introduced by the respiratory route. In the human cases pulmonary lesions were usually present. The sputum, though scantly, contained the virus, and the patients coughed a good deal, yet transmission from man to man was rare; cases of psittacosis could be nursed in the open ward with ordinary precautions without danger to the other patients. What the explanation of this might be, he was unable to say. It might be that the act of coughing did not expel sufficient virus to make these cases a menace to others and in support of this was the scantiness of the sputum. On the other hand, there was a possibility that as the result of sojourn in the human tissues the virus lost some of its invasiveness.

The other problem concerned the source of infection in zoster. There was considerable evidence that the viruses of zoster and chicken-pox were the same. A large percentage of susceptibles exposed to cases of zoster contracted chicken-pox, in fact clinical records of this occurrence were so numerous as to rule out the possibility of it being a coincidence. If zoster ever gave rise to zoster in contacts it did so rarely, and it seemed as though it arose in individuals already carrying the virus. The designation, symptomatic zoster, indicated the recognition of this probability in some cases, but the inability to trace the source of infection in the so-called idiopathic case suggested that, here also, the same held true. If this were so and if one could assume the identity of the viruses of zoster and chicken-pox—two rather large assumptions perhaps—then it would seem that the seeds of zoster were sown in a previous attack of chicken-pox. During recovery from an attack of chicken-pox the virus would be overcome but not exterminated; a state of symbiosis would be set up and the most likely place for this to take place would be in the central nervous system. Later in life, when possibly the immunity acquired by the attack of chicken-pox was weakening, some factor such as trauma or poisoning by one of the metals, might upset the balance between host and virus, to the detriment of the former. The dormant virus would be lit into activity and an attack of zoster would result.

Mr. R. E. Glover referring to the experiments on the transference of fowlpox by mosquitoes said that under natural conditions the severest outbreaks of this disease often occurred during the winter and early spring months when it was most unlikely that transmission by mosquitoes could take place.

Dr. J. O. W. Bland said that he had not himself worked on the problem of the means by which viruses gained entrance to the body but he had been interested in the way in which, having entered, they subsequently penetrated the cells in which they multiplied. He had observed that in the case of psittacosis virus growing in tissue cultures of chick-embryo, colonies of the organism could be found: (a) within macrophages; (b) within cells of epithelial type migrating from cultures of lung, and (c) within cells of fibroblast type migrating from cultures of muscle. Now it could be presumed that the virus gained entrance to macrophages by means of the phagocytic activity of the cells and the same might be true perhaps of the lung epithelium. Fibroblasts, however, were not considered to have much power of phagocytosis, if any. How then did the virus gain entrance to these cells? He had hoped to be able to observe, in living cultures, either directly or in cinematograph films, this penetration by the virus. Up to the present, however, this had not been possible and the problem remained unsolved.

Mr. John Bunyan said that one possible site of entry of viruses appeared to have received no attention—i.e. the periodontal tissues. Periodontal disease in varying degrees was present in nearly every mouth, causing the periodontal membrane, which had a good nerve supply, to be opened to infection. It had been noted that patients who had had their mouths thoroughly overhauled, and particularly in cases where overhanging fillings had been eliminated, were much less liable to contract heavy colds or influenza.