EXPERIMENTAL STUDIES on ENCEPHALITIS LETHARGICA and HERPES FEBRILIS

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Contents.

Foreword.	Page	3.
Part 1 - Introduction.	Page	5a.
Part 2 - The Diagnostic value of Cerebral Lesions in the Domestic Rabbit.	Page	26.
Part 3 - Experiments on the Survival of the so-called Virus of Encephalitis Lethargica and the Virus of Herpes Febrilis *in vitro*.	Page	52.
Bibliography.	Page	87.
Illustrations.	Page	94.

FOREWORD.

I was very fortunate in being introduced to the study of the viruses of encephalitis lethargica and of herpes by Dr. Simon Flexner of the Rockefeller Institute for Medical Research, New York. Dr. Flexner himself has done a large amount of work on these viruses, and from him I learned technique, obtained material, and received much valuable advice and criticism.

Owing to the vast literature which has accumulated on a subject about which so little is definitely known, it is impossible to deal in detail with all the evidence recorded. Much of it is contradictory, and it is difficult, if not impossible in many cases to determine the actual truth. After a consideration of practically all the main articles, my impression is that a great bulk of the work has not been properly controlled, and that the conclusions will be found to be erroneous and valueless. With so important and so difficult a subject it is only possible to take up one or two particular points for detailed study. In this Thesis the results of investigations of two problems are recorded. The first deals with the brain lesions found in the domestic rabbit, and the results show that much

experimental work on encephalitis lethargica in this animal, particularly by Scandinavian and American observers, is open to very grave question, and their results must therefore be of little use. Briefly I have shown that the perivascular and other brain lesions in the rabbit, regarded by many workers as pathognomonic of experimental encephalitis, may frequently be found in uninoculated animals, in some cases in as many as 75 per cent ofthe rabbits examined. These results are based on the examination of brain sections from 372 rabbits. Therefore the results of work in which the histological picture of the brain of the rabbit has been taken as the criterion for a positive transmission of the virus to that animal cannot be accepted.

The second point of study has been the attempt at cultivation of the viruses of encephalitis lethargica and herpes febrilis. Several observers have claimed the isolation and cultivation of what they consider to be the etiological agent, but the organisms described differ widely, with each worker, in their morphological and biological characteristics. An attempt has been made by the writer to investigate the claims of the various workers. None of these observations have been confirmed, and certain investigations along deductive lines have shown most

of the previous work to be uncontrolled and fallacious. Two papers relating to the Diagnostic Values of Brain Lesions in the Domestic Rabbit and Experiments on the Survival of so-called Encephalitic and Herpetic Viruses in vitro are already in the press, having been accepted for the Journal of Experimental Medicine.

The whole subject of the so-called "filterable viruses" is obscure; practically nothing is known of their morphology, little is known of their biology, and it is only by carefully controlled work along many lines of investigation that further knowledge can be gained.

Part 1.

INTRODUCTION.

ENCEPHALITIS LETHARGICA.

Von Economo (1) in 1917 first showed that encephalitis lethargica was a definite clinical entity, and since that time a great deal of experimental work has been carried out by large numbers of investigators in many countries.

In this survey, which is merely introductory to the experimental work submitted in this thesis, it is manifestly impossible to review in order each of the many papers that have appeared on so-called experimental encephalitis lethargica. Moreover, lengthy discussion would have to be included, and therefore, for the sake of brevity and clearness, the main accepted position has been summarised in the following pages.

It is chiefly through the work of Levaditi and his collaborators (2 to 20), and Doerr and Schnabel (21, 22), that our knowledge has been gained, and the general conclusions are here presented.

The work of Levaditi and his collaborators.(2 to 20).

Difficulty was at first encountered in transmitting the disease to animals, but rabbits have been successfully inoculated with brain material from a fatal human case. Once passage had been secured, it

was comparatively easy to re-transmit the virus, not only to rabbits, but also to guinea pigs and white mice. Monkeys, however, are naturally immune, which is in contrast with another disease of the central nervous system, namely, acute poliomyelitis. The earliest experiments were done using intra-cerebral inoculations, but once transmission had been secured, animals could be infected in a variety of ways :- by corneal scarification, intra-ocularly, or through the nasal mucous membrane after injury. By repeated passage through the rabbit the virus becomes capable of causing infection in these animals by inoculation intraperitoneally, intramuscularly, into the sciatic nerve, and also by dermal scarification. Attempts to transmit the virus by intravenous, subcutaneous and alimentary routes were unsuccessful.
Scarification of the cornea and inoculation with virus (rabbit brain) produces in rabbits a severe keratitis. Later (7-13 days) the animal shows symptoms of involvement of the central nervous system and death supervenes. (These symptoms will be described later in connection with my work on cultivation experiments).

Resistance of the virus to various bactericidal agents.

Exposure to a temperature of 55-70°C. for 30 minutes

kills the virus. Bile, and methylene blue also destroy its infectivity very quickly : 1 per cent phenol requires application for 3 days to eliminate the virus. Infective brain material, dried in vacuo over sulphuric acid, retains its activity for periods up to 6 weeks.

Nature of the virus.

The nature of the virus is as yet unknown. It is ultra-microscopic and filterable. Attempts at cultivation by Levaditi and by Doerr and their collaborators have been unsuccessful. Attempts by other observers and the writer are described later.
The virus remains potent in 50 or 33 per cent solutions of glycerine for several months.
Minute bodies have been described by Da Fano (23-25) in brain sections from human cases of encephalitis lethargica. They can be demonstrated by various stains such as Unna's polychrome methylene blue, Mann's stain, Giemsa's stain, etc. These minute bodies vary in size and appear to be surrounded by a halo. Similar bodies have been described by Levaditi and his collaborators, who, however, regard them as acidophilous degenerations of the nucleus caused by the virus.

Immunity.

Acquired. Subcutaneous inoculation with killed or living encephalitis lethargica virus never protects against a subsequent intra cerebral inoculation of the living virus, but previous subcutaneous injection with active material prevents the appearance of keratitis. Vaccination with killed virus produces only a very partial immunity even to corneal inoculation.

Blood serum of immunised rabbits does not neutralise active virus in vitro, and the blood serum of human recovered cases only shows slight evidence of immune bodies several months after onset of the disease. Serum from recent human cases has no neutralising effect whatever on fresh virus.

With regard to immunity from corneal inoculation in rabbits, several interesting facts arise. Whereas the animal may withstand subsequent intra-cerebral inoculation with virus, the other cornea is still susceptible.

Sources of the virus.

The first unmistakably positive transmission of the virus from human material was that of Levaditi and Harvier (2,3,), who in February 1920 inoculated one

monkey and two rabbits with brain emulsion from a fatal human case of encephalitis lethargica. One rabbit died 8 days later while the other rabbit and the monkey remained well. It is from this one strain that the experiments were made from which the main conclusions of Levaditi have been drawn. Levaditi and his collaborators have been unable, up to the present, to obtain positive transmission effects in the rabbit by the sub-dural inoculation of cerebro-spinal fluid from human cases of encephalitis lethargica. These observers have, however, obtained a similar virus though in an attenuated form from the saliva of apparently healthy persons, presumed therefore to be carriers of the infection (14). This virus isolated by corneal scarification is probably that of herpes and not that of encephalitis lethargica.

The same general conclusions have also been reached by Doerr and Schnabel (21,22) working independently and their experimental results are inclose agreement with those of Levaditi and his co-workers.

McIntosh and Turnbull (27) have claimed to have transmitted the virus of encephalitis to the monkey. Flexner (26), on several grounds regards their work as very unconvincing . In a critical examination of

the literature on experimental encephalitis lethargica Flexner points out that Doerr and his associates (21, 22) after many unsuccessful attempts finally in 1921 recorded the successful inoculation of the rabbit with cerebro-spinal fluid from a fatal human case of encephalitis. In 1922 Doerr (28) recorded two more successful transmissions, one from human brain material direct, and one from human brain tissue from a rabbit which succumbed from an intercurrent staphylococcal infection of the trephine wound thus making in all 3 successful transmissions. Doerr's associate, Schnabel, (29), working independently in the Robert Koch Institute in Berlin, reported in 1922 (the first case in Germany) that he had isolated a virus similar to the previous ones from the cerebro-spinal fluid from a case of acute epidemic encephalitis.

Thus from the vast amount of work done in Europe on experimental encephalitis lethargica, it would appear that the successful transmission of encephalitis virus from human material to the rabbit has only been accomplished in 5 instances, once in France (Levaditi), 3 times in Switzerland (Doerr) and once in Germany (Schnabel). Flexner (26) has since recorded the successful inoculation of encephalitis

from human cerebro-spinal fluid to the rabbit. This strain, "Beckley", was used with others in the experiments described later in this thesis.

This difficulty of transmitting encephalitis virus to lower animals has apparently not been encountered by Loewe and Strauss (30,31), of the Mount Sinai Hospital; New York. As early as May 1919 these observers recorded the transmission of encephalitis to the monkey. Later they stated that the rabbit and guinea pig could also be affected. They further claimed the isolation, by means of the Noguchi technique, of a specific micro-organism, details of which will be considered later. Loewe and Strauss advocate that the rabbit should be used for diagnosing doubtful cases of encephalitis lethargica. They claim 12 positive inoculations out of 16 different samples of cerebro-spinal fluid (75 per cent positive), and 11 successful transmissions from the nasopharyngeal washings from 14 patients (78 per cent positive). The faulty premises on which these conclusions are based are pointed out in Part 2 of this thesis.

It is thus seen that the so-called virus of epidemic encephalitis has probably only been isolated by four workers and that it has very definite pathogenic and biological characteristics. It is an invisible

filter passing micro-organism which up to the present has not been cultivated outside the body.

AUSTRALIAN "X" DISEASE.

A peculiar disease occurred in New South Wales, Queensland and Victoria in 1917. At first it was thought to be an atypical form of poliomyelitis but was later recognised as a hitherto unknown complaint and designated Australian "X" disease.

Flexner (26) who examined specimens of the central nervous system from patients dying of this disease, is of the opinion that the lesions show a resemblance to poliomyelitis but are distinct from encephalitis. However the high mortality (70 per cent) of Australian "X" disease, and the small number of recoveries in which there is residual paralysis (2 out of 35), and two more in which there were signs of mental disorder, are quite unlike poliomyelitis. 30 per cent of the patients were over 15 years of age and it was noted that infants and young children when affected were more likely to survive than old children and adults, which would suggest that Australian "X" disease may be due to poliomyelitis virus of exalted virulence.- Transmission experiments show that the disease is easily communicable to monkeys, and the following series of successful inoculations have been reported by Cleland and Campbell (32). Human to monkey;

monkey to monkey for several generations; monkey to sheep; sheep to sheep for several generations; sheep back to monkey; monkey to one horse and one calf. It is of interest to note that rabbits resist inoculation. It would appear that the virus of Australian "X" disease is quite different from, but of the same type as poliomyelitis and encephalitis lethargica.

HERPES LABIALIS (Syn. HERPES FEBRILIS).

Löwenstein (33,34), was the first to record that the virus of herpes labialis is transmissible to rabbits although, as pointed out by Da Fano (35), the first successful transmission was made by Grüter in 1912-1914. His results, which were not published till 1920 (36), showed that it was possible to infect the cornea of the rabbit and that the condition could be transmitted from animal to animal. With the fluid from experimental herpetic keratitis of the rabbit, Grüter succeeded in producing typical herpetic lesions on the eyes of blind men.

Kraupa (37) confirmed Grüter's work but again his article appeared after Löwenstein's.

Löwenstein by scarifying the cornea of the rabbit and then infecting with material from herpes labialis, produced a severe keratitis with lesions similar to human herpes corneae. The experimental condition could be transmitted from rabbit to rabbit. Even dilution of the infective material 1 in 200 did not appreciably diminish its virulence. The material was rendered non-infective by heating to 56°C. for 30 minutes or by keeping it in the incubator at 37°C. for 24 hours. Blood from herpetic cases was not capable of producing corneal lesions in rabbits, and

vesicle material from cases of scalding and pemphigus was likewise non-infective.

The nature of the lesion produced after corneal inoculation with herpetic material is, after 48 hours, an opacity along the lines of scarification. The area of opacity rapidly becomes increased in size, and the whole cornea becomes filmed over. The eyelids remain closed and on forcing them apart a purulent exudate is found and the typical appearance of an intense keratitis is noted.

Although he failed to infect with Berkfeld filtrates of herpetic material, Löwenstein, from general considerations, was of the opinion that the virus is a filter passer.

Löwenstein's results have been confirmed by Doerr (38), Stocker (39) and Baum (40).

Doerr made the very important observation that rabbits inoculated corneally with human herpetic material showed not only the usual eye lesions which may be observed after 48 hours, but symptoms indicating involvement of the central nervous system. These peculiar cerebral symptoms which appeared 5 to 7 days after inoculation consisted of convulsions, movements in circles, trismus, transitory paresis of the extremities and profuse salivation. In the majority

of such cases death ensued although recoveries were noted. In a further paper, Doerr, in collaboration with Vöchting (41), confirmed these observations and extended the work of Löwenstein. It was found that after inoculation of the one cornea, the other became resistant to infection, and the degree of immunity was in proportion to the length of time between the two inoculations, thus showing a general immunity.

Moreover, by intravenous inoculation of infective material from experimental keratitis, a general herpetic condition could be produced in the rabbit. Having drawn attention to the cerebral symptoms following experimental herpetic keratitis, Doerr and Vöchting showed that the brain material was infective when introduced subdurally into rabbits, and the virus could be passaged by such inoculations. They thus showed that herpes virus has a distinct affinity for the central nervous system and suggested a possible relationship between that virus and the virus of encephalitis lethargica. Doerr and Vöchting, however, could not reproduce keratitis from brain material, but this was shown to be possible by Blanc (42). This observer confirmed the work of Doerr and Vöchting and showed that rabbits may die with cerebral symptoms

5 days after corneal inoculation with herpetic material. Moreover, brain emulsion from such animals could cause herpetic keratitis in other rabbits, some of which died with symptoms of encephalitis. Blanc also points out the similarity of the experimental encephalitis produced by herpetic virus, and by material from cases of encephalitis lethargica. Blanc and Caminopetros (43) and Doerr and Schnabel (21) showed that rabbits recovering from herpetic keratitis were subsequently immune to subdural inoculation with infective brain material.

With regard to the susceptibility of other animals to herpetic virus, Doerr and Vöchting (41) showed that the infection could be transmitted from the rabbit to the guinea pig, and this observation was confirmed by Luger and Lauda (44). Teissier, Gastinel and Reilly (45) found that the rat was susceptible to intra-cerebral inoculation, but not, or only slightly so to corneal infection.

Blanc and Caminopetros (42,46), have shown that the dog, pigeon, toad and monkey are immune to corneal infection, whereas the white mouse is susceptible.

The question of filterability of the virus is still controversial, but most observers are agreed that the etiological agent of herpes is a filter passer.

HERPES GENITALIS.

With regard to herpes genitalis, Blanc and Caminopetros (46), Lipschütz (47), Fontana (48), Ravaut and Rabeau (49), showed that the vesicular fluid could produce keratitis in rabbits. Moreover, Fontana showed that material from such an experimental keratitis could produce a typical herpetic vesicle on human skin, and confirmed the observation of Lipschütz that herpes genitalis is transmissible from man to man by skin inoculation.

HERPES ZOSTER.

In discussing herpes zoster, Da Fano (35) hesitates to state that it is caused by the same virus as herpes labialis and herpes genitalis.

Truffi (50) and Meineri (51) claim to have transmitted a virus from herpes zoster to the rabbit and guinea pig respectively, but their results are not conclusive. Luger and Lauda (52) state that vesicle fluid from cases of herpes zoster is non-infective for the rabbit.

With regard to the etiological agent of herpes, numerous investigators have described minute particles both in human vesicles and in the experimental disease in the rabbit, which they consider to be the incitant.

Histological investigations as to the nature of herpes virus.

Löwenstein noted in sections of vesicles from the rabbit, stained by Giemsa's method, numerous very minute particles, resembling bacilli or diplococci. In preparations stained by Pappenheim's panchromatic method (Jenner-Giemsa), he noted different sized particles arranged in pairs, reddish-purple in colour and frequently surrounded by a halo. Similar bodies were also noted in human herpetic vesicles and in human serum to which vesicle fluid had been added. All attempts to culture these bodies failed. Later Löwenstein (53) examined film preparations from experimental herpetic iritis and noticed in Jenner-Giemsa specimens that the leucocytes contained minute bodies arranged in pairs and staining reddish, while blue coloured granules surrounded by a halo were observed in the cytoplasm of epithelial cells.
In 1921 Blanc and Caminopetros (46) noticed in Romanowsky preparations from the conjunctival secretions of rabbits suffering from herpetic keratitis, small deeply staining granules.
Da Fano (35) in an important paper in 1923 described a detailed study of the brains of rabbits infected intracerebrally with herpetic virus. In the

nervous system he finds "granular structures morphologically similar to the encephalitic neuro-corpuscles of Levaditi, Harvier and Nicolau, morphologically and tinctorially identical with the minute bodies found within and without the central nervous system of cases of lethargic encephalitis." Da Fano names these structures "minute herpetic bodies" and suggests that they might be the virus itself or particles of organic matter to which the virus tenaciously clings.

Lipschütz (54,55), describes in the vesicles of herpes zoster, and also in experimental herpes zoster in the rabbit, minute intra-nuclear inclusions. Similar bodies were also found in the original and experimental lesions of herpes labialis and herpes genitalis. Such inclusions, termed α and β bodies, vary in size and may be as large as 2 μ, when they almost entirely fill the nucleus of the epithelial cell. They are bright red or purple red structures and are said to be easily distinguishable from the nucleus and nucleolus. Lipschütz considers them to be a specific reaction to the virus by the cell and nucleus. Owing to the resemblance of these structures to the "Clamydozoa" of Prowazek, the name "clamydozoonoses" has been suggested. Luger

and Lauda (52) found similar bodies but do not regard them as specific.

Cultivation experiments with herpetic virus have failed to reveal the etiological agent.

Kooy [56], claims to have isolated from human and experimental herpetic conditions , a short pleomorphic bacillus, with which he claims to have produced herpetic conditions. Luger and Lauda (57), have been unable to confirm Kooy's results. Other workers have consistently failed to find any definite etiological agent, and the present opinion is that the incitant is an unknown new species and probably filterable.

THE RELATION BETWEEN THE SO-CALLED VIRUS OF ENCEPHALITIS LETHARGICA AND THE VIRUS OF HERPES LABIALIS.

Doerr and Vöchting (41), as previously mentioned, had noted that corneal scarification of herpetic material in rabbits is followed about a week afterwards by symptoms indicating involvement of the central nervous system, and suggested that the virus of herpes was allied to that of encephalitis. Later Levaditi and Harvier (12) also showed that herpetic virus was capable of giving rise to encephalitic symptoms when inoculated into rabbits. These observations were confirmed by Blanc (42), Blanc and Caminopetros (43), Doerr and Schnabel (21). It was shown that the so-called encephalitis virus was able to produce definite herpetic vesicles when inoculated on the scarified cornea of a rabbit.

Cross immunity tests further showed that the viruses were very closely allied to each other, if not identical. A rabbit recovered from a weak strain of herpes, is completely protected against a massive dose of encephalitis virus inoculated intracerebrally. Similarly, immunity produced by the encephalitic strain protects the animal against a virulent strain of herpes virus.

Levaditi, Harvier and Nicolau (20) came to the conclusion that the virus of herpes and that of epidemic encephalitis are of the same nature; the former being only a less virulent variety of the latter.
Doerr and Schnabel (21,22) although expressing the same opinion were much more guarded in their statement. Schnabel (29) has shown that encephalitis virus as contained in the brain of the rabbit can induce herpetic vesicles on the human skin.
At the present time it is impossible to state definitely the precise relationship of the viruses of encephalitis lethargica and herpes febrilis. In view of the published facts and in view of some experiments which I made with the viruses available (see part 3 of this thesis) I am of the opinion that, until proved otherwise, they should be regarded as identical. In view of the difficulty of transmission of encephalitis virus from the human to the rabbit our present knowledge as to the precise nature of that virus is still very scanty and when further facts have been obtained then the exact relationship between the two viruses may become more definitely established.

Part 2.

THE DIAGNOSTIC VALUE OF BRAIN LESIONS IN THE DOMESTIC RABBIT.

The use of the rabbit as an experimental animal for the study of diseases of the central nervous system has been greatly extended during the last few years. Recently a large amount of work has been done on the experimental transmission of the virus of encephalitis lethargica and of herpes labialis to the rabbit by means of intra-cerebral inoculations, the presence of certain cerebral lesions being the criterion for a positive transmission. The description and illustrations of the changes in the brains of such experimental animals are similar to those noted by Bull (58) and Oliver (59) as occurring in uninoculated stock rabbits, and to the lesions described by Reasoner (60) as being characteristic of experimental cerebral syphilis in the rabbit.

As a number of observers have based their conclusions solely on the results of thr microscopic examination of the brains of these animals, Dr. Flexner suggested that I should re-investigate the question as to the diagnostic value of certain rabbit brain lesions, employing a large amount of material which happened to be available at the time. In this way the frequency and severity of such changes could be ascertained in animals which had not been inoculated intra-cerebrally, and, in addition, the observations

of other workers could be controlled.

The value attached to the sole presence of cerebral lesions is shown in the following brief review of the literature.

Literature.

Loewe, Hirschfeld, and Strauss (61,62,) diagnose the presence of encephalitic virus in experimental rabbits from the histological lesions and point out that in doubtful cases the histological picture suffices for a diagnosis. These observers state that the changes consist of a marked meningitis with an infiltration of mononuclear leucocytes. The small blood vessels in the cortex and in the basal ganglia show a perivascular infiltration with mononuclear cells and there may be areas of mononuclear infiltration surrounding a zone of necrosis. These lesions are stated to be similar to those found in the human brain in encephalitis lethargica and to be diagnostic of the experimental disease. Moreover, they have claimed the isolation of a micro-organism which they regard as the etiological agent of encephalitis lethargica, (63,30,31) and when cultures are inoculated intracerebrally in rabbits, lesions similar to the above are found. Based on these results, they assert that the culture causes encephalitis lethargica.

From their published experiments, it is noted that rabbits dying the day after intracerebral injection with suspected encephalitis material may

show marked meningitis with infiltration of mono-nuclear leucocytes, perivascular infiltration with monocuclears, and hemorrhages into the cortex.

With regard to similar lesions being found in the brains of non-inoculated rabbits, Strauss (64) writee: "The rabbit has been found to have diseases, especially diseases of the blood vessels similar to arterio-sclerosis, which has negatived the work done in the production of arterio-sclerosis in rabbits, but I know of no other lesion of any importance that has been found by experimental workers in rabbits, especially when using careful methods, which is like that produced by our inoculations." Loewe (65) states : "Throughout our extensive animal experimentation, we have never met the spontaneous production of the characteristic lesions." These observers have been supported by Thalimer (66,67,) who affirms that "virus from the central nervous system has been injected into rabbits and has produced the disease -- has produced the microscopic lesions of the disease." He also regards the perivascular and meningeal infiltration in the brain of the rabbit as being "diagnostic of the disease."

Kling, Davide, and Liljenquist (68,69,70,71,) in addition, emphasize the presence of brain lesions

as evidence of the experimental transmission of encephalitic virus. They point out that rabbits dying many months after intracerebral inoculation may show these changes. In one experiment, 2 rabbits were injected intracerebrally with filtrates from nasopharyngeal washings. One rabbit died 7 months later without exhibiting any symptoms, but a meningo-encephalitis was found in the brain on microscopic examination. The brain of this animal was inoculated intracerebrally into a second rabbit, which died 4 months later. This animal also showed a pathological picture similar to those previously described. In other experiments, one rabbit died 3½ months and another 10½ months after inoculation. From the meningeal and perivascular infiltration in all these rabbits, the authors considered the material used for inoculation to contain the virus of encephalitis lethergica. They remark: "It is astonishing that so pronounced an inflammation can be produced without giving rise to any apparent cerebral symptoms." Recently these authors have attempted to perform cross-immunity tests between herpes and encephalitis lethargica on animals which have been inoculated intracerebrally 6 to 7 months previously. They draw conclusions that the encephalitic virus

does not immunize against the herpetic. The criteria, however, for determining the encephalitic infection were solely the perivascular and meningeal infiltrations with mononuclear cells.

McIntosh (72) reports the inoculation of human cerebrospinal fluid into a rabbit which died 7 months after injection. On the basis of "extensive infiltration in brain", he makes the diagnosis of encephalitis lethargica. McIntosh asserts that in encephalitis lethargica, the cellular infiltration consists mainly of lymphocytes and glial cells. Supporting Kling's work, he suggests that a higher percentage of encephalitis cases would be obtained from cerebrospinal fluid if rabbits which showed no signs after intracerebral inoculation were killed 10 to 20 days later and the brain examined histologically. McIntosh further emphasizes that the most important features of the lesions of epidemic encephalitis in rabbits are: (1) the site, namely the meninges and around the blood vessels. (2) The nature of the cells -- lymphocytes and glial cells -- and (3) The presence of small foci of cells in the brain substance.

Reasoner (60), in experimental syphilis of the rabbit, noted in the brains of affected animals

meningeal and perivascular infiltration with mononuclear cells. He also describes focal necrotic lesions in the cortex with marked proliferation of plasma cells and lymphocytes.

Plaut, and Mulzer (73) find similar lesions in the brains of rabbits inoculated with Treponema pallidum and regard the microscopic findings as evidence of cerebral syphilis. Brown and Pearce (74), however, when considering changes in the brain in experimental syphilis in the rabbit, emphasize that lesions analogous to those which might be produced by a syphilitic infection may be found in supposedly normal rabbits.

Other observers have also drawn attention to the fact that lesions similar to those just described may be found in non-inoculated rabbits or in rabbits suffering from other diseases.

Thus Bull (58) pointed out that the perivascular changes in the brains of rabbits inoculated with streptococci from cases of poliomyelitis could be found in stock rabbits dying of bacillary septicemia and concluded that these pathological findings were not peculiar to poliomyelitis. He also noted "areas of necrosis of nerve cells surrounded by infiltration of cells of the lymphocytic type and adjacent to

blood vessels showing perivascular infiltration extending usually from the meninges."

Later, Oliver (59) drew attention to the presence in stock rabbits of a chronic meningo-encephalitis, namely, infiltration of the meninges of the perivascular areas with mononuclear cells. He points out their similarity to the brain lesions of human encephalitis lethargica. In addition to these lesions are described areas of necrosis filled with cellular débris and also focal areas of infiltration. The number of animals examined and the existence of minor infections such as snuffles are not mentioned.

This brief review of the literature shows that caution is necessary in the interpretation of experimental results when cerebral lesions in rabbits are solely used as criteria.

An investigation was undertaken to determine the frequency and nature of cerebral lesions in (a) uninoculated, supposedly normal rabbits, (b) those inoculated (not intra-cerebrally), with various materials, and (c) those suffering from spontaneous intercurrent infections. The results of the examination of 372 rabbits will form the substance of this paper. The pathological findings will be described first and the incidence among different groups of animals will be shown later, and finally the significance of the results will be discussed.

Material used. In order that as much material as possible should be collected, Doctors Brown and Pearce of the Rockefeller Institute kindly placed at my disposal sections of brains of a number of animals, some having been inoculated with a transplantable rabbit tumour, others early cases of experimental syphilis, and the remainder normal animals.

The animals employed in the tumour and syphilis experiments were carefully selected and kept in rigid quarantine in individual cages before being

used. Only those which showed no evidence of any disease whatsoever were used for inoculation. The normal animals were control to this series, and their freedom from disease was verified by autopsy. In addition to this material, the brains from stock animals with snuffles, from normal rabbits killed to obtain kidney tissue, and from stock animals of different ages chosen at random, were sectioned and examined. In some of the animals complete transverse sections of the cerebrum only were obtained, but in the others, sections of forebrain, the complete cerebrum, the mid-brain, and the medulla were secured. The material from Doctors Brown and Pearce was fixed in Zenker's fluid and the remainder in 10 per cent formalin in normal saline solution. All sections were stained by hematoxylin eosin, while some showing lesions were stained by Giemsa's method. In addition, frozen sections of the brains from rabbits with snuffles were cut at once, and if lesions were present, emulsions of brain substance were inoculated intracerebrally into small (800 - 900 gram) rabbits, and cultures made in various aerobic and anaerobic media.

Results of Examination. The lesions found in the brains of these rabbits are characterized by

the presence of mononuclear leucocytes and may be classified into 4 types.

1. Perivascular infiltration.
2. Infiltration of the meninges.
3. Infiltration under the ependyma of the lateral ventricles.
4. Infiltration into the brain substance.

This classification does not separate the lesions into hard and fast groups, as in many cases lesions in all 4 sites are present. Usually the perivascular, meningeal, and interstitial lesions are found in the same case, but in others one of the types may predominate.

The lesions described in detail are as follows :

1. Perivascular lesion. This is the commonest and most striking lesion and may vary from a single layer of mononuclear cells to a thick, dense sheath of cells almost obscuring the blood vessel. Often, the so-called Virchow-Robin spaces surrounding the vessel may be filled with such cells. In hematoxylin-eosin preparations, the cells are small, round, and deeply staining.

2. Meningeal infiltration. This lesion is not quite so common as the perivascular and consists

of infiltration into the pia-arachnoid of mononuclear cells. Often the lesion is so marked that there is a broad band of cells on the surface of the brain and there are finger-like masses of cells dipping into the brain tissue surrounding the blood vessels. The lesion may occasionally be slight, but definite, and appear as a few cells lying apparently between the pia and the brain surface proper.

3. Infiltration under the ependyma of the lateral ventricles.

This is the least common lesion and may only be found in the fore-brain. Care has to be taken in differentiating the mononuclear cells from nerve cells which may be found in this region. The nerve cells are larger, less densely stained, and have an open chromatin network in the nucleus. Careful examination with the high powers of the microscope will prevent any confusion as to the nature of the cells.

4. Infiltration into the cerebral substance.

This lesion is more common than the meningeal infiltration and only slightly less frequent than the perivascular change, and is usually associated with the latter. This infiltration may be diffuse with mononuclear cells scattered throughout the section, but usually there are aggregations of cells

distributed throughout the brain. These focal collections of mononuclear cells are sometimes large enough to be seen with the naked eye in the stained specimen. The neighbouring blood vessels, as a rule, show marked perivascular lesions.

In addition, there is frequently to be seen what has been described in non-encephalitic rabbits by Reasoner (60), Bull (58), and Oliver (59), as focal areas of necrosis, but are thought by Kling (71) and others (61,62,64) to be diagnostic of experimental encephalitis lethargica. It usually consists of an area in which the centre is definitely necrotic and containing granular débris. Around this is an area of cells having a large, faintly-staining nucleus with a well-defined nucleolus and an open chromatin network.

The cells in relation to the necrotic centre are phagocytic and may be seen filled with deeply staining granular débris. Around this cellular zone is a mononuclear-celled infiltration and the adjacent blood vessels show perivascular rings of similar cells. Under higher magnification may be seen an increase of glial tissue surrounding the lesion and forming more or less of a capsule. If the section is not taken through the centre of the necrotic

focus, the larger, epithelial-like cells and the ring of mononuclear cells only are seen.

In other sections, smaller foci are observed, while in others a process of healing with definite scar tissue may occur. Occasionally groups of 4 or 5 epithelial-like cells, without any mononuclear infiltration, may be noted as small islands in the brain substance.

These areas of necrosis have been noted in 15 per cent of the total number of brains examined.

The following table shows the incidence of these lesions and the type of rabbit in which they were found. The lesions are classified as marked and slight. The marked lesions are easily discernible while the slight lesions are definite and indicate distinct pathological change.

TABLE 1

Distribution and Incidence of Cerebral Lesions.

	Source of Rabbits	Marked	Slight	Negative	Total
(1)	Transplantable Tumour	63	10	73	146
	Per cent	43	7	50	
	Total " " positive		50		
(2)	Treponema pallidum inoculation.	28	5	49	82
	Per cent	34	6	60	
	Total " " positive		40		
(3)	Uninoculated stock	23	8	21	52
	Per cent	44.5	15.5	40	
	Total " " positive		60		
(4)	Miscellaneous diseases	30	5	15	50
	Per cent	60	10	30	
	Total " " positive		70		
(5)	Snuffles	32	0	10	42
	Per cent	76		23	
	Total " " positive		76		
	Total	176	28	168	372
	" PER CENT	47.5	7.5	45	
	" " " POSITIVE		55		

From this table it is noted that the lesions of meningo-encephalitis are present in the brain of about half the number of rabbits. The incidence of cerebral changes in the tumour, syphilis, and normal rabbits is approximately similar in each case (40 to 60 per cent). In the animals suffering from intercurrent disease, excluding snuffles, the percentage is higher and rises to 70 per cent, while in the case of the snuffles rabbits, 76 per cent are affected.

Thus out of a total of 372 rabbits which have not received intracerebral injection, more than half (55 per cent) have definite lesions present in the brain, and in 47.5 per cent, the lesions are marked. The presence of intercurrent infections may increase the proportion up to 76 per cent.

Results of animal inoculation. 15 young rabbits (800 to 900 grams) were inoculated intracerebrally with 0.25 to 0.35 cc. of suspensions of ground brain tissue from 10 rabbits having cerebral lesions determined by frozen sections prior to use. In none of these animals were any symptoms noted. They were killed at periods from 14 to 30 days after inoculation and the brains sectioned and examined. Three exhibited typical cerebral lesions, but no

conclusions could be drawn as uninoculated animals of the same size and weight used as control also showed a similar pathological condition.

Results of culture. Brains were removed from rabbits under aseptic conditions and frozen sections were made. If lesions were present, cultures were made of the brain, in broth, on blood agar plates, in Smith-Noguchi medium, and on anaerobic rabbit-blood agar plates. In none of these media was any growth obtained.

DISCUSSION.

It appears that approximately one-half of the 372 rabbits examined show particular lesions in the brain. They consist of mononuclear infiltration, either surrounding the blood vessels, under the ventricular wall, throughout the meninges and in the cortex, or, more frequently, in more than one of these areas. In addition, peculiar necrotic structures are present in the cortical brain substance. This percentage is maintained not only in animals which are normal by a complete histological examination, but also in rabbits inoculated with a non-infective, transplantable tumour, and in those in the early stages of experimental syphilis. The proportion of animals showing such lesions is increased in the miscellaneous disease group: in the snuffles series more than three-fourths may exhibit evidences of a meningo-encephalitis. It has been shown in another paper(75) that in ordinary stock animals free from nasal discharge, about 25 per cent exhibit chronic sinusitis. On the other hand, snuffles is not the only factor, as animals free from any sinus or mastoid infection and in which no evidence of disease can be found macroscopically may show pronounced cerebral changes. Furthermore, in the

disease group, which does not include snuffles but other infections, such as coccidiosis, subcutaneous abscesses and pneumonia, there is also a higher percentage of brain involvement than in the normal. However, the presence of a transplantable tumour, or early experimental syphilis, does not materially affect the proportion in which these changes are found. Attempts at transmission and cultivation of brain tissue showing these lesions have failed to yield positive results.

The frequent occurrence of spontaneous and accidental lesions of a wide variety, in the brain of the laboratory rabbit, should have an important bearing on the interpretation of experimental results based on the presence of similar cerebral changes in this animal. This is strongly emphasized by Doctor Flexner (26).

For example, Reasoner (60) noted in experimental syphilis of the rabbit, meningeal and perivascular infiltration with mononuclear cells, associated with focal necrotic areas in the cortex. Indeed, these changes are described as specific for this experimental disease. In Fig. 6 of Reasoner's paper, a meningitis is shown; in Fig. 7 a focal necrotic lesion; and in Fig. 8 a perivascular

infiltration labelled "characteristic syphilitic blood vessel." If a comparison be made between these photographs and Figs. 2 to 7, 11 to 14, and 16 of the present article, it appears that normal rabbits, those inoculated with a transplantable tumour, and those suffering from snuffles or other diseases, reveal practically the same lesions. Moreover, Bull (58) presents photographs, Figs. 12 to 17, of brains from rabbits which succumbed to bacillary infection or were killed during a streptococcus infection, in which the perivascular infiltration, the meningitis, and the focal necrotic lesions are definitely exhibited. So does Oliver (59), whose illustrations of meningeal, perivascular, and cerebral necrotic lesions, Figs. 1 and 2, were taken from supposedly healthy rabbits of the laboratory stock. It is therefore apparent that these cerebral changes can hardly be considered as specific manifestations of experimental syphilis in the rabbit.

Recently a number of investigators have reported the experimental production of lesions of the meninges and brain by inoculating rabbits with the so-called viruses of epidemic (lethargic) encephalitis and of febrile herpes. Their conflicting results are probably due to a lack of recognition of

spontaneous or accidental cerebral lesions in this experimental animal.

For example, Loewe and Strauss (30,31,61,62, 63,64), report that the cerebral changes which consist chiefly of a meningeal and perivascular mononuclear infiltration associated with areas of necrosis surrounded by a zone of mononuclear cells, indicate the action of the so-called virus of encephalitis lethargica. They obtain similar results with their supposed cultures of this material. They point out that in doubtful cases of the disease in man, the intracerebral inoculation of the patient's spinal fluid in the rabbit results in the same lesions which suffice for a diagnosis of epidemic encephalitis. These observers are supported by Thalimer (67). Now, if a comparison is made of Loewe and Strauss' photographs of the meningitis (Fig. 8 of their article 31), the perivascular infiltration (Figs. 9, 10, 12, 13, 14, and 15 of the same article); Fig. 4 of another of their papers (30), the focal infiltration with mononuclear cells (Figs. 11 and 13 of the first article 31), the focal necrotic areas (Fig. 10 of the same article), together with Figs. 4 to 8 of Thalimer's series (67), with Figures 1 to 16 illustrating the present paper, it will be

observed that the same lesions can occur in healthy animals, or in those employed for a variety of experimental purposes, but none for intracerebral inoculation. A further comparison may be made with the photographs of similar conditions in animals free from the so-called encephalitic virus presented by Bull (58) and by Oliver (59) and to which reference has already been made.

Kling and his co-workers (68,69,70,71,76,77), also emphasize the presence of the cerebral lesions in the rabbit as evidence of the experimental transmission of epidemic encephalitis and of febrile herpes. In describing the histopathology, they state: "Epidemic encephalitis is characterized not only by perivascular lesions but also by chronic foci having a necrotic centre with epithelial cells and surrounded by lymphocytes"(69). Kling [76] reports that from 50 to 60 per cent of the inoculated rabbits reveal these cerebral changes. On the other hand, of the present series of 372 brains from a supposedly normal, laboratory stock of animals and from those not inoculated intracerebrally or with encephalitic or herpetic materials, 55 per cent show a similar histopathologic condition. This can readily be seen by comparing the photographic reproductions of what

Kling and his co-workers regard as specific encephalitic changes as shown in their Figs. 1, 3, 4, 5, 8 (77), Figs. 1, 2 and 4 (70), and others (68), with Figs. 1 to 7, 13 and 16 of this article. In the latter can be seen precisely similar meningeal and perivascular infiltrations, and focal mononuclear lesions. The peculiar necrotic areas which they consider as pathognomonic of experimental encephalitis (their Fig.1 (69) and which consists of a central necrotic area containing epithelial-like cells surrounded by a zone of mononuclear cells, was found in 15 per cent of the rabbits, supposedly normal or not inoculated with encephalitic materials. An identical lesion can be observed in Figs. 11 to 16, presented herewith, and taken from a supposedly normal stock rabbit, from one with snuffles, from three with a transplantable tumour, and from one in the early stages of experimental syphilis.

This study leads to the conclusion that the cerebral lesions found so frequently in the domestic rabbit are pre-existing, that is, they are present before any experimental procedure is begun. Their origin is still an unsolved problem. Doerr and Zdansky (78) suggest that these pathological changes which are independent of the lesions of epidemic

encephalitis in man may be due to parasitic affections of the rabbits. Recently Twort and Archer (79) have reported experiments which support their view that certain spontaneous brain lesions in this animal are caused by the action of some unidentified filter-passing virus. It is important to note that their experimental animals with cerebral involvement show no symptoms indicating disease of the nervous system.

CONCLUSIONS.

Lesions of meningo-encephalitis were found in 55 per cent of 372 rabbits comprising the laboratory stock regarded as healthy, others with snuffles or dying from different affections while being kept under observation, and still others which were employed for experimental purposes, such as tumour transplantation and Treponema pallidum inoculation. None was injected intracerebrally. The lesions consist in the main of infiltration with mononuclear cells occurring around the blood vessels, in the meninges, in the cortex, and under the ependyma of the lateral ventricles, together with particular focal necrotic areas in the cortex. The incidence of these histopathological changes varies in different series of animals: in those supposedly normal and

rabbits inoculated with a transplantable tumour or with Treponema pallidum material, the percentage of positives was from 40 to 60; in those suffering from miscellaneous diseases, such as pneumonia, septicemia, etc., the percentage was 70, and in rabbits ill with snuffles, as many as 76 per cent were affected. Marked lesions were observed in 47.5 per cent of the total.

The histopathological picture observed in these rabbits corresponds to those offered by a number of investigators as the sole evidence of the transmission of certain nervous diseases of man to this animal. The spontaneous and accidental cerebral lesions in the rabbit of a wide variety and of frequent occurrence are to be regarded as pre-existing before any experimental procedure is begun. Their recognition is of the utmost importance in the interpretation of experimental results based on the presence of similar changes in this animal.

Part 3.

EXPERIMENTS ON THE SURVIVAL OF THE SO-CALLED VIRUS OF ENCEPHALITIS LETHARGICA, AND THE VIRUS OF HERPES FEBRILIS *in vitro*.

Much interest centres at present about the subject of the etiology of epidemic lethargic encephalitis and of febrile herpes. In both conditions a virus has been described as the incitant, but in each the virus possesses properties so undistinguishable that they cannot be separated by animal tests. There is a general agreement among investigators, however, regarding the qualities of the active agent of febrile herpes and the invisible infecting micro-organism associated with the lesions characterising this condition. In the case of the so-called virus of encephalitis lethargica, many workers have isolated organisms varying widely in their morphological and cultural characteristics, each of which has been regarded as the incitant of the disease.

At the request of Dr. Simon Flexner I undertook to repeat certain experiments on the cultivation of the so-called virus of epidemic encephalitis outside the body. These experiments failed to confirm the previous reports, and in addition some new facts have been secured which may be of value in any further cultivation tests which may be undertaken.

LITERATURE.

Von Wiesner (80), Rosenow (81), and Brasher

and his associates (82) isolated Gram-positive streptococci or diplostreptococci from brains obtained from cases of encephalitis lethargica in man. Morse and Crump (83) have described a staphylococcus-like microorganism occurring in similar material. Bradford, Bashford and Wilson (84) have cultivated in Smith-Noguchi medium a filter-passer from such tissues, which is similar in some respects to the microorganism recovered by Loewe and Strauss (30,31), to be described presently. Others have obtained in cultures a Gram-positive coccus and a Gram-negative bacillus (85,86,87,88).

Loewe and Strauss (30, 31) have reported the isolation of a filter-passer from the brain, spinal fluid, filtered nasopharyngeal secretions, and blood from encephalitis lethargica in man, from the brain of inoculated rabbits and monkeys, and from the nasopharyngeal mucosa of inoculated rabbits. This microorganism, they state, can induce in rabbits the disease resembling that of man. It resembles the globoid bodies of poliomyelitis, and is filterable. Growth is obtained in the Smith-Noguchi medium and is usually evidenced on the 5th to 7th day by clouding of the medium. Transfers to a semisolid medium are only possible with later generations of the organism

when a general clouding occurs in which colonies can be observed by magnification. Growth, however, in semisolid medium is not obtained directly from the infectious material.

Loewe and Strauss state that intracerebral inoculation of cultures reproduces the disease in rabbits. The incubation period is from 2 to 42 days, when 50 per cent of the rabbits succumb with typical lesions, and in a later series of 20 rabbits "12 succumbed with typical lesions" (60 per cent). They believe that an apparent natural immunity exists in rabbits. In turn, cultures from the brain and spinal fluid of inoculated rabbits have produced lesions in 5 of 12 animals (42 per cent). It is important to mention that the criterion for establishing a positive transmission is the presence of lesions in the brain noted after histological study. In only one animal are clinical symptoms described, in which paralysis of the posterior extremities occurred 5 weeks after inoculation and then progressing to the anterior limbs. Histological examination of the brain revealed "typical pathological lesions". These consist, in brief, of a meningeal and perivascular infiltration with mononuclear cells and small focal necroses.

Loewe and Strauss' results have been confirmed by Thalimer (67, 64), who emphasizes the importance of the changes in the brain as a critical sign of positive transmission. He pointed out that the lesions were sometimes confined to a small portion of the brain and cord, and many sections were required so as not to miss them in some specimens. Sometimes only an occasional blood vessel showed perivascular infiltration while in others nothing more than marked congestion of the brain was found.

The cultural work of Loewe and Strauss and of Thalimer has not been confirmed by Amoss (64).

From this review of the literature it is apparent that the question of the incitant of encephalitis lethargica is still open. The results to be reported in this paper are based on experiments undertaken at the request of Dr. Simon Flexner to study anew this problem.

The first part of this study relates to cultivation tests with the so-called virus of encephalitis lethargica and of herpes with the object of isolating, if possible, the microorganism of Loewe and Strauss for which certain proofs of its relationship to the disease have been presented.

It was necessary first to establish the presence of an infective agent in the materials employed for cultivation before search for a specific incitant could be begun.

Materials.

In the following experiments, two strains of so-called encephalitis and one of herpetic virus were employed.

THE SO-CALLED ENCEPHALITIS VIRUSES.

1. Strain Levaditi. This strain was obtained by Doctor Levaditi from a case of encephalitis lethargica, and forwarded to Doctor Flexner. The virus survives in 50 per cent glycerol for at least 6 months. Intracerebral inoculation into rabbits produces, after an incubation period of 4 days, elevation of temperature (41°C. to 43°C. = 106°F. to 109°F.), retention of urine, profuse salivation, gnashing of the teeth, marked tremor of the extremities, wild, uncontrolled movements, and impairment of vision; running in circles and intermittently rising up on the posterior extremities and falling forward or backward. Later the animal becomes lethargic and paretic, and dies in a convulsive attack, usually 7 - 9 days after inoculation. Scarification and inoculation of the cornea produce marked conjunctivitis, with vesicle formation along the line of scarification 6 to 8 days later, and keratitis and general symptoms are noticeable from the 8th to 12th day, followed a few days later by death.

After intracerebral inoculation, all animals die with typical symptoms, but after corneal infection about 50 per cent recover. This virus had passed through 12 animal passages in the Rockefeller

Institute before the present series of experiments was begun. The m.l.d. of the virus is 0.02 cc paper filtrate of a 5 per cent emulsion of fresh rabbit brain in saline.

2. Strain Beckley (26). This strain was isolated by Doctor Flexner from cerebrospinal fluid obtained from a patient diagnosed as "cerebral syphilis". This virus is the most potent of the three, being more intense than the herpetic strain H.F.

Intracerebral inoculation produces symptoms as noted above in 2 days and death in 4 days. Corneal inoculation produces marked local lesions in 3 to 4 days and death with typical symptoms in 7 to 9 days.

No recoveries have been noted either after intracerebral or corneal inoculation.

Death is invariably produced by the brain injection of 0.001 cc. of a paper filtrate of a 5 per cent emulsion of fresh rabbit brain in saline. When the present series of experiments was begun, this virus had been propagated through 12 successive animals.

HERPETIC VIRUS.

Strain H.F. This virus was obtained from a case of Herpes labialis by Dr. Flexner. It is highly potent, and survives in 50 per cent glycerine for at least 9 months. Intra-cerebral injection produces marked symptoms as described above in 2 to 3 days. Scarification of the cornea and subsequent infection produces a marked local lesion in 4 to 6 days and death in 7 to 12 days.

The symptoms are similar to those obtained with the Levaditi virus, but are more intense.

No recoveries have been noted either after intracerebral or corneal inoculation, and the m.l.d. on brain injection is 0.001 cc. of a paper filtrate of a 5 per cent emulsion of fresh rabbit brain in saline. Up to the time of being received, the virus had undergone 10 animal passages.

It is emphasized that in each of these 3 strains where virus was known to be present, intracerebral inoculation of the rabbit with the infected material invariably produced typical symptoms followed by death. No natural immunity in the rabbit as described by Loewe and Strauss was noted.

Criteria for establishing the experimental disease in the rabbit. It is necessary to state at this point the criteria which must be considered as essential in establishing experimental encephalitis lethargica in the rabbit. For reasons to be stated shortly, the following are the requirements for the definition of the experimental disease:

1. The period of incubation. This varies according to the virus used, and its method of introduction. The intracerebral method only has been employed in these studies and with fresh virus, under these conditions, symptoms may be observed in 2 to 5 days. Under circumstances where the virus has been attenuated, a period up to 12 days may elapse before clinical symptoms are noted. Owing to the potency of the viruses and the small amounts necessary to produce the disease, it is felt that the presence of the infective agent should be demonstrable in rabbits within 2 weeks.

2. Symptoms. The first symptoms to be noted are elevation of temperature and retention of urine. The former is usually 40.5°C. to 41.8°C., but often reaches 42.3°C., occasionally 43°C.(109°F.). This temperature is sustained throughout the course of the disease until just before death when it is rapidly lowered and may become subnormal. The retention of urine is a very frequent sign and may be demonstrated by gently grasping the animal round the loins and using very slight pressure. Then as much as 300 cc. of urine may be expressed from the animal.

The fever may continue for 24 to 48 hours, when general symptoms relative to involvement of the central nervous system become evident. The animal is hypersensitive, makes wild, dashing movements, which owing to disturbance of vision are often terminated by the animal hitting the wall or other obstacles. After a few moments of quiescence, the animal bends back its head, lifts one or both fore paws off the ground, then stands up on its hind legs and finally falls backwards, forwards or sideways. Sometimes the animal runs in circles, at other times it may rush forward until it is stopped by some obstacle. Salivation is very frequently observed. The saliva runs freely from the mouth, wetting the

fur around the jaws and neck. Grinding or gnashing of the teeth is also very frequently noted. The animal sits with its head elevated and the gnashing is distinctly audible. A few hours later, the animal becomes weak and paretic and lies on its side. At this stage, the temperature is lowered and the breathing laboured. A convulsive attack follows in which the animal dies.

3. At autopsy. the viscera are found to be normal. Care must be taken to exclude pneumonia, rabbit septicemia, or other affections. The brain usually shows slight congestion. There is no apparent meningitis present.

Cultures made from heart blood, lungs and brain show no growth in ordinary media, thereby eliminating septicemia, pneumonia, or meningitis, or other known affections as the cause of death.

4. Transmissibility of the virus from rabbit to rabbit by means of intracerebral injections of suspensions of affected brain can be effected indefinitely..

With respect to the microscopic lesions in the brain, it has been already shown by the writer that of 372 rabbits, not inoculated, either intracerebrally or with encephalitic or herpetic virus,

more than half (55 per cent) show brain lesions which correspond to the description given by certain investigators as the sole criterion for determining experimental encephalitis lethargica in rabbits. Since these lesions appear in control animals, it was believed that their presence could not be accepted as a criterion for the determination of the experimental disease, and, furthermore, the consideration of these changes as the only evidence of a positive transmission rests on faulty premises.

To summarize, the 4 requisites for defining experimental encephalitis lethargica in rabbits are : a) the incubation period, b) the typical symptomatology, c) the absence of macroscopic changes in the organs except for slight congestion of the brain, d) absence of concomitant or secondary infections by ordinary bacteria (pneumonia, peritonitis, abscesses, rabbit septicemia, or other diseases, and absence of growth of bacteria from these organs or blood in ordinary media, and e) transmissibility of the virus from rabbit to rabbit indefinitely.

The first experiments related to cultivation tests employing for this purpose the Smith-Noguchi technique.

Cultivation in Smith-Noguchi Medium.

The technique employed was as follows:

The material used consisted of pieces of rabbit brain which contained the virus. The animal was sacrificed when in extremis and the brain immediately removed with strict aseptic precautions. The complete autopsy was finished to exclude intercurrent infections. The cerebrum was carefully cut into portions about 1 cm. square. Such fragments were added to Smith-Noguchi medium and as controls similar pieces were inoculated into broth, and also smeared on rabbit blood agar plates, the latter being incubated under both aerobic and anaerobic conditions. In addition, a 5 per cent emulsion of brain was made in saline and 0.25 cc. was inoculated intracerebrally into a rabbit as a control for the presence of the virus. The Smith-Noguchi medium was in accordance with the requirements of Loewe and Strauss. The ascitic fluid was free from bile, clear, and had a high specific gravity.

Cultures were examined every day and those showing contamination were discarded. Using an accurate technique, it was only very occasionally that such accidents occurred. In no instance was contamination of the broth or blood agar plates noted. The Smith-Noguchi tubes were carefully examined for clouding about the tissue, as indicative of growth.

When such clouding did occur it happened that a similar condition was noted in the uninoculated tubes. Films were made from all tubes, however, on the 5th, 7th, 10th, and 14th day of incubation at 37°C., and stained by polychrome methylene blue, Gram's and Giemsa's methods. At the end of 10 days, sub-plants were made into fresh Smith-Noguchi medium, which, in turn, was similarly sub-planted 10 days later. In other words, 3 sub-cultures were made before a final result was noted.

Using Levaditi and Beckley strains of so-called encephqlitic virus and the H. F. strain of herpetic virus, no macroscopic evidence of growth was obtained in any media, incubated either aerobically or anaerobically. Stained film preparations failed to reveal any microorganism, even on the third subplant.

These experiments were repeated, using variations of the Smith-Noguchi medium by substituting for the ascitic fluid, rabbit serum, undiluted, or diluted with Ringer's solution or with dextrose broth. The results were similarly negative.

Since no living, multiplying agent could be detected on these cultures, we then proceeded by the deductive method to determine the viability of the virus in various media in the hope of finding a suitable pabulum for multiplication of the incitant.

Survival of the virus in collodion sacs implanted intra-abdominally in rabbits. It has been shown that the virus of typhus fever retains its infectivity for one month (28 - 31 days) within a collodion sac placed intra-abdominally in guinea pigs (89). Similar experiments were made with brain tissue in collodion sacs implanted in rabbits.

Sacs were prepared according to the

directions of Gates (90). Small fragments of rabbit brain containing the virus in Ringer's solution or in broth were introduced into the sacs which were then sealed. Using aseptic precautions, one or 2 sacs were introduced into the peritoneal cavity of each rabbit. After a varying number of days, the rabbits were killed, the sacs removed, the contents examined for evidences of contamination, and 0.25 cc. of the brain fragments and fluid injected intracerebrally into rabbits. Experiments were done using the 3 strains of viruses mentioned.

The virus does not survive 5 days when placed with either Ringer's solution or broth in collodion sacs implanted abdominally in the rabbit. It is therefore apparent that this method is unsuitable for cultivation purposes.

Survival of the virus in anaerobic and aerobic media. The next experiments concern the survival of the virus in anaerobic and aerobic media. If as Loewe and Strauss state, the virus of encephalitis lethargica multiples in the Smith-Noguchi medium, then pari passu the culture material should remain active for a long period of time. Since we failed to isolate the microorganism which they described, it was thought that information as to the time the virus can survive in this medium would indicate whether it had merely survived, had died or even had multiplied within the cultures, and would thus control our negative results. The viability of the virus was tested by inoculating rabbits intracerebrally with the fluid material surrounding the tissue and with the brain tissue itself. In this way we could determine not only the presence of the virus or its multiplication in the surrounding medium, but also the length of time that it survived in the brain itself.

The ascitic fluid of the Smith-Noguchi culture was poured off and centrifuged lightly to throw down particles of brain, and the supernatant fluid retained. The fragment of the brain tissue remaining in the culture was weighed in a sterile capsule and a 10 per cent emulsion made in normal saline.

For control, similar pieces of brain from the same rabbit were placed in tubes of 10 cc. of 1 per cent dextrose broth (pH 7.4) and incubated aerobically. The supernatant broth and the remaining brain tissue were treated in the same manner as in the case of Smith-Noguchi medium series.

A uniform amount was always injected, namely, 0.25 cc., and young rabbits of similar size and weight (1,000 grams) were used. Intracerebral inoculation was invariably employed. The skull was trephined at the mid-point of the parietal bone, midway between the centre of the supra-orbital margin and the ridge lying between and anterior to the ears, and the material was injected by means of a 1 cc. syringe furnished with a short needle 1/4 inch long.

The following protocols (Tables I and II) show the results of a typical experiment.

TABLE I.

Survival of Encephalitic Virus, Strain Beckley in Smith-Noguchi medium.

Incubation at 37°C.

(Days)	Material	Result
2	Centrifuged fluid	No reaction after 21 days.
2	Brain	Typical symptoms. Died in 5 days.
4	Centrifuged fluid	No reaction.
4	Brain	Typical symptoms. Died in 6 days.
6	Centrifuged fluid	No reaction.
6	Brain.	Typical symptoms. Died in 7 days.
8	Centrifuged fluid.	No reaction.
8	Brain	" " .
10	Centrifuged fluid	" " .
10	Brain	" " .
12	Centrifuged fluid	" " .
12	Brain	" " .
14	Centrifuged fluid	" " .
14	Brain	" " .

TABLE LL.

Survival of Encephalitic Virus, Strain Beckley, in Aerobic, 1 per cent Dextrose Broth.

Incubation at 37°C.

(Days)	Material.	Result.
2	Centrifuged broth	Typical symptoms. Died in 5 days.
2	Brain	Typical symptoms. Died in 5 days.
4	Centrifuged broth	Typical symptoms. Died in 5 days.
4	Brain	Typical symptoms. Died in 5 days.
6	Centrifuged broth	Typical symptoms. Died in 6 days.
6	Brain	Typical symptoms. Died in 5 days.
8	Centrifuged broth	No reaction.
8	Brain	Typical symptoms. Died in 6 days.
10.	Centrifuged broth	No reaction.
10.	Brain.	Typical symptoms. Died in 6 days.
12.	Centrifuged broth	No reaction.
12.	Brain	Typical symptoms. Died in 5 days.
14.	Centrifuged broth	No reaction.
14.	Brain	" " .

These experiments were repeated several times with each of the three strains of viruses with similar results. Thus the so-called virus of encephalitis lethargica and that of febrile herpes contained in the rabbit brain and incubated in the Smith-Noguchi medium could not be shown to have diffused into, or multiplied in the surrounding medium after a period of two days. The viruses could, however, be shown to be still viable in the brain itself up to the sixth and occasionally the eighth day; the larger the piece of brain, the longer the survival. On the other hand, when placed in dextrose broth and incubated under aerobic conditions, they are found in the surrounding fluid up to the sixth day, and 0.25 cc. of the supernatant broth produces symptoms as severe as those induced by the fresh viruses. The brain fragments in this medium were still infective on the tenth to the twelfth days.

It is therefore apparent that the three samples of viruses did not multiply in the Smith-Noguchi medium. They survived longer in aerobic broth, in which they were demonstrated both in the supernatant fluid and in the brain fragment, long after they had become inactivated in the Smith-Noguchi medium, which therefore must be regarded as

exerting an unfavourable effect.

A further confirmation of this conclusion is afforded by the next experiment in which virus was added in the form of an emulsion of ground brain tissue to the Smith-Noguchi medium and to aerobic broth, in order that a wide surface of the inoculum would be exposed to the media. Three sets of tests were made with each of the three viruses with practically uniform results.

Experiment A. The brain containing fresh virus was ground up in a sterile mortar with sand. Saline solution was added to form a 20 per cent emulsion. After lightly centrifuging to deposit particles of brain, 1 cc. of the emulsion was added to 15 cc. of Smith-Noguchi medium, and 1 cc. to a like amount of dextrose broth.

Before incubation at 37°C., material was removed from each tube and 0.25 cc. inoculated intracerebrally into control rabbits, which developed typical symptoms and died after 5 days. Fluid was withdrawn from the tubes on the 2nd and 4th days of incubation, at 37°C., and the test does (0.25 cc). injected intracerebrally into a series of rabbits. It was found that the aerobic broth material caused typical symptoms leading to death in the same time as

the controls, whereas similar material in the Smith-Noguchi medium was entirely without effect.

The material injected was carefully controlled by cultivation on various media under both aerobic and anaerobic conditions. In no case was any growth obtained.

These experiments show that in the Smith-Noguchi medium at 37°C., the viruses, as contained in the ground brain tissue, are either killed or rendered inactive within 2 days, while in aerobic broth cultures at 37°C., they remain infective after 4 days incubation.

Acidity of Medium in Relation to the Destruction of the Virus.

The problem now concerned the factor in the Smith-Noguchi medium which prevents the survival of the virus. The first consideration was that of acidity or hydrogen-ion concentration. It was thought that the autolysis of the kidney tissue might produce enough acid to destroy any virus which might diffuse into the surrounding fluid, whereas the virus in the brain would be protected. Accordingly a series of experiments was carried out in which the pH of the medium was determined by means of the

hydrogen-ion electrode. (*)

* The determinations of the hydrogen-ion concentrations were kindly made by Dr. J. H. Northrop.

Experiment B.

Control ascitic fluid kidney tissue medium with a vaseline seal after 7 days incubation showed a pH of 7.2 in the upper part of the medium, and a pH of 7.1 in the fluid surrounding the kidney tissue. Smith-Noguchi medium to which brain tissue was added showed a pH of 7.24 at the upper and 7.2 at the lower part of the fluid. Furthermore, varying amounts of sodium phosphate buffer solution were added to Smith-Noguchi medium containing brain tissue. It was found that 2 parts of ascitic fluid and 1 part of sodium phosphate solution (pH 8.0) added to fresh kidney and brain tissue showed, after 7 days incubation at 37°C., a pH of 7.4 in the fluid at the bottom of the tube. This point was chosen because the dextrose broth in which the virus survives has a pH of 7.4. It was supposed that the mixture of ascitic fluid and buffer solution would form an adequate medium because the virus will survive in dextrose broth which is of relatively simple composition.

A series of experiments with this modified ascitic fluid kidney tissue medium, using each of the different viruses, was carried out. In no case could any virus be detected in the fluid around the

brain and kidney tissues.

In order to eliminate the acidity as the determining factor in the destruction of the virus, the pH determination of broth and a piece of brain, incubated aerobically, was determined. In addition, it is known that encephalitic virus and herpetic virus can survive in 50 per cent glycerin for periods up to 10 months. The pH of 50 per cent glycerin used for preserving the virus was therefore determined.

The results are tabulated in Table III.

TABLE III

Determination of pH of Dextrose Broth and Brain, Incubated Aerobically at 37°C.

Day	Top of Tube pH	Bottom of Tube pH
2nd	6.7	6.6
4th	6.22	6.22
6th	6.42	6.42

pH of 50 per cent glycerin, 5.54.

It is thus seen that the broth is considerably more acid than the Smith-Noguchi medium, while the 50 per cent glycerin in which the viruses survive for long periods is even still more acid.

It would appear then that the pH value of the medium is not the determining factor for the survival

of the herpetic and encephalitic virus.

Oxygen Tension in Relation to the Viability of the Virus.

The next question to be determined was the part played by oxygen tension in stimulating or inhibiting the growth of the active agent in the virus of encephalitis lethargica and Herpes labialis.

Experiment C.

12 tubes of dextrose broth were inoculated with virus in the form of pieces of fresh brain tissue all from the same rabbit. The tubes were divided into 3 batches and four tubes were incubated aerobically, four anaerobically in the Brown jar, (91), and the remainder under a vaseline seal (92).

On the second and fourth days of incubation, the broth from two tubes of each batch was centrifuges and 0.25 cc of supernatant clear fluid from each tube was inoculated intracerebrally into rabbits. All 12 animals thus inoculated shewed typical symptoms and death occurred within the same time as in the control animals similarly inoculated with fresh virus.

In all, 3 series of 12 tubes were used employing each of the three viruses for inoculation.

From this experiment it was concluded that oxygen tension as such does not play a material part in deciding either survival or destruction of the viruses.

We may conclude, therefore, that the destruction of the virus in the Smith-Noguchi medium is not due to the development of acidity, nor to the

anaerobic condition which prevails. That this occurrence is constant is supported by the fact that several different specimens of ascitic fluid and kidney tissue from a number of rabbits were employed in constructing this medium. Furthermore, the samples of ascitic fluid all conformed to the standards set originally by Loewe and Strauss.

Finally, it may be stated that as yet we have been unable to recover from the centrifuged material of the aerobic broth cultures of the encephalitic or herpetic virus, which is highly infective for rabbits, any of the micro-organisms described previously by others as the incitants of these affections.

SUMMARY.

This paper is concerned with the study of the questions of the survival and multiplication of the so-called virus of encephalitis lethargica and the virus of febrile herpes in artificial cultures.

Two main sets of experiments were carried out. In the first set an attempt was made to repeat the published reports of Bradford, Bashford and Wilson, and Loewe and Strauss, and Thalimer. These observers claim to have succeeded, by the use of the Smith-Noguchi anaerobic tissue method of cultivation, in obtaining from the so-called virus of encephalitis lethargica, growths of a micro-organism which is visible under the microscope, and active when inoculated into rabbits.

We have been unable to confirm these published reports: on the contrary, our experiments show conclusively that the Smith-Noguchi medium is not favourable even for the survival of the virus, much less for its multiplication or growth. The same general fact is true for the virus of febrile herpes. However, it could be shown that while the viruses diffusing into the fluid part of the Smith-Noguchi medium rapidly lost their activity at 37°C., (in less than 2 days), within the brain fragment they

remain active for 6 days. In no instance could any formed bodies be detected in the medium which could be regarded as micro-organisms, although of course, because of the composition of the medium, detritus of very fine texture is always present which to the untrained eye might possibly be so construed.

While we found that the Smith-Noguchi medium was inimical to the survival of the viruses, it was ascertained that aerobic broth is far less destructive to the active material.

Under the conditions of our experiments, it was determined that the viruses diffusing into the surrounding broth remained active for six days, while within the brain fragment itself they survived for twelve days.

In connection with these studies we investigated whether it was a difference in the oxygen tension or in the pH, which determined the destruction of the viruses in the Smith-Noguchi medium or their survival in aerobic broth. Changes in oxygen tension, and variations in the hydrogen-ion concentration between the limits of pH 7.4 and pH 6.2, do not exert any appreciable effect, and we have not been able to determine the precise nature of the injurious action of the Smith-Noguchi medium.

Not only do our experiments fail to uphold Bradford, Bashford and Wilson, Loewe and Strauss, and Thalimer, but they contradict the published reports of Wiesner, Rosenow and others, who identify the encephalitis virus with certain common bacteria. It can be stated unequivocally, that with an aseptic technique the so-called virus of encephalitis lethargica and the virus of febrile herpes can be passed from brain to brain of the rabbit, under conditions in which no visible micro-organism can be made to appear or grow, either in aerobic or anaerobic cultures.

The difference in the results of the present work and that of the previous investigators may be ascribed to the different criteria used for the establishment of the experimental disease in rabbits. The following five requisites are necessary before a diagnosis of the experimental disease in rabbits can be made: a) definite incubation period, b) typical symptomatology, c) absence of gross lesions in the organs, except for congestion of the brain, d) absence of concomitant or secondary infections by ordinary bacteria, and e) transmissibility of the virus from rabbit to rabbit indefinitely. Since histopathological lesions in the brains of rabbits, indistinguishable from those described by previous

investigators as the sole criterion for determining the experimental disease, have been found in from 40 per cent to 76 per cent of animals uninoculated with the encephalitic or herpetic virus, these cerebral changes cannot be considered as an essential requirement. We believe, therefore, that the interpretation of the sole presence of histopathological changes in the brains of inoculated rabbits, as positive evidence of experimental encephalitis lethargica or Herpes labialis, has been erroneous.

CONCLUSION.

Our experiments fail to confirm the statements previously made that micro-organisms of the class of the "globoid bodies" of poliomyelitis may be cultivated from the so-called virus of encephalitis lethargica in the Smith-Noguchi medium. The experiments indicate first, that this medium is very unfavourable to the survival of the virus, and second, that ordinary broth under aerobic culture conditions is relatively a favourable medium for maintaining the activity both of the encephalitic virus and the virus of febrile herpes. Probably no multiplication of either takes place, but only survival for a maximum period of 6 days in the broth itself, and 12 days in

the fragment of brain tissue. Our experiments with the so-called virus of encephalitis lethargica lead us also to state that activity in the rabbit has nothing whatever to do with the presence of the cocci and other bacterial forms described by Wiesner, Rosenow, Bastai, and others.

BIBLIOGRAPHY.

(1) Von Economo, C., Wien. klin. Wchnschr., 1917, xxx, 581.

(2) Levaditi, C., et Harvier, P., Jour. med. franc., 1920, ix, 121.

(3) Levaditi, C., et Harvier, P., Bull. Acad.de méd., 1920, lxxxiii, 365.

(4) Harvier, P., et Levaditi, C., Bull. et mem. Soc. Med. d. hop. de Par., 1920, xliv, 179.

(5) Harvier, P., et Levaditi, C., ibid, 1920,xliv,583.

(6) " " ibid, 1920,xliv,1487.

(7) Harvier, P., Rev. de med., 1920, xxxvii, 353.

(8) Levaditi, C., et Harvier, P., Ann. de l'Inst. Pasteur, 1920, xxxiv, 911.

(9) Levaditi, C., et Harvier, P., Compt. rend. Soc. de biol., 1920, lxxxiii, 385.

(10) Levaditi, C., et Harvier, P., ibid, 1920, lxxxiii, 674.

(11) Levaditi, C., et Harvier, P., ibid, 1920, lxxxiii, 1140.

(12) Levaditi, C., et Harvier, P., ibid, 1921, lxxxlv, 300.

(13) Levaditi, C., Harvier, P., et Nicolau, S., ibid, 1920, lxxxiv, 524.

(14) Levaditi, C., Harvier, P., et Nicolau, S., ibid, 1921, lxxxiv, 817.

(15) Levaditi, C., Harvier, P., et Nicolau, S., ibid, 1921, lxxxiv, 957.

(16) Levaditi, C., Harvier, P., et Nicolau, S., ibid, 1921, lxxxv, 287.

(17) Harvier, P., Paris med., 1921, xl, 485.

(18) Levaditi, C., Bull. de l'Inst. Pasteur, 1921, xix, 289, 337.

(19) Levaditi, C., Compt. rend. Acad. d. sc., 1921, clxxiii, 370.

(20) Levaditi, C., Harvier, P., et Nicolau, S., Ann. de l'Inst. Pasteur, 1922, xxxvi, 61.

(21) Doerr, R., und Schnabel, A., Schweiz. med. Wchnschr., 1921, li, 469, 562.

(22) Doerr, R., und Schnabel, A., Ztschr.f.Hyg. u. Infectionskrankh., 1921, xciv, 29.

(23) Da Fano, C., Brit. Med. Jour., 1921, i, 153.

(24) " " Jour. Physiol., 1920-21, liv, 114.

(25) Da Fano, C., and Ingleby, H., Proc. Roy. Soc. Med., 1918-19, xii, 42.

(26) Flexner, S., Jour. Amer. Med. Assn., 1923, lxxxi, 1688, 1785.

(27) McIntosh, J., and Turnbull, H.M., Brit. Jour. Exper. Path., 1920, i, 89.

(28) Doerr, R., 1922, quoted from (26).

(29) Schnabel, A., Klin. Wchnschr., 1922, i, 1685.

(30) Loewe, L., and Strauss, I., Jour. Infect. Dis., 1920, xxVii, 250.

(31) Loewe, L., and Strauss, I., Proc. N.Y. Path. Soc., 1920, xx, 18.

(32) Cleland, J.B., and Campbell, A.W., Med. Jour. Australia, 1919, i, 234. and Brit. Med. Jour., 1919, i, 663.

(33) Löwenstein, A., Münch. Med. Wchnschr., 1919, lxvi, 769.

(34) Löwenstein, A., Klin. Monatsbl. f. Augenh., 1920, lxiv, 798, 983.

(35) Da Fano, C., Jour. Path. and Bact., 1923, xxvi, 85.

(36) Grüter, Klin. Monatsbl. f. Augenh., 1920, lxv, 398.

(37) Kraupa, E., Münch. Med. Wchnschr., 1920, lxvii, 1236.

(38) Doerr, R., Klin. Monatsbl. f. Augenh., 1920, lxv, 104.

(39) Stocker, F., ibid, 1920, lxv, 298.

(40) Baum, O., Derm. Wchnschr., 1920, lxx, 105.

(41) Doerr, R., and Vőchting, R., Rev. Gén. Ophthalm., 1920, xxxiv, 409.

(42) Blanc, G., Compt. rend. Acad. Sc., 1921, clxxii, 725.

(43) Blanc, G., et Caminopetros, J., Compt. rend. Soc. de biol., 1921, lxxxiv, 629, 767.

(44) Luger, A., u. Lauda, E., Ztschr. f. Hyg. u. Infectionskrankh., 1921, xciii, 206.

(45) Teissier, P., Gastinel, P., et Reilly, J., Compt. rend. Soc. de biol., 1922, lxxxvi, 73, 75.

(46) Blanc, G., et Caminopetros, J., Compt. rend. Soc. de biol., 1921, lxxxiv, 859.

(47) Lipschütz, B., Derm. Wchnschr., 1921, lxxiii, 798, 983.

(48) Fontana, A., Pathologica, 1921, xiii, 321, 406.

(49) Ravaut et Rabeau, Compt. rend. Soc. de biol., 1921, lxxxv, 1132.

(50) Truffi, M., Pathologica, 1922, xiv, 565.

(51) Meineri, P.A., ibid, 1922, xiv, 772.

(52) Luger, A., u. Lauda, E., Ztschr. f. d. ges. Exper. Med., 1921, xxiv, 289.

(53) Löwenstein, A., Klin. Monatsbl. f. Augenh., 1920, lxv, 399.

(54) Lipschütz, B., Wien. Med. Wchnschr., 1921, lxxi, 231.

(55) Lipschütz, B., Arch. f. Derm. u. Syph., 1921, cxxxvi, 428.

(56) Krooy, J.M., Klin. Monatsbl. f. Augenh., 1921, lxvi, 75.

(57) Luger, A., u. Lauda, E., Wien. Klin. Wchnschr., 1921, xxiv, 132, 251, 259.

(58) Bull, C. G., Jour, Exp. Med., 1917, xxv, 557.

(59) Oliver, J., Jour, Infect. Dis., 1922, xxx, 91.

(60) -Reasoner, M.A., Jour. Amer. Med. Assn., 1916, lxvii, 1799.

(61) Loewe, L., Hirschfeld, S., and Strauss, I., Jour. Infect. Dis., 1919, xxv, 378.

(62) Loewe, L., and Strauss, I., Jour. Amer. Med. Assn., 1920, lxxiv, 1373.

(63) Loewe, L., and Strauss, I., ibid, 1919, lxxiii, 1056.

(64) Acute Epidemic Encephalitis. An Investigation by the Association for Research in Nervous and Mental Diseases, New York, 1921, p. 214.

(65) Ibid, p. 198.

(66) Ibid, p. 224.

(67) Thalimer, W., Arch. of Neur. and Psychiatry, 1922, viii, 286.

(68) Kling, C., Davide, H., and Liljenquist, F., Compt. rend. Soc. de biol., 1921, lxxxv, 815, 823.

(69) Kling, C., Davide, H., and Liljenquist, F., ibid, 1922, lxxxvii, 1179.

(70) Kling, C., Davide, H., and Liljenquist, F., Hygiea, 1921, lxxxiii, 566.

(71) Kling, C., Hygiea, 1922, lxxxiv, 894, 913.

(72) McIntosh, J., Brit. Jour. Exper. Path., 1923, iv, 36.

(73) Plaut, F., and Mulzer, P., Münch. med. Wchnschr., 1922, lxix, 1779.

(74) Brown, W.H., and Pearce, L., Arch. Derm. and Syph., 1920, ii, 635.

(75) McCartney, J.E., and Olitsky, P.K., Jour. Exper. Med., 1923, xxxviii, 591.

(76) Kling, C., Wien. Arch. f. Innere Med., 1923, vi, 121.

(77) Kling, C., Davide, H., and Liljenquist, F., Hygiea, 1921, xxi, 705.

(78) Doerr, R., and Zdansky, E., Schweiz. Med. Woch., 1923, iv, 349.

(79) Twort, C.C., and Archer, H.E., Lancet, 1923, i, cciv, 1102.

(80) Von Wiesner, R.R., Wien. klin. Wchnschr., 1917, xxx, 933.

(81) Rosenow, E.C., Towne, E.B., and Wheeler, G.W., Jour. Amer. Med. Assn., 1916, lxvii, 1202.

(82) Brasher, C.W.J., Caldwell, J.R., and Coombe, E.J., Brit. Med. Jour., 1919, i, 733.

(83) Morse, P.F., and Crump, E.S., Jour. Lab. and Clin. Med., 1920, v, 275.

(84) Bradford, J.R., Bashford, E.F., and Wilson, J.A., Quart. Jour. Med., 1918, xii, 88.

(85) Stafford, C.M., Jour. Lab. and Clin. Med., 1918-1919, iv, 691.

(86) Bastai, P., Rev. critica di Clin. Medica, 1920, xxxi, 139.

(87) Bastai, P., Bull. d. Inst. Sierotherap., Milan, 1921, ii, 197.

(88) Ottolenghi, D., D'Antona, S., and Tonietti, F., Policlinico, Sec. Pract., 1920, xxvii, 1075.

(89) Olitsky, P.K., and McCartney, J.E., Jour. Exper. Med., 1924, (in press).

(90) Gates, F.L., Jour. Exper. Med., 1921, xxxiii, 25.

(91) Brown, J.H., Jour. Exper. Med., 1921, xxxiii, 677.

(92) Gates, F.L., and Olitsky, P.K., Jour. Exper. Med., 1921, xxxiii, 51.

ILLUSTRATIONS.

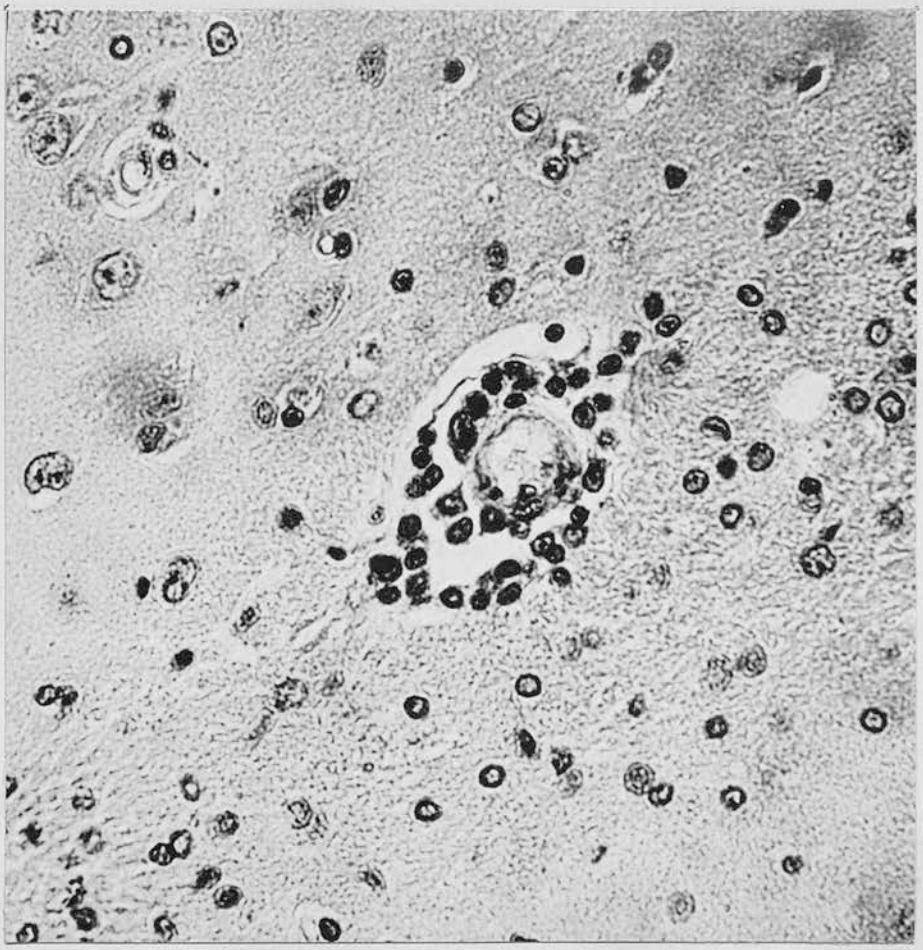


Fig. 1. Section of brain of rabbit injected intratesticularly with Treponema pallidum material, showing perivascular infiltration with mononuclear cells in the Virchow-Robin space. X640.

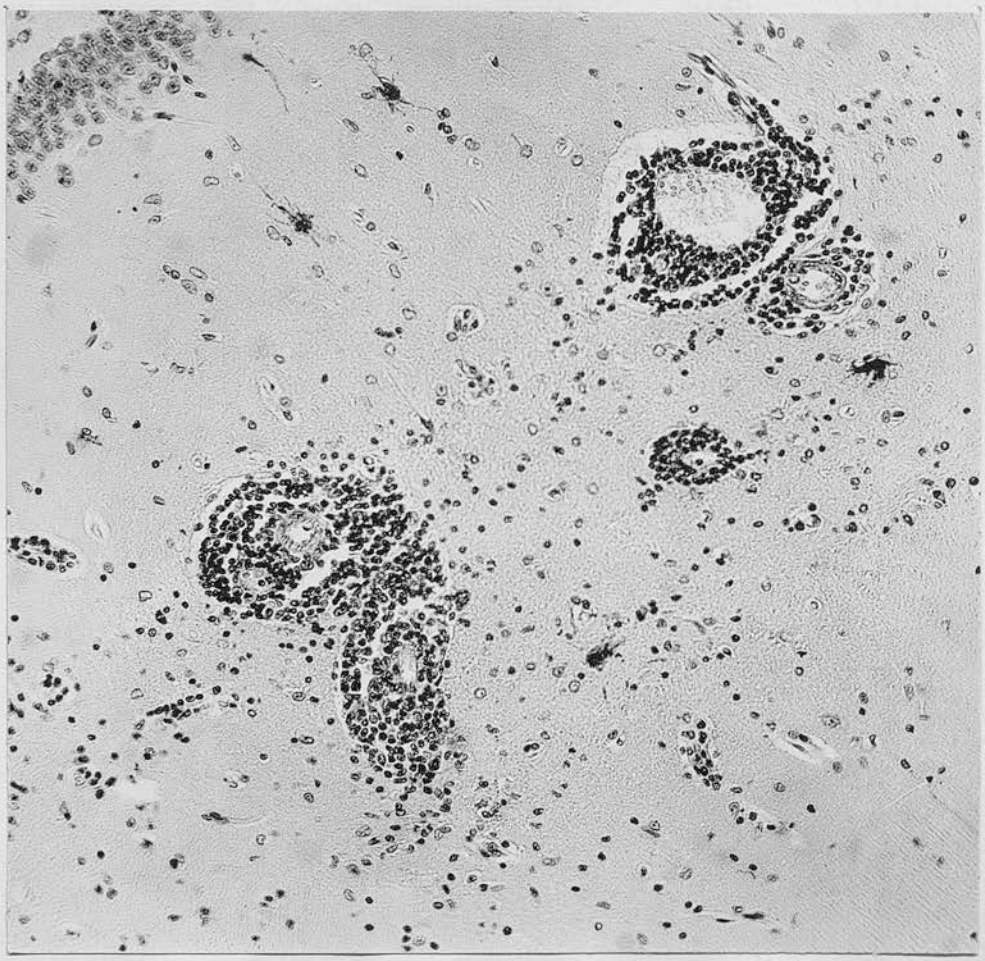


Fig. 2. Section of brain of a supposedly normal stock rabbit. To be noted are the numerous perivascular lesions. X 200.

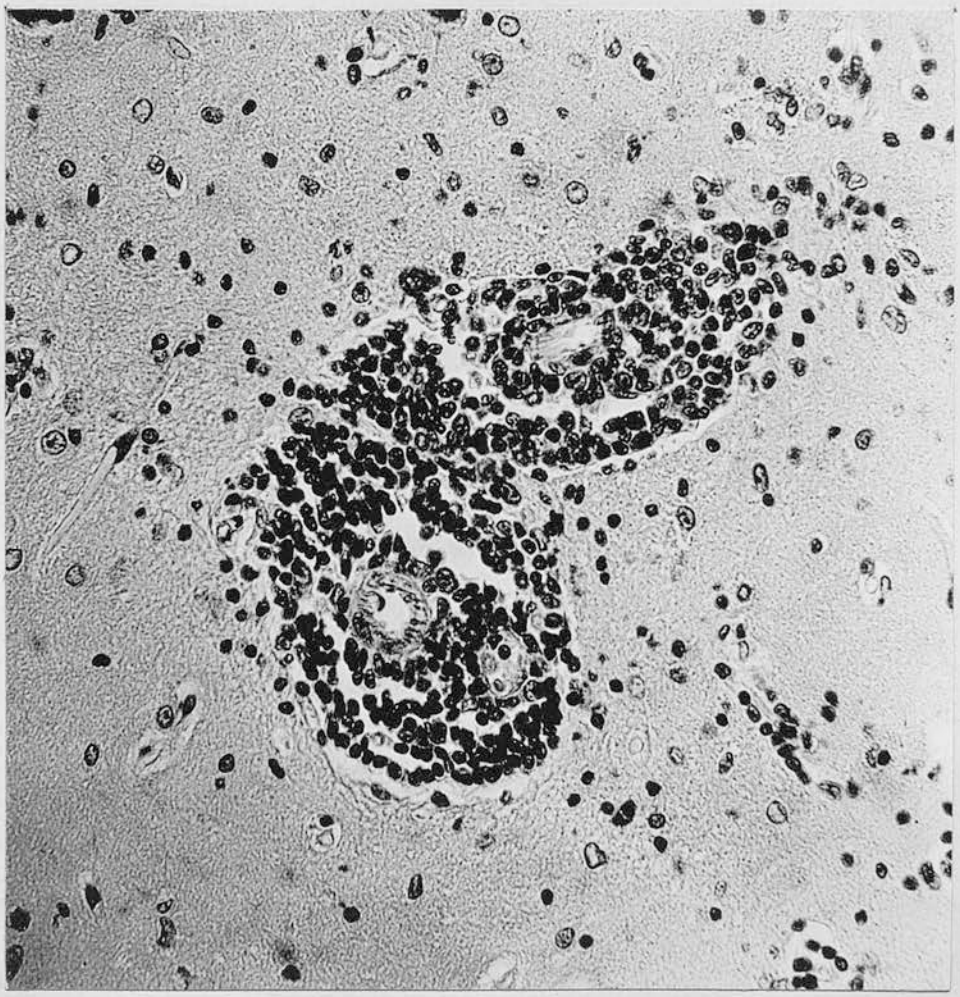


Fig. 3. The same field as Fig. 2 under higher magnification, illustrating nature of the mononuclear cells. X 365.

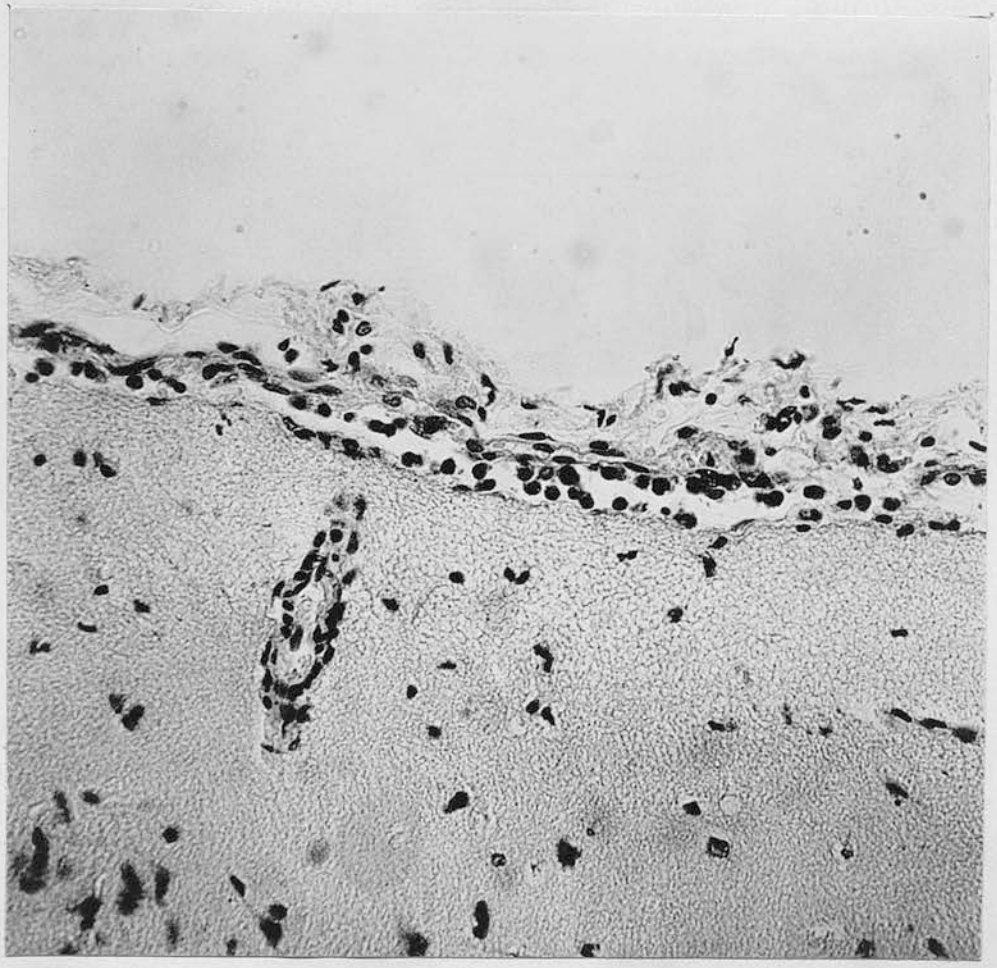


Fig. 4. Section of rabbit brain from an early case of snuffles, showing slight meningeal infiltration with mononuclear cells. There is some perivascular infiltration around the small blood vessel. X 365.

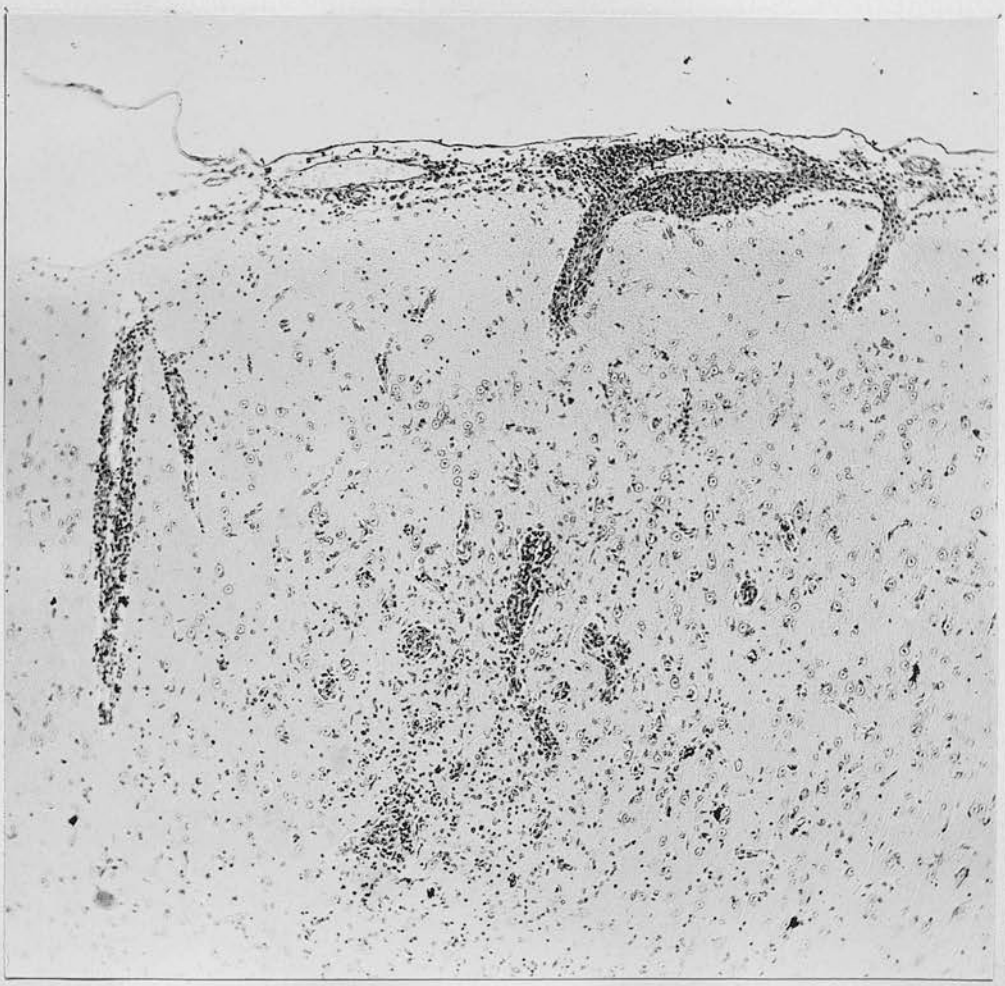


Fig. 5. Section of brain of rabbit inoculated intratesticularly with a transplantable tumour, showing meningeal, cerebral, and perivascular infiltration. X 100.

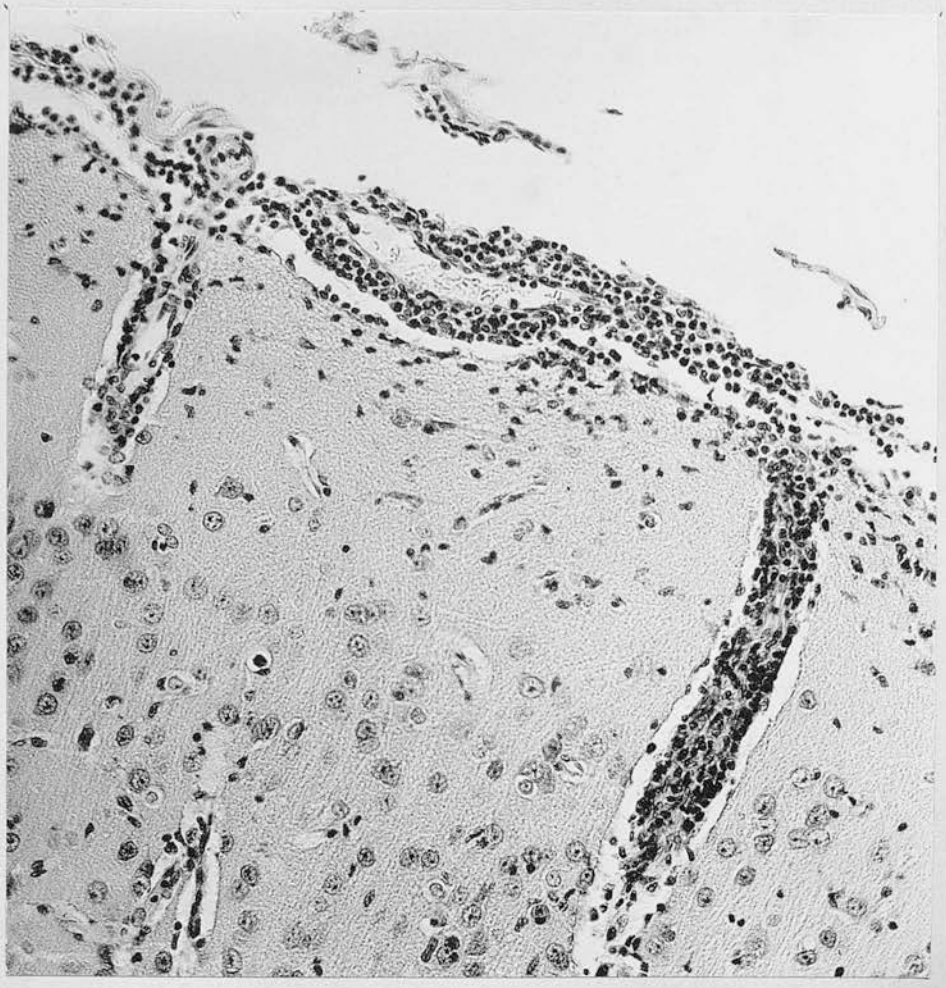


Fig. 6. Section of brain of rabbit with snuffles, containing meningeal and perivascular infiltrations. X 250.

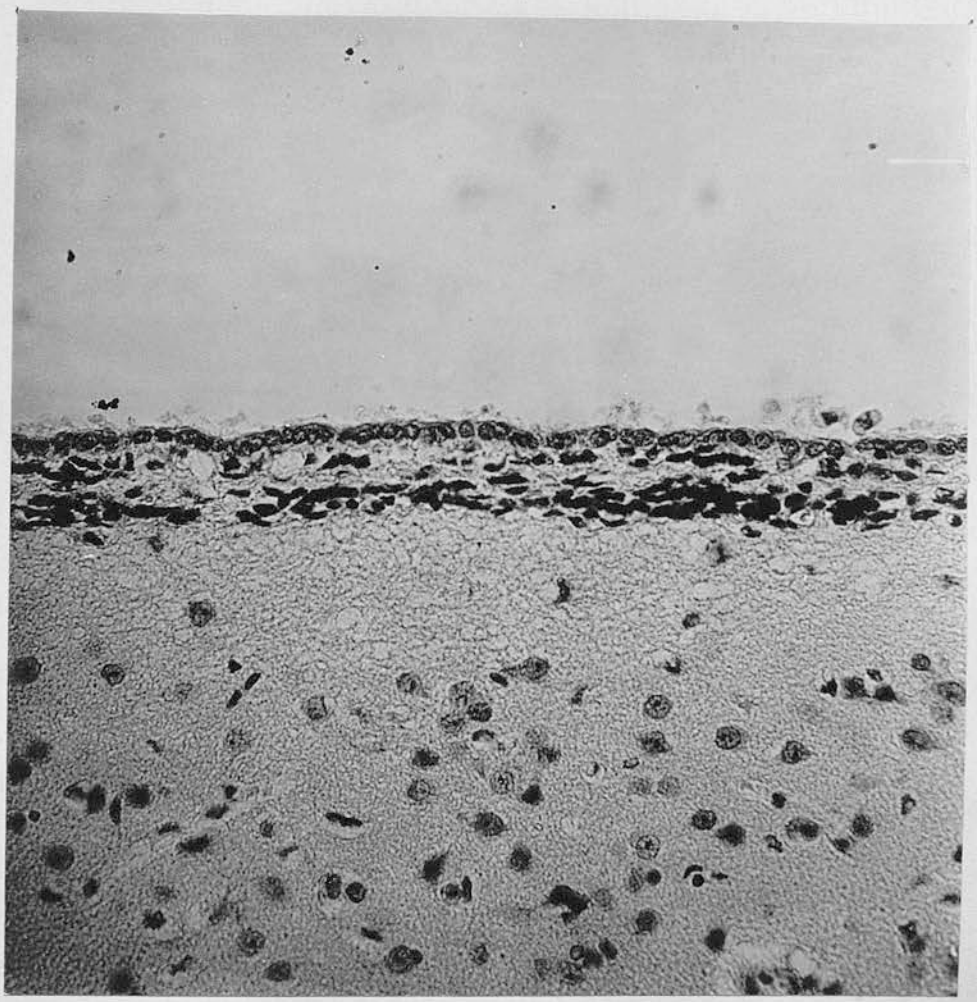


Fig. 7. Section of brain of rabbit with early snuffles, revealing slight mononuclear infiltration under the ependyma of the lateral ventricle. X 210.

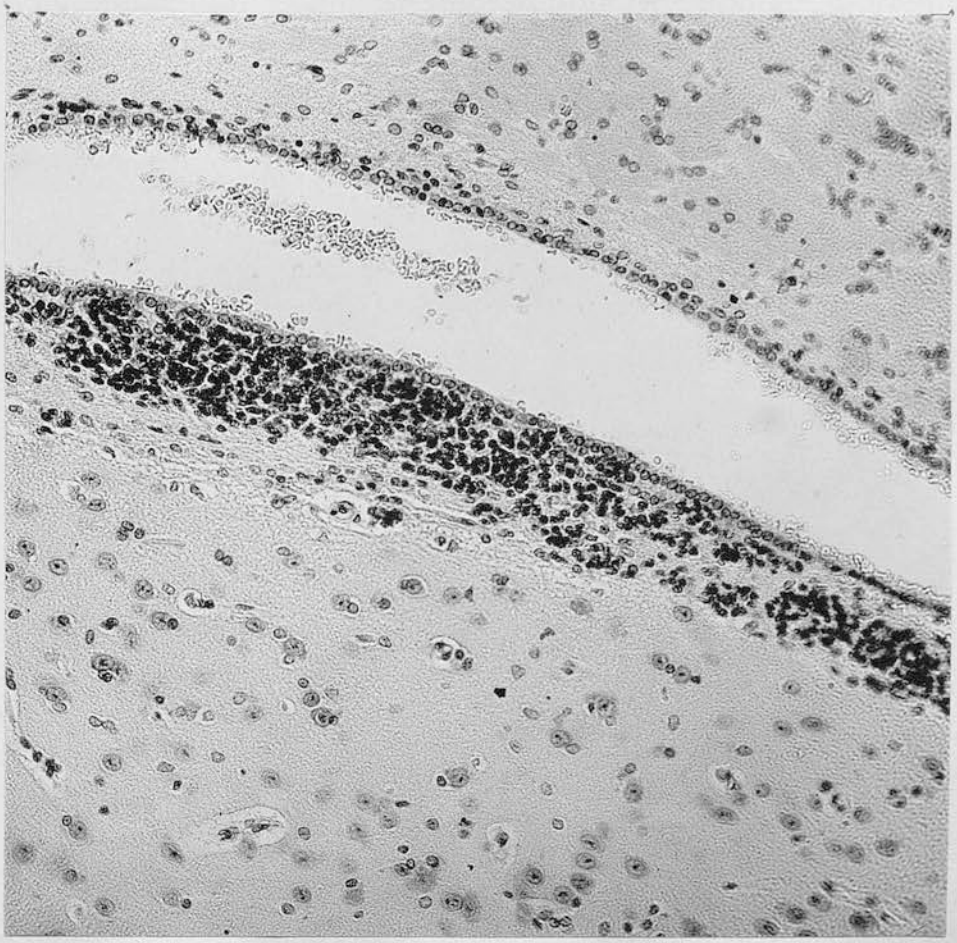


Fig. 8. From a similar rabbit, but with more marked infiltration with mononuclear cells under the ependyma if the lateral ventricle. X 250.

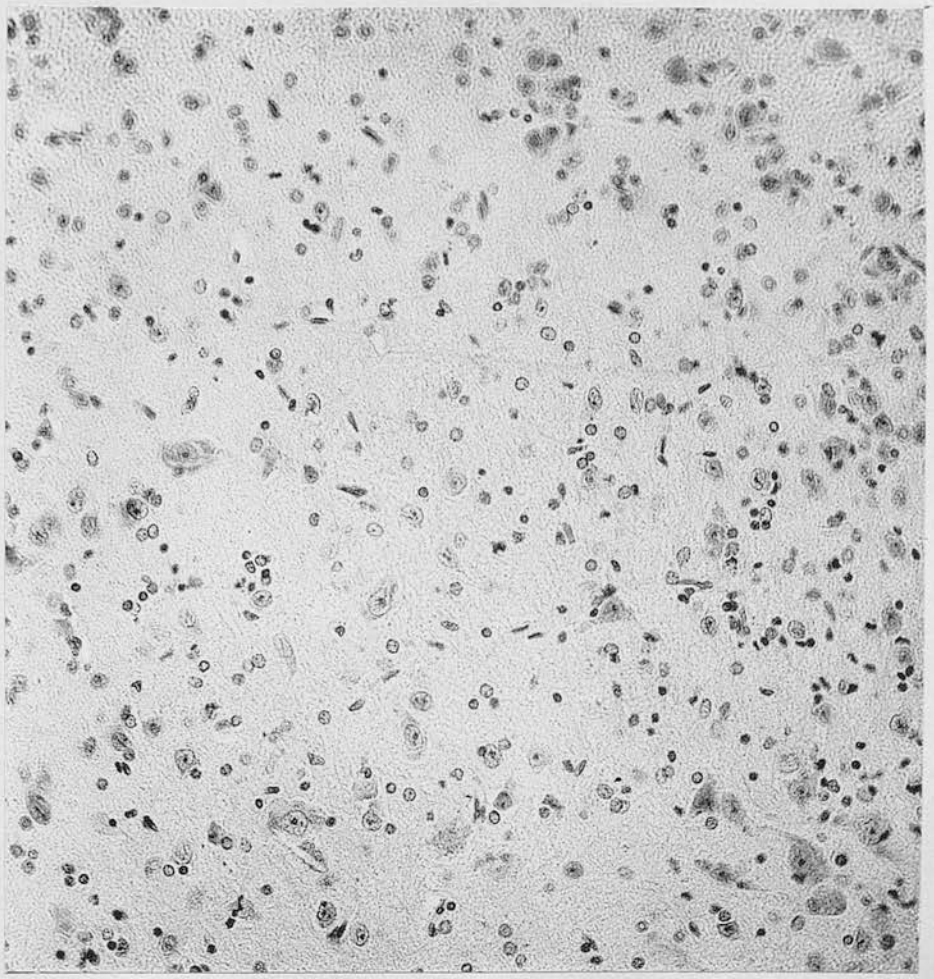
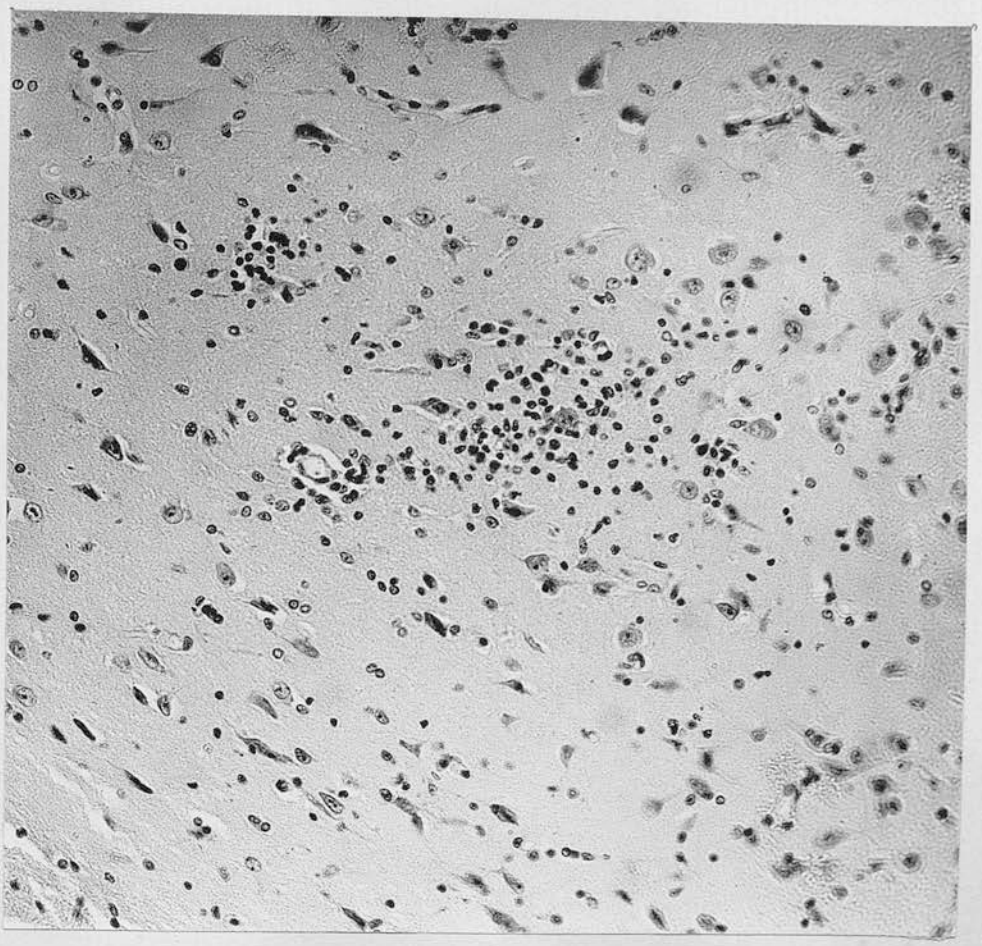


Fig. 9. Section of brain of rabbit with a subcutaneous abscess. Diffuse infiltration with mononuclear cells into the cerebral substance is seen. X 280.



intra-testicularly

Fig. 10. Brain of rabbit inoculated with a transmissible tumour, showing small groups of mononuclear cells in the brain substance. X 250.

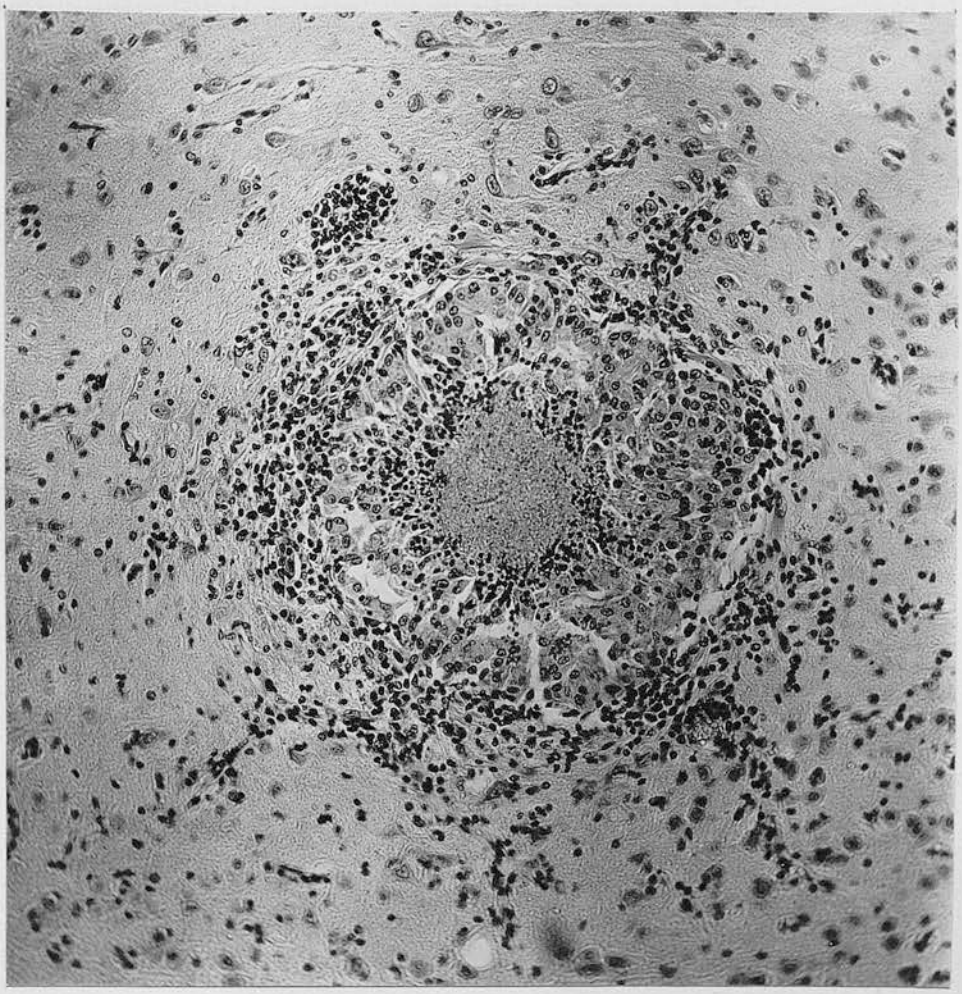


Fig. 11. Brain from a similar rabbit with a well-marked area of focal necrosis. To be noted are the necrotic centre, large, epithelial-like cells, and the surrounding mononuclear infiltration. X 190.

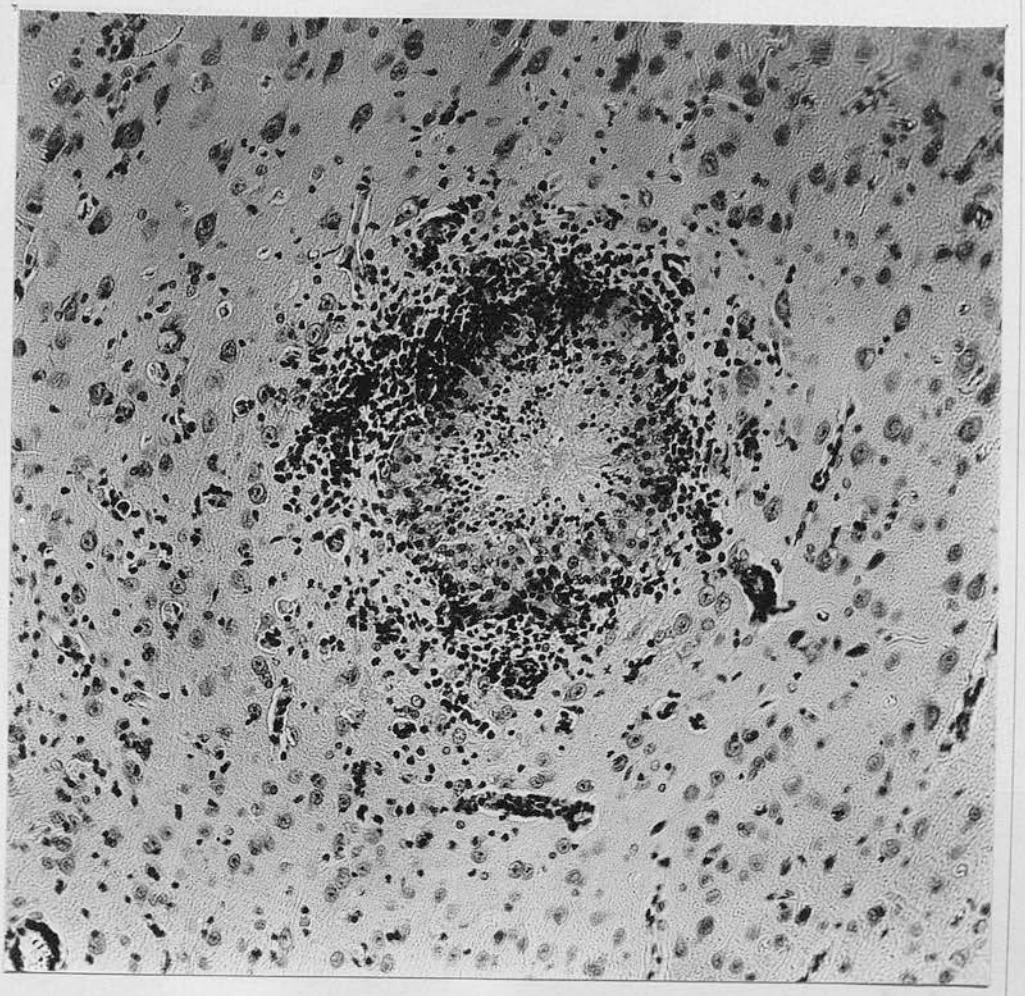


Fig. 12. A similar area of necrosis found in a supposedly normal stock rabbit. X 190.

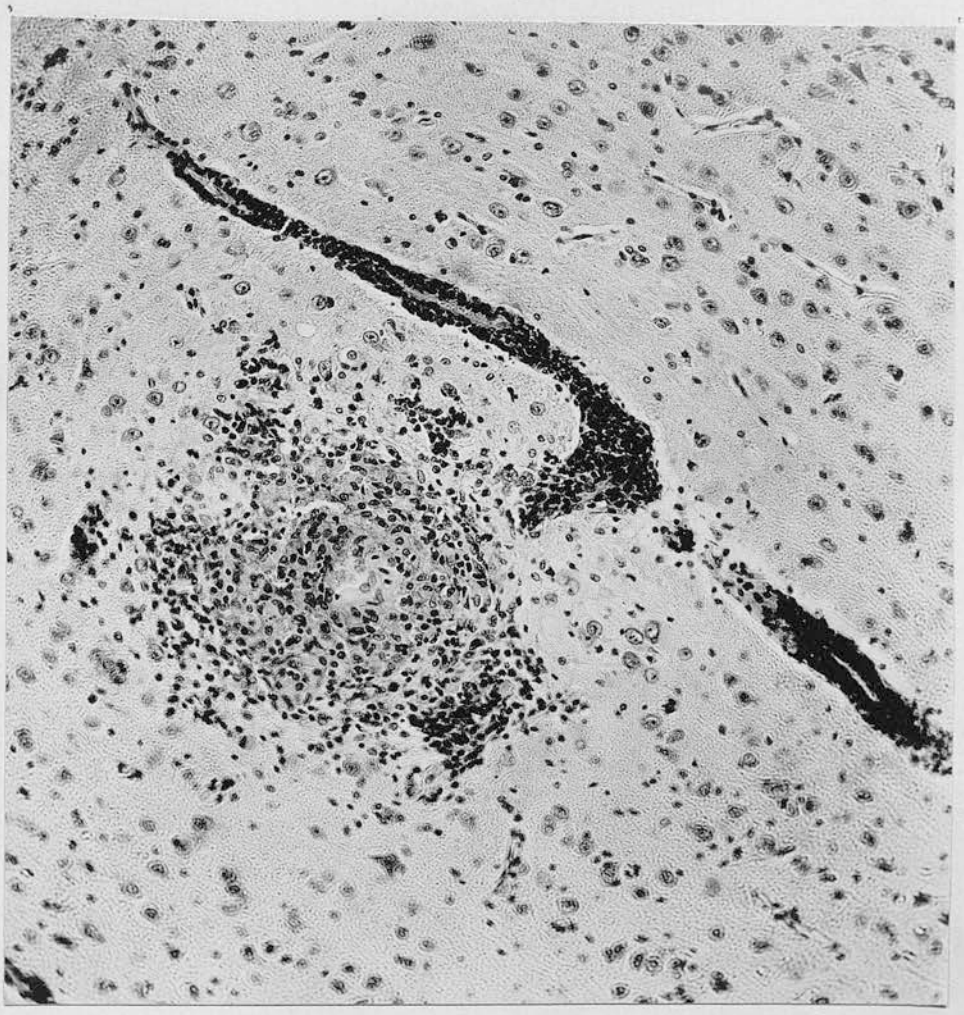


Fig. 13. Section of brain from a snuffles rabbit, showing a similar area of focal necrosis, but not cut through the centre. The blood vessels reveal marked perivascular infiltration. X 190.

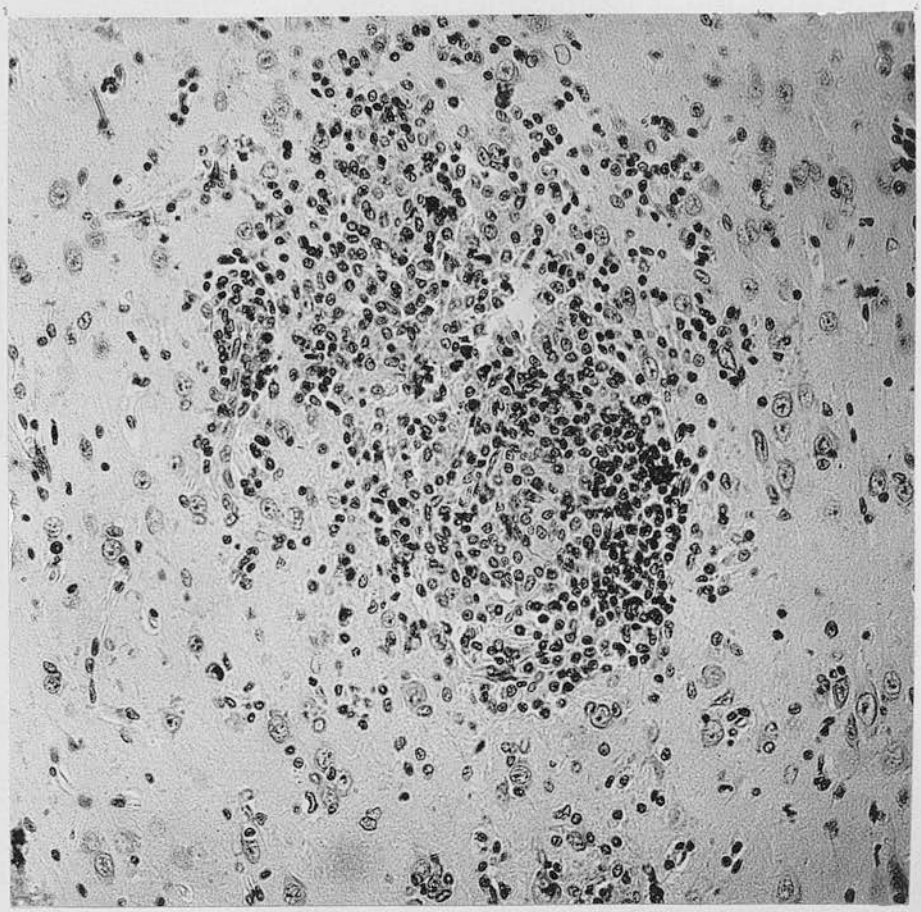


Fig. 14. Brain from a rabbit inoculated intratesticularly with a transmissible tumour. A focal necrotic area with infiltration by mononuclear cells is seen. X 270.

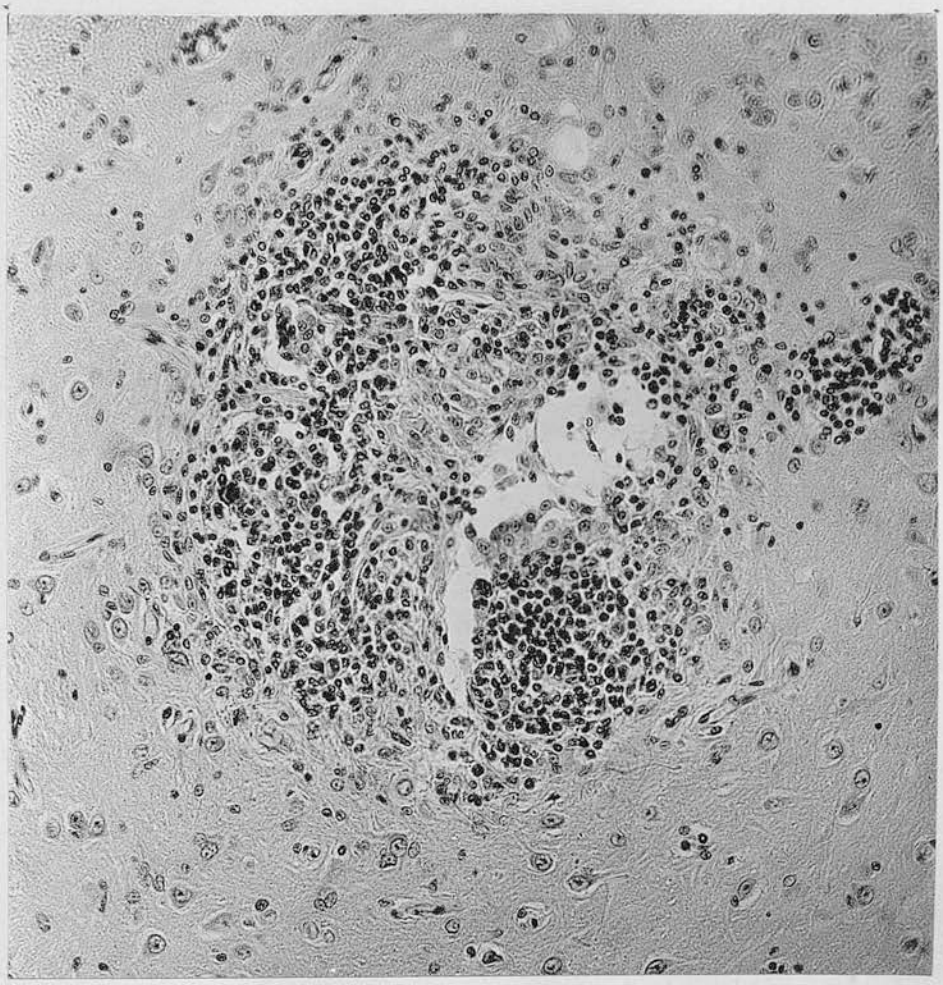


Fig. 15. Section of brain of rabbit injected intratesticularly with Treponema pallidum material, showing an area of focal necrosis in process of healing. X 250.

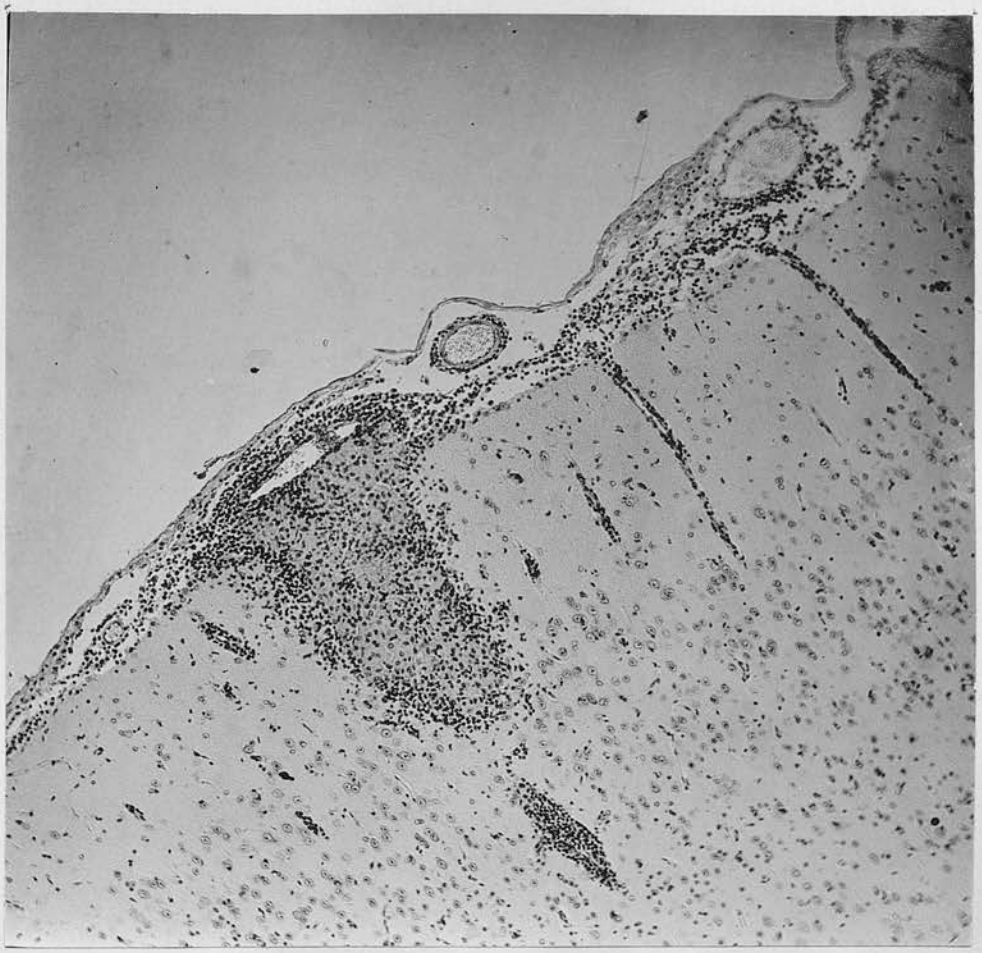


Fig. 16. Brain of rabbit inoculated intratesticularly with a transmissible tumour. To be noted is an area of necrosis at the surface of the brain, with meningeal and perivascular infiltration. X 110.

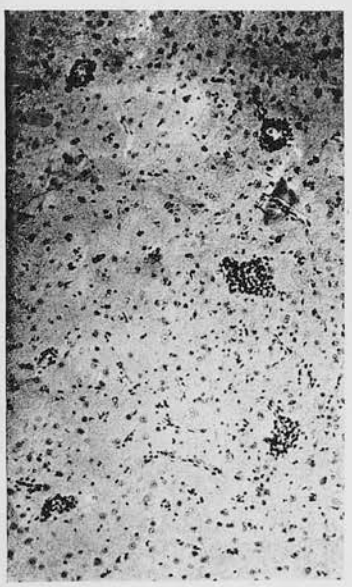


FIG. 12.

Fig. 17. Reproduced from Fig. 12, article by Bull (58).

Compare with Figs. 1, 2, 3 and 5.

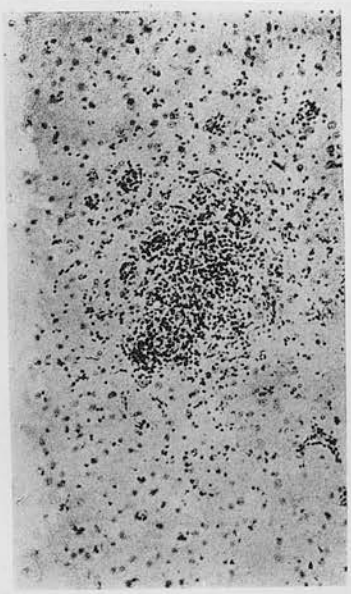


FIG. 13.
(Bull: Streptococci from Poliomyelitis.)

Fig. 18. Reproduced from Fig. 13, article by Bull (58).

Compare with Figs. 5, 11, 12, 13 and 14.

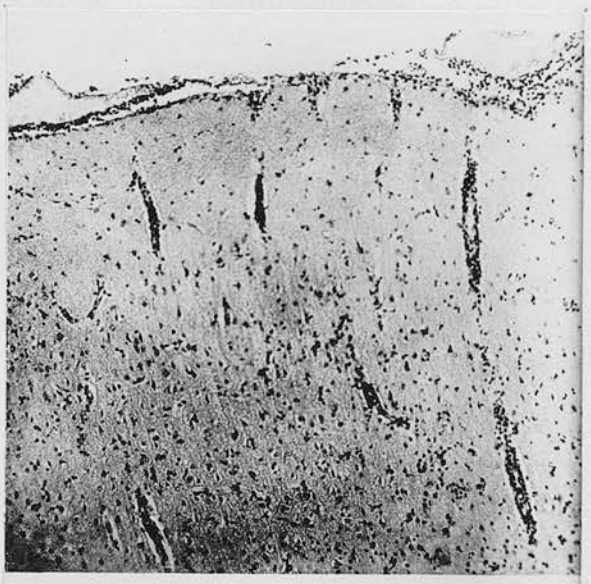


FIG. 14.

Fig. 19. Reproduced from Fig. 14, article by Bull (58).

Compare with Figs. 2, 5 and 16.

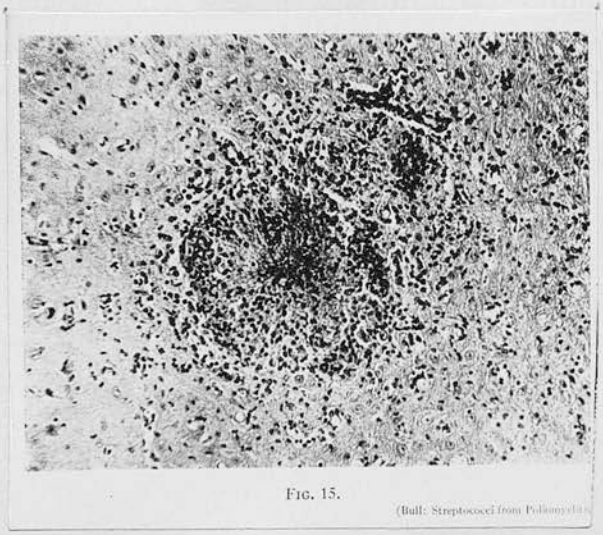


FIG. 15.

(Bull: Streptococci from Poliomyelitis

Fig. 20. Reproduced from Fig. 15, article by Bull (58).

Compare with Figs. 11, 12, 13, 14 and 15.

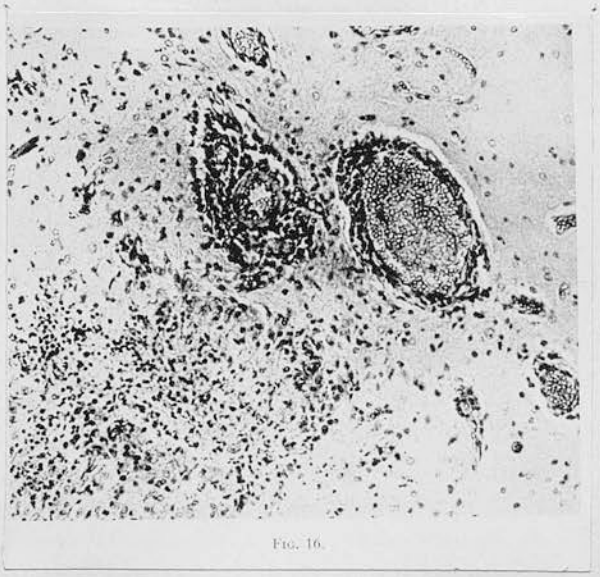


Fig. 16.

Fig. 21. Reproduced from Fig. 16, article by Bull (58).

Compare with Figs. 1, 2, 3 and 5.

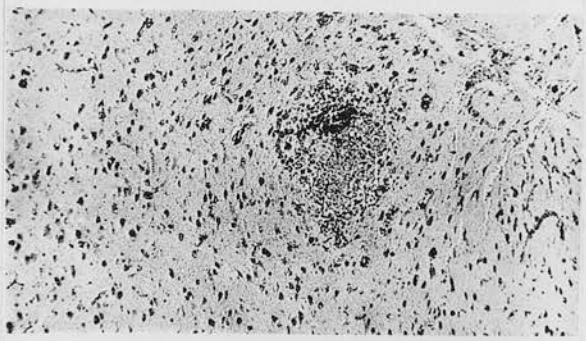


FIG. 17.

(Bull: Streptococci from Poliomyelitis.)

Fig. 22. Reproduced from Fig. 17, article by Bull (58).

Compare with Figs. 5, 12 and 14.

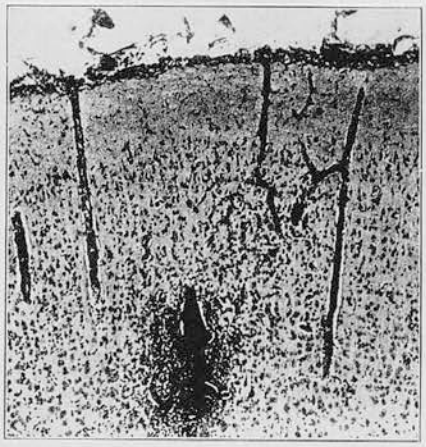


Fig. 1.—Cerebral cortex showing infiltration of pia and small vessels with lymphocytes. In the lower part of the section appears an area of focal infiltration.

Fig. 23. Reproduced from Fig. 1, article by Oliver (59).

Compare with Figs. 5, 6, 12, 13 and 16.

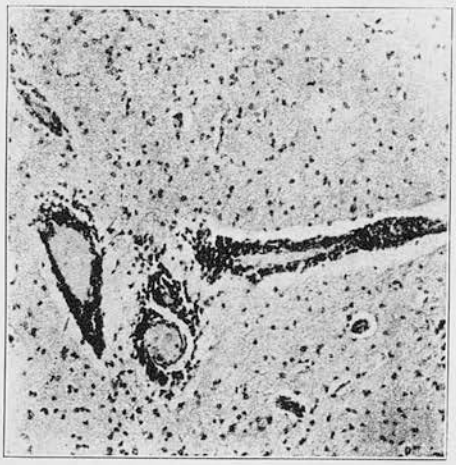


Fig. 2.—Perivascular infiltration with lymphocytes around vessels in neighborhood basal ganglions.

Fig. 23. Reproduced from Fig. 2, article by Oliver (59).

Compare with Figs. 1, 2, 3, 5 and 13.

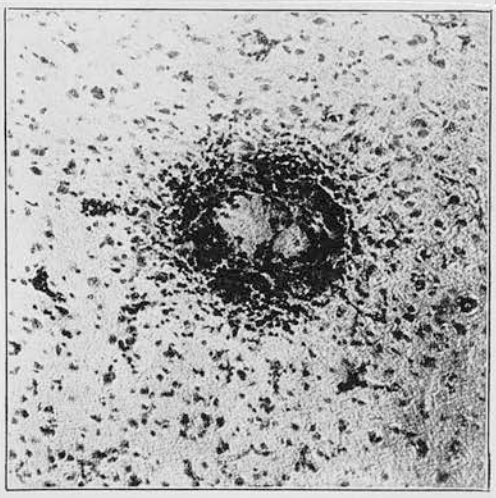


Fig. 7.—Focal lesion with necrotic center in Rabbit 11/7-1.

Fig. 24. Reproduced from Fig. 7, article by Reasoner (60).

Compare with Figs. 3, 11, 12 and 13.

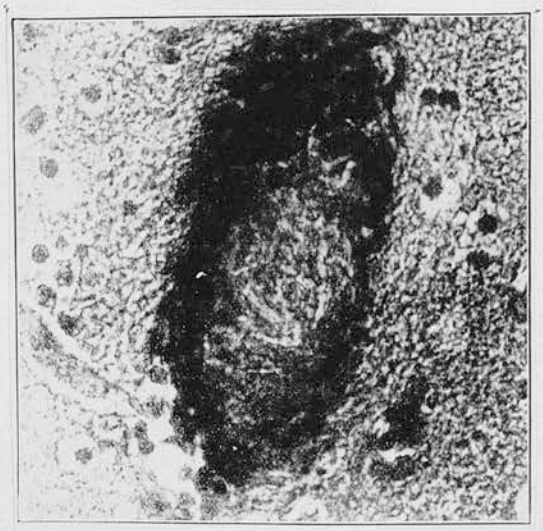


Fig. 8. Characteristic syphilitic blood vessel involvement in Rabbit

Fig. 25. Reproduced from Fig. 8, article by Reasoner (60), described as "Characteristic syphilitic blood vessel involvement."

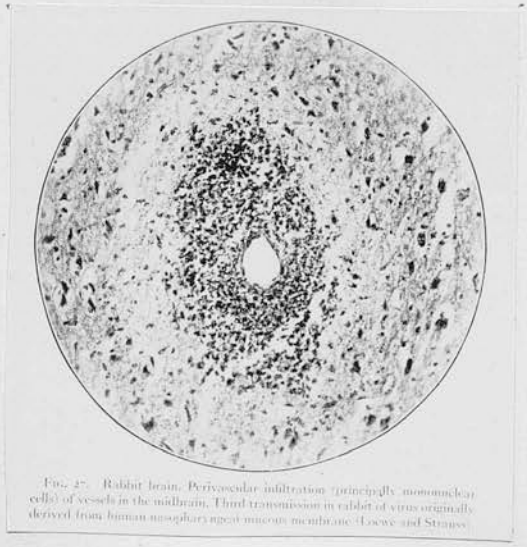


Fig. 27. Rabbit brain. Perivascular infiltration (principally mononuclear cells) of vessels in the midbrain. Third transmission in rabbit of virus originally derived from human nasopharyngeal mucous membrane (Loewe and Strauss).

Fig. 26. Reproduced from articles by Loewe and Strauss, (64), Fig. 27, and (31), Fig. 9.

Compare with Figs. 1, 2, 3 and 16.

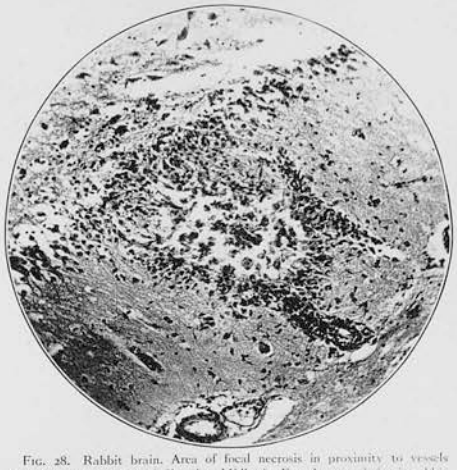


FIG. 28. Rabbit brain. Area of focal necrosis in proximity to vessels showing the perivascular infiltration. Midbrain. Fourth transmission in rabbit of virus derived from human nasopharyngeal mucous membrane (Loewe and Strauss).

Fig. 27. Reproduced from articles by Loewe and Strauss, (64), Fig. 28, and (31), Fig. 10.

Compare with Figs. 11 and 12.

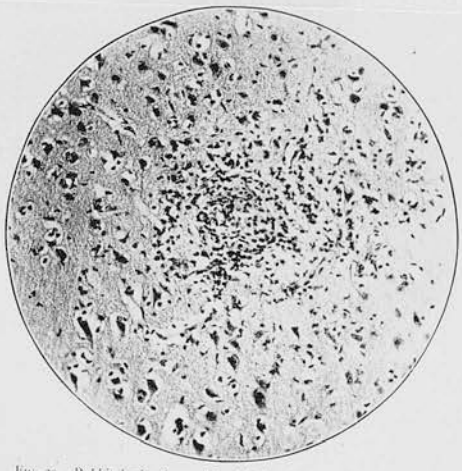


Fig. 29. Rabbit brain. Area of focal infiltration with mononuclear cells in the basilar ganglia. Animal injected with Berkefeld filtrate of brain of monkey successfully inoculated with virus derived from human nasopharyngeal mucous membrane, and which had been transmitted through four generations in rabbits (Loewe and Strauss).

Fig. 28. Reproduced from articles by Loewe and Strauss, (64), Fig. 29, and (31), Fig. 11.

Compare with Fig. 10.

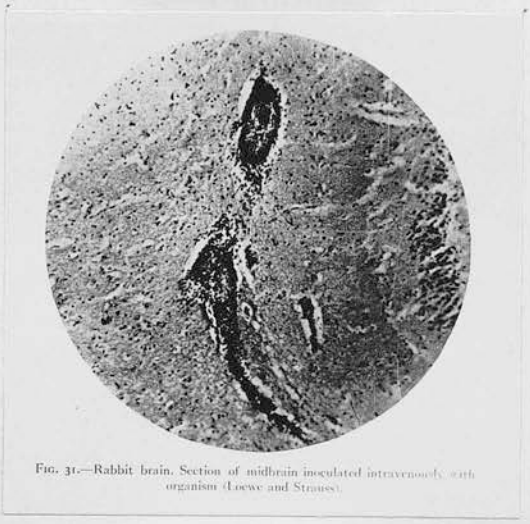


Fig. 31.—Rabbit brain. Section of midbrain inoculated intravenously with organism (Loewe and Strauss).

Fig. 29. Reproduced from article by Loewe and Strauss, (64), Fig. 31.

Compare with Figs. 3 and 13.

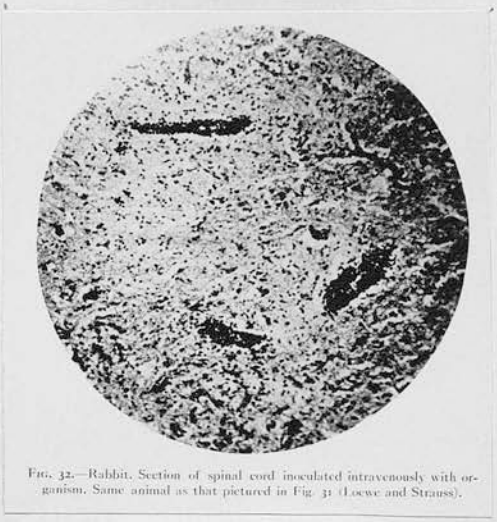


Fig. 32.—Rabbit. Section of spinal cord inoculated intravenously with organism. Same animal as that pictured in Fig. 31 (Loewe and Strauss).

Fig. 30. Reproduced from article by Loewe and Strauss, (64), Fig. 32.

Compare with Figs. 2, 5, 13 and 16.

FIG. 34. Rabbit brain. Cortex of cerebrum showing focal infiltration with round cells in proximity to vessel showing mononuclear cell infiltration of the adventitia. Rabbit injected with same inoculum as animal pictured in Figs. 29 and 30.

Fig. 31. Reproduced from articles by Loewe and Strauss, (64), Fig. 34, and (31), Fig. 13.

Compare with Fig. 5.

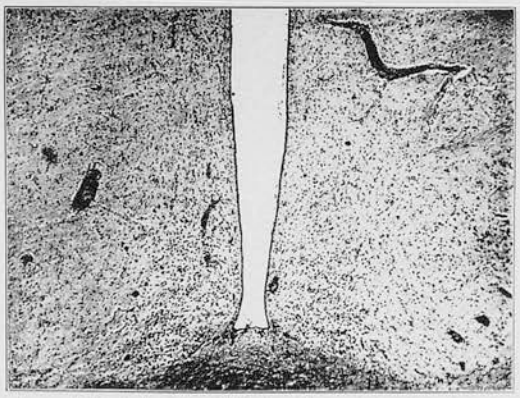
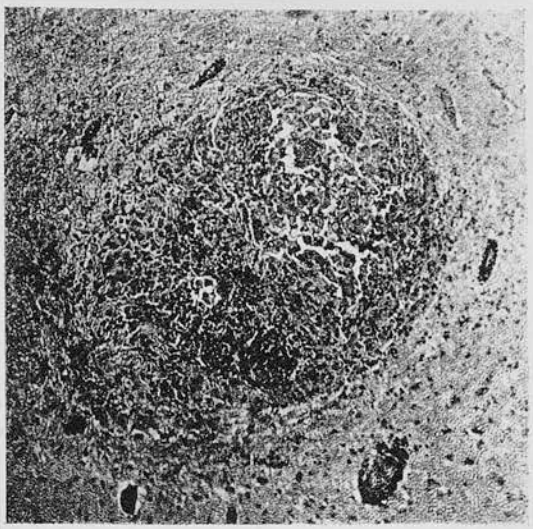


Fig. 31. Rabbit brain. Mononuclear cell infiltration of the parenchyma and of the adventitia of vessels in the basilar ganglia. Animal injected with Berkefeld filtrate derived from nasopharyngeal mucous membrane of animal pictured in Figs. 29 and 30.

Fig. 32. Reproduced from articles by Loewe and Strauss, (64), Fig. 35, and (31) Fig. 14.

Compare with Fig. 5.



Microphotographie 1.

Fig. 33. Reproduced from article by Kling, (69), Fig. 1.

Compare with Figs. 11, 12, 13, 14, 15.