INTRODUCTION

A study of the histopathology of the central nervous system in schizotrypanonis has already several times been attempted.

CHAGAS mentions the production of paralysis in cats.

In the acute form of CHAGAS' disease, GASPAR VIANNA showed up foci of inflammation all over the nervous tissue of the brain, cerebellum, basal nuclei, pons, medulla and spinal cord in its various segments. The trypanosome-harbouring cells he took to belong to the neuroglia; he was also the first to consider the process as one of meningencephalo-myelitis.

In puppies inoculated with schizotrypanum, but having shown no paralysis, MAGARINOS TORRES and VILLAÇA founds a mild encephalo-myelitis of an acute nature and with proliferation of the neuroglia cells of the "Körnchenzellen" type. The foci were made up of hypertrophic, neuroglia cells, "Abruunzellen" of neuroglia origin, neuroglia cells with regressive processes. Plasma-cells were also to be seen in varying numbers.

CROWELL saw at an autopsy of an acute case of CHAGAS' disease a slight thickening of the leptomeninges over the hemispheres and base of the brain with a discrete infiltration by mononuclears and lymphocytes. This process was nowhere intense but fairly uniform and unmistakeable. In the nervous tissues groups of cells of the neuroglia type were seen together with mononuclears; others with the appearance of
neuroglia cells contained many trypanosomes.

Experimental paralysis caused by *Schizotrypanum* infection may be said not to have attracted much attention until EURICO VILLELA read before the Brazilian Society of Biology (C. R. de la Soc. de Biologia, Oct. 31, 1924) a note on paralysis obtained in adult dogs by inoculating a virulent strain of *Schizotrypanum cruzi* obtained from a spontaneously infected armadillo (*Tatus novemcinctus* L.).

This paralysis could, with few exceptions, be systematically reproduced by reinoculation. The present paper is meant to complete that note by giving the results of the histopathological examination of the central nervous system of the paralysed dogs. In studies we undertook about ten years ago, paraplegia was obtained in a bitch which died in clonic and tonic convulsions. Histologic examination of the spinal cord and brain showed inflammatory foci in which *Leishmania*-shaped trypanosomes were to be seen. Later, (1916-1917), while attempting to prove the intra-uterine transmission of the experimental disease, one of the writers (E. VILLELA) obtained paraplegia in a bitch which bore three puppies. Of these, two became paraplegic without any new inoculation, so that infection must in all likelihood been congenital. Trypanosomes were however not found in the blood of any of them. As the material was not kept for examination, the paralysis could not be proved to be due to the congenital infection.

The last set of experiments, from which material for this study was drawn, was also undertaken with a view to observing intrauterine transmission, its conditions and the congenital disease. Our attention however was called to the severity of the disease which leads to abortion and death within a short time. Convulsions, paralysis, titubation, disorders in locomotion etc. showed that the nervous system was considerably affected.

In spite of the studies mentioned many interesting questions awaited a satisfactory solution, among others, the true nature of the cells which contain trypanosomes in the nervous system, the histopathologic details of experimental paralysis, the pathogenic aspect of the process, which had already been undertaken by GASPAR VIANNA and needed to be taken up again.

The present investigation is not meant to be final solution of all these questions and we hope, on the contrary that it may serve as a starting point for further research. We venture to point out the interest attached to the enquiry into the pathologic lesions of the neuroglia seen in the paralysis of dogs, and principally of the lesions of the "third element", as seen by the use of modern neurohistopathological methods as well as the behaviour of the conducting fibres in this process of diffuse encephalo-myelitis.

And again the pathology of the nervous system in CHAGAS' disease, this complex and fascinating question is a field as yet unexplored which might well attract the attention of the neuro-pathologist.

The strains of *Schizotrypanum cruzi* used in our experiments were obtained from naturally infected armadillos collected around Lassance (State of Minas Gerais), where they are common and show a large percentage of infection. The virulence of strains from the armadillo is already considerable and can be yet increased by passage inoculation through puppies and guinea-pigs. In most cases the trypanosomes had undergone frequent reinoculation but in one case it was obtained from the second passage through guinea-pigs.

Two to five cubic centimetres of blood, taken either from puppies, gui-
nea-pigs or paralysed dogs were given by subcutaneous injection. The period of incubation was of at least five or six days, but varied. A systematic examination was not always made so as to determine the term of incubation. The accompanying table shows the date on which the blood was examined for trypanosomes with a positive result and not the day the trypanosomes actually appeared in the blood. The fact is that often this examination was only undertaken so as to make sure of infection and was made at the time when infection was most probable to occur. In most cases the trypanosome is to be found by examination of fresh blood; afterwards it disappears. Not infrequently it is no longer to be found when paralysis sets in, and, to demonstrate it, it is necessary to inoculate the blood in a sensible animal. Sometimes however it maintains itself constant during the whole evolution of the disease; there is however no necessary correlation between the number of trypanosomes in the peripheric circulation and the severity of the infection. Of course it may be mentioned that the infections with many trypanosomes in the blood are often accompanied by considerable oedema. The virulence of the strain is indicated by the shortness of the term of incubation, considerable number of trypanosomes in the blood, the severity of the infection which in most case quickly leads to death. Many of the animals were killed deliberately but only those which our experience led us to believe would die within 24 hours. A reproduction of the paralyses in whole sets of animals, its practically constant appearance allow one to believe in a special neuropathism of the strain used. Whether the blood was taken from puppies, guinea-pigs or paralysed adult dogs, nervous symptoms were obtained in the same constant manner.

Paralysis is shown principally in adult dogs. In puppies the disease seems to evolve quicker and with a greater severity, so that the symptoms of septicaemia predominate. There is not time enough for the appearance of clinical symptoms of the involvement of the nervous system with definite localisation. And yet the pathological examination of the nervous system, shows an encephalo-myelitis. The same happens sometimes with adult dogs. Inoculation 65 (cf. accompanying table) is, however, an instance of paralysis in a puppy.

Guinea-pigs, which also have intense septicaemia, never show paralysis. Lambs (of one day to one month of age) never get paralysis, although they are infected; as a matter of fact, the number of trypanosomes in the blood of these animals is never considerable.

SOUZA CAMPOS inoculating the intestinal contents of Triatoma which had been allowed to suck infected animals and inoculating cultures of the same strain in rabbit blood-agar obtained the same results; he also obtained paralytic rabbits and white mice.

Might this be due to a special neuropathism of the strain or does it merely depend on a greater degree of virulence?

This is a question which E. VILELA and EVANDRO CHAGAS are trying to elucidate. One fact already ascertained by them is that dogs inoculated at the same time with strains from human beings did not become paralysed and that in one of them a reinoculation with a strain from the armadillo gave positive results. An exact appreciation of the fact remains as yet unattained.

All the material for study is from dogs. Animals were obtained from the street service of «Limpeza Publica», which gathers them of the thoroughpares. Street dogs, adult mongrels of different sizes and of ill defined
breeds, they remained in the laboratory kennels for at least a fortnight before being inoculated. As we were studying intra-uterine transmission, we at first inoculated bitches for preference; later on male and female dogs were inoculated indifferently.

As adult dogs and as street dogs we took it for granted that they had resistance, natural or acquired, against the most common infectious diseases, namely piroplasmosis and distemper.

At times however canine itch and multiple ulcerations of unknown nature to us were present.

So as to ascertain the possible concomitance of distemper we made a few experiments which are given in the addendum and were all negative as to results. The *Schizotrypanum* found in the lesions, of all cases leaves no doubt as to the aetiology of the meningo-encephalomyelitis.

Post-mortem examination was always made soon after death and in no case more than 12 hours later.

When death was foreseen to occur within a short time so that the autopsy would not be carried out in time, the animal was killed by chloroform and immediately autopsied.

The central nervous system would be removed and fixed in the following reagents:

Alcohol 96%.
ZENKER's fluid.
Formaldehyde 20%.
Formaldehyde-Ammonium Bromid.

For these fluids we chose segments of the motor zones of the brain and the hippocampus, of the cervical and lumbar expansions, of the spinal cord and different zones of the cerebellum. The rest of the material was kept in formaldehyde 10% for further use.

1) Material of alcohol at 96% was included in celloidin and the following stains were applied:

   a) UNNA-PAPPENHEIM's methyl-green-pyronin.
   b) NISSL's stain, with UNNA's blue and with toluidin-blue.

II) Material from ZENKER's fluid was stained for preference in haematoxylin-VAN GIESON, and eventually by MANN's method and MALLORY's phosphotungstic haematoxylin.

III) the material fixed in 20% formaldehyde was used for silver impregnation by BIELSCHOWSKY's method.

IV) formaldehyde-ammonium bromid material was used for gold-sublimate impregnation (CAJAL's method).

The formaldehyde 10% material was used for the stains I-VI and VI of ALZHEIMER; of LHERMITTE for the fibrillar neuroglia; of WEIGERT-PAL and WRIGHT for the myelin as well as for the routine haematoxylin-eosin stain.

Of the 39 dogs observed, material from eleven was submitted to a careful and detailed microscopic examination. It was taken from dogs which either died naturally or were killed by chloroform, and in which the disease had lasted for different lengths of time. The description of the histopathology of the process and the conclusions which we feel entitled to submit are based on the examination with different methods of the central nervous system of these eleven dogs.

**SYMPTOMATOLOGY.**

When trypanosomes make their appearance in the peripheral circulation, there are usually no evident symptoms. These only appear about 20 days after inoculation and are: lack of appetite, emaciation and sadness.

At times there is purulent secretion of the conjunctiva and keratitis (cf. Fig. 6, Plate 40). Diarrhoea, with mucosanguinolent catarrh is not rare. In some cases there is oedema, which may be very intense.
Nervous symptoms appear early. Emaciation and sadness are accompanied by locomotory disorders, so that movements halting, are unsteady, with a tendency to falling and marked asstasia (cf. Fig. 1, Plate 38). Weakness goes over into paralysis (cf. Fig. 7, Plate 41), which evolves rapidly, involving the four limbs so that the animals lie on the ground (cf. Fig. 8, Plate 41), generally with intense dyspnoea, as well.

Paralysis has a predilection for the hind limbs (cf. Fig. 4, Plate 39) and offends either one or both of them. It is less common in the fore limbs.

Beside the halting gait there is also titubation and lack of cerebellar coordination; the animals stand with legs wide apart and oscillating; they walk in a zig-zag fashion. At other times there is a tendency to adopt a circular motion pending to one side, or to fall, always on the same side, to which the head is turned. Tonic and clonic convulsions (cf. Fig. 2, Plate 38 and Fig. 3, Plate 39) followed by repeated fits of coma are frequent. In some cases the atrophy of the paralysed muscles is very striking.

Paralysis increases rapidly and death ensues within a few days. There are some cases however which last longer showing alternative turns for better or for worse. In rare instances the animal gets well and recovers its movements and general state of health.

The sphincters do not seem to suffer since there is no difficulty in retaining urine and faeces.

Paralysis is seen most commonly between the 25 and the 35th days and death generally occurs between the thirtieth and the fortieth days.

In the last re-inoculations with repeated passages there is a tendency to greater regularity in the fixation of the time at which paralysis occurs, the period becoming shorter.

Paralysis occurred in 39 of the dogs inoculated and only two survived. The accompanying table gives a synopsis of the observations made on the 39 dogs which became paralysed and points out the most interesting occurrences.

DIFFERENTIAL DIAGNOSIS

Knowing that so wide-spread a disease as canine distemper includes a paralytic form, it will be easily understood that our first care was to make sure that in the animals under observation there was no association between distemper and schizotrypanosis.

The animals experimented on were kept each in its own kennel on the grounds of the Hospital OSWALDO CRUZ (cf. Fig. 9, Plate 42).

As has been stated before these were adult dogs captured by the street service of «Limpze Publica» in the thoroughfares of Rio de Janeiro. In these kennels we kept animals inoculated with Schizotrypanum, as well as others not inoculated left as controls. The latter never showed any paralysis, as they should have if there had been any distemper of a particular virulence about the kennels, or any other epizootic disease. These animals were inoculated later on with Schizotrypanum; only after that inoculation did they show the paralysis, which appears in inoculated animals with regularity within a month, more or less.

In spite of these precautions we still made the following experiments.

Exp. 1.—The central nervous system of a dog which became paralytic after being inoculated with Schizotrypanum was used for intracranial injection in 4 rabbits. Rabbits are sensitive to the virus of canine distemper. One of the rabbits died after four days, another on the 10th day. The other two survived. None of them showed any cata-
rhinal inflammation of conjunctivae or pituitary membrane, or any paralysis.

Exp. 2.—The central nervous system of a dog inoculated with Schizotrypanum and having become paralysed (Dog n. 44) was used for subcutaneous injection. The animal chose was a puppy raised in the laboratory and guarded from contamination with distemper. The inoculation was made on Sept. 6, 1922. Up to Sept. 19 he had shown no coryza, conjunctivitis or cough, nor any of the nervous symptoms of distemper.

On Sept. 19th, trypanosomes were to be found in the blood, on Sept. 28 in large numbers. On Jan. 31, 1923 death supervened.

Exp. 3.—A litter of three 15-day old puppies (an age at which they should be very receptive to distemper) were painted on the snout and nasal cavities with the nasal discharge of a paralysed dog inoculated with Schizotrypanum (Dog n. 44).

They survived without showing any symptoms of distemper.

Exp. 4.—A litter of 4 puppies of less than 8 days of age and their mother were left together with a bitch inoculated with Schizotrypanum and paralysed (Dog n. 67) and together with Dogs n. 87, also inoculated, from Sept. 9, 1922 on to Dec. 15, 1922.

These dogs showed nothing till Sept. 22 when two of them n. 3 and 4, were inoculated with the blood of another puppy (n. 5 of inoculation 89) containing trypanosomes.

On Sept. 27, 1922, these puppies, n. 3 and n. 4, had trypanosomes in the peripheral circulation. By Dec. 4, 1922, n. 3 was very thin and n. 4 was found dead. The two puppies not inoculated were quite fit.

On Dec. 5, 1922, puppy n. 3, which is by now rather emaciated, somnolent and indifferent but has no paralysis, having plenty of trypanosomes in the blood, is killed. Up to Dec. 15, 1922, the puppies not inoculated showed nothing out of the way, except an abscess on the leg of n. 1.

Exp. 5.—On Aug. 16, 1922 an adult bitch is inoculated with 20 c. c. of fluid obtained by filtering through a BERKEFELD filter the blood of a dog inoculated with Schizotrypanum and having shown paralysis (dog n. 41).

Up to Jan. 30, 1923 no paralysis or catarrhal discharge from mucous membranes had been seen. No trypanosomes were found in the blood.

On this date, Jan. 30, 1923, i. e. five and a half months after having been inoculated with the filtered blood and having been always exposed to other paralytic dogs, this animal was given an injection of 5 c. c. of the blood of a guinea-pig (inoc. 109) containing Schizotrypanum cruzi (armadillo-strain).

On Feb. 8, 1923, Schizotrypanum was to be found in the peripheral circulation.

On March 14, 1923, emaciation and paralysis of hind limbs was seen.

On March 17, 1923 the paralysis was more pronounced as well as convulsive seizures. The animal is killed. Histologic examination shows lesions of the nervous system in which Schizotrypanum is to be found. This bitch is in the table with the indication Inoc. 55.

Exp. 6.—An adult dog was superimmunised against distemper so that it should furnish serum against this disease (Dr. O. C. MAGALHÃES).

This animal did not show any paralysis; five months later it was given to us by the doctor who had made use of it. It was then inoculated with 3 c. c. of blood from a paralytic bitch (Dog n. 152). This animal then became paralysed, and is included in our table with the indication Inoc. n. 154.

From the foregoing experiments it may be concluded that the search for
distemper through inoculation of the central nervous system of paralysed dogs to receptive animals (rabbit, puppy as yet not contaminated) never gave any results.

Canine distemper is caused by a filterable virus, yet the inoculation of the filtered blood of one of our paralysed dogs in another dog gave negative results. This same dog later injected with guinea-pig blood containing *Schizotrypanum*, became paralysed and its central nervous system showed lesions in which the parasite was to be seen.

Besides all this, the nasal mucus of paralysed dogs (a very virulent material when the disease is distemper) did not produce distemper on puppies of a few days, which ought to have been very receptive to distemper and on which it was rubbed. The same negative results were obtained by exposing to contagion from paralysed dogs puppies of a few days of age.

**PATHOLOGY.**

**MACROSCOPICAL EXAMINATION.**

Macroscopic examination of the central nervous system gave always negative results: no oedema, congestion or haemorrhage, whether meningeal or parenchymatous, were to be seen.

In one case alone, oedema and congestion with a cloudy appearance of the meninges were to be found, but as there was also secondary infection, arising from infected ulcers, this could hardly be considered an exception to the rule.

**MICROSCOPIC LESIONS**

I. **INFLAMMATORY LESIONS.**

The lesions shown in this type of encephalo-myelitis are essentially made up of small foci, in the neighbourhood of prae-capillary or capillary vessels, distributed without system in the white and gray matter of the brain and spinal cord; the cerebellum is less often affected; the foci, when seen in the cerebellum, are also found indifferently in any layer, and have been found in the molecular stratum, in that of small granular cells and in the white matter.

The structure of each of these foci necessarily varies with its age. In each dog, however, the foci usually show uniform characters, indicating that they arose about the same time.

The foci which appear to us to be in their first stages are made up of relatively large cells, with a rounded or slightly curved and almost always excentric nucleus, displaying its chromatin disposed in quite visible granules. The protoplasm is voluminous and reveals pseudopodous processes. The whole morphology corresponds therefore to that of the endothelial leucocyte or macrophagous polyblast (cf. Fig. 29, Plate 31). In these foci, lymphocytes and above all plasma-cells are also to be found, generally close to the vessels. Examination of preparations stained by the usual methods is misleading as concern the importance of plasma-cells in the process studied, and would make them seem rather scarce. In preparations stained with UNNA-PAPPE-NHEIM's Methyl-green-Pyronin, however, the cells are plentiful in the exudation. This is specially so in the case of the process seen in the leptomeninges, in which the perivascular infiltration by plasma-cells makes a most interesting picture in preparations stained by the UNNA-PAPPE-NHEIM stain (cf. Fig. 17, Plate 47 and pags. 20 to 23, Plate 49). It must be pointed out, however, that in no case is this type of cell dominant, as SPIELMEYER describes it in sleeping-sickness.

In foci made up of relatively few
cells (6 to 15 in the field of a histologic section) the parasite is rarely found; there is no desintegration of the nervous substance nor haemorrhage.

Of the macrophages we described in the foci, part, at least, must arise from cells of the blood (mesodermic macrophages, or endothelial leucocytes); this would seem undeniable in view of the close proximity of foci and vascular lesions (cf. Fig. 10, Plate 43 and fig. 13, Plate 45). Certain characters have led us to believe that they were in some extent of another origin. As a matter of fact the transformation of these macrophagous cells into cells with fatty granules (Körnchenzellen, Gitterzellen, Abräumzellen) could be seen to occur with regularity in our different preparations and took place in a considerable scale. The fatty contents of the cells could be made clear in preparations stained with Scharlach Red (Scharlachrot) (Cf. Figs. 18 and 19, Plate 48) and by Alzheimer's VI method (Cf. Figs. 18 and 19, Plate 50).

Recent research, specially that undertaken by RIO HORTega, METZ and SPATZ and by PENFIELD, shows that these cells with fatty granular contents arise mostly out of the microglia, a variety of cells which HORTega includes among the cells pertaining to the third element of the neuroglia.

In the process of encephalomyelitis present in experimental schizotrypano- s is fatty-granular cells are found in the foci in a constant manner at a certain period of the lesion, and would seem to arise form the microglia. They might be called microglial macrophagous cells.

Although no direct proof is available, such as might be obtained by the application of HORTega's method, it may be said that some facts we have observed bear out this point of view.

Thus we had our attention imme- diately called to the absence of Schizotrypanum (leishmaniform bodies) in the cytoplasm of the endothelial leucocytes (macrophages) of the infiltrated leptomeninges, standing in sharp contrast to the great numbers of these parasites seen in the cytoplasm of cells of the same appearance in the foci found in the nervous tissue. This fact led us to suppose that the macrophages of the nervous tissue were of the same extent different of the mesodermic macrophage or endothelial leucocyte.

Many parasites (leishmaniform bodies) are seen, with a normal appearance, within these (microglial) macrophages, in which they evidently undergo a development, so as to become fusiform and attain the flagellate form, and, it would seem that within these cells they find very favourable circumstances for living as is the case with lepra bacilli in the lepra cell.

This facile and convenient parasitism is all the more striking, if borne in mind that the endothelial leucocytes which, with lymphocytes and plasma-cells go to make up the exudate in myocarditis caused by Schizotrypanum are seldom parasited, and the few exceptions contain a small number of parasites undergoing degenerative process, which indicate the destructive action of the endothelial leucocyte on the parasite.

In foci at a more advanced stage, the transformation of macrophages in granular-fatty cells becomes general; the latter are clearly dominant. At this stage there is an evident désintégration of the nervous substance to judge from the formation of fissures or cavities in the fundamental substance. Granular-fatty cells are well evidenced by ALZHEIMER's methods I and VI.

At a later stage of its development, the focus of encephalo-myelitis shows a dispersion of the fatty-granular cells; the focus attains a considerable size,
with indistinct limits and an unclear outline. Fig. 18, Plate 48 of a preparation stained by Scharlachroth gives an idea of this aspect.

The oldest foci we had a chance to see were made up of cells with a fusiform, elongate nucleus and a branching protoplasm and apparently corresponding to the fibrous neuroglia. No macrophages are then seen; the parasites are found at times.

**VASCULAR LESIONS**

Inflammatory lesions also affect the vessels of the brain and spinal cord; these vessels take an active part in the process of encephalo-myelitis studied. Progressive vascular lesions, however involving an apparent or real formation of new vessels are no prominent feature of the process under consideration.

As a matter of fact, when a more affected part of the nervous system is examined under low magnification (Fig. 11, Plate 44) the small vessels appear more prominent than they would under ordinary conditions. But this is merely due to perivascular or lymphatic infiltration of the vessels and not to a numerical increase of vessels.

Vascular lesions show a remarkable constancy in their location in the vicinity of the foci of encephalo-myelitis. Whatever the age of the focus there is to be seen either an infiltration of the adventitia, or of this and the lymphatic sheath of the vessel or even an infiltration affecting the parenchyma as well and making up a really perivascular infiltration. The infiltrating cells, endothelial leucocytes, lymphocytes and plasma-cells are at first lodged in the adventitia of the vessels and the lymphatic space then appears as a clear halo round the vessels. As the progress of the inflammation should be more intense from the outstart, the lymphatic sheaths are themselves invaded by the exudate cells, among which the endothelial leucocytes are always dominant (cf. Fig. 13, Plate 45). Parasites (*Schizotrypanum cruzi* with the morphology of a leishmaniform body) are rarely to be seen in the interior of these endothelial leucocytes infiltrating the adventitia or the lymphatic spaces of vessels, in evident contrast with the intensely parasitized (microglial ?) macrophages often seen in the foci of encephalo-myelitis.

This vascular infiltration is never seen with the appearance of the diffuse dissemination met with in sleeping-sickness, or in some zones of the central nervous system in encephalitis lethargica. It is limited, in a single histologic section, to one or another vessel and to a certain segment of the vessel and is always in the neighbourhood of a focus of encephalo-myelitis.

Another lesion not uncommonly seen in the small vessels located at the edge of or within the foci is the proliferation of the endothelium and the partial or complete obliteration of the lumen of the vessel.

**II. LESIONS OF THE NERVOUS CELL.**

The cells most commonly displaying lesions are PURKINJE’s cells in the cerebellum and, next, the big pyramidal cells of the cortex.

The lesions of PURKINJE’s cerebellar cells are so intense in some animals as to immediately suggest some connection between them and the disturbances of equilibrium which are one of the most prominent and precarious symptoms of infected dogs.

The lesions of PURKINJE’s cells consist in swelling and globosity of the cell, with perinuclear chromatolysis and considerable nuclear lesions such as disappearance of the nuclear membrane, swelling and vacuolisation of the nucleolus. In other cases the cell
is reduced, retracted and the arborisations can be followed along a great extension (cf. Fig. 31, Plate 51).

These lesions are sometimes seen in a row of neighbouring cells and not in the following, or then they are seen in the cells of one stratum.

The lesions of the pyramidal cells just consist of chromatolysis; another common lesion of the cortex is neuropaphagia.

The multipolar cells of the spinal cord are usually well preserved: it is even an interesting fact to be seen, a nervous cell in the spinal cord, lying in the neighbourhood of foci of encephalomyelitis and showing a picture of perfectly normal NISSL's bodies.

III LESIONS OF THE NEUROGLIA.

We will not return to the lesions of the microglia, one of the kinds of cells known as the 'third element' of the neuroglia, since we have referred to them before.

These cells, 'the most numerous in the brain centres after the nervous cell itself', according to HORTEGA, are usually the origin of the fatty-granular cells, which may, on a lesser scale, develop from mesodermic cells or even from the fibrous neuroglia (astrocytes).

As it is, the part played by lesions of the microglia in the sense of an intense transformation into fatty-granular cells is of considerable importance and is perhaps even the most striking process seen in the encephalo-myelitis caused by experimental schizotrypanosis (cf. Fig. 13, Plate 48 and fig. 20, Plate 49).

Cells of the protoplasmic neuroglia are increased in number and in size in the neighbourhood of the foci (cf. Figs. 15 and 16, Plate 46). These cells remain isolated and as if limiting the focus. In our material neuroglia cells were also seen undergoing amoeboid transformation, as is shown by the figures. In figures 25 and 26, Plate 50 amoeboid neuroglia cells stained by ALZHEIMER's method IV are depicted. Fig. 27, Plate 50 shows one of these cells stained by the same specific method and containing parasites. *Schizotrypanum cruzi* is thus found, in the central nervous system, either within (microglial ?) macrophages, (cf. figs. 28, 33, 34 and 36, Plate 51) either within amoeboid neuroglia (cf. Fig. 27, Plate 50).

IV. MENINGEAL LESIONS.

The dura-mater showed a normal structure in every case in which it was examined, which in nearly every case was in sections of the spinal cord. It has already been stated that macroscopically no haemorrhages (haemorrhagic pachymeningitis) was to be seen.

The leptomeninges are on the other hand often the seat of a discrete inflammation, which is at times (principally over the cerebellum) of a haemorrhagic nature.

In no case does the process of leptomeningitis occupy a large extension, and usually it is seen in one or another point of the preparation. No purulent leptomeningitis was ever seen.

The picture seen is generally that of an infiltration of the meshes of the leptomeninges by an exudation of mononuclears occupying small areas. In the exudation, the endothelial leucocyte (mesodermic macrophage) certainly prevails. Besides this type of cell, lymphocytes, plasma-cells and, at times, as has been pointed out, red blood-corpuscles are found.

The lesions of the vessels of the leptomeninges are more extensive. It is not difficult, on longitudinal sections of the spinal cord, to follow a small arterial or venous vessel, with its adventitia considerably infiltrated by endothelial leucocytes and plasma-
cells, along a great extension. (Cf. Figs. 2 and 22, Plate 49).

Sometimes the lesions of the leptomeninges are found facing a superficially located focus of encephalo-myelitis, just as if there had been a discrete propagation of the inflammation. As a rule they do not appear to be dependent on the inflammation of the nervous substance. This is specially evident in the case of the cerebellum where there are usually few if any foci, while leptomeningitis, at times haemorrhagic leptomeningitis, is common.

In one preparation of the spinal cord, foci with endothelial leukocytes and Schizontopyanum was seen in the substance of one of the anterior roots.

The process of leptomeningitis of dogs with experimental schizotrypanosisis is exactly similar, as regards cellular constitution and location, to that seen by us in some human cases of the acute form of CHAGAS’ disease. We never saw any lesion of haemorrhagic nature in the human process, however.

The following protocols are some of the dogs selected from different stages of the process, and on which the general description that has just been given is based.

**Dog. n. 23.**

Killed on the 53rd day of the disease.

Section I. Spinal cord (Hæmatoxylin-eosin). The section shows a number of foci, both in the white and grey matter. On one side 7 foci in the white and 5 in the grey matter were counted, whilst on the other side there were 1 focus in the white and about 5 in the grey matter. In the grey matter the foci are located mostly in the anterior horns. The outlines are rather nuclear, and when the foci are neighbouring each other the exsudate becomes rather diffuse in nature. The small arterial vessels, even in the grey matter, show a pronounced mononuclear infiltration of the lymphatic spaces. The great number of parasites in the foci is surprising. The leptomeninges show a discrete infiltration; of a non-perivascular nature. In the immediate vicinity of the small arterial vessels no infiltration is recognised.

In the foci the fatty-granular cells (Gitterzellen) prevail. In the plasma of these as in that of the endothelial leukocytes there are parasites.

Section II. Spinal cord (Hæmatoxylin-eosin). Five foci in the white matter; about 7 in the grey, closely connected and difficult to separate. Gitterzellen, macrophages and many parasites. Leptomeninges unaffected, ependyma normal.

Section III. Spinal cord (Phosphomolybdic hæmatoxylin method, with previous fixation in WEIGERT’s Giæbeize with formaldehyde).

Proliferation of the neuroglia cells round the foci. Some of them attain large dimensions. The fatty-granular cells are shown up well by this method. Cells of the ameboid neuroglia are recognised, some of them containing parasites.

Section IV. Cerebral cortex (Hæmatoxylin-eosin). No infiltration of the leptomeninges which appear normal. In the nervous substance about 9 foci are to be seen out clearly. They are found in the innermost part of the grey matter and in the white matter in the immediate vicinity of the small vessels.

Section V. Cerebral cortex (Hæmatoxylin-eosin). 14 small foci mostly in the deepest layer of the cortex (stratum of polymorphous cells?) and in the white matter. None in the stratum of pyramidal cells. The foci are all small. In a larger one the cells are made up of macrophages and fatty-granular cells. The parasite, in the shape of a leishmaniform body is found in many of the cells. Each one of the macrophages, in the field, contains the parasite. Infiltration of the vessels is perivascular and in the lymph-spaces but is not very marked. In certain parts of the white substance there seems to be a discrete diffuse infiltration. One of the foci remains in contiguity with the lymph-space of a praecapillary vessel, and is remarkable by the numbers of parasites inside the macrophages (which in this cases are perhaps endothelial leukocytes or cells from the adventitia). Leptomeninges with no lesions.

Section VI. Brain (Ammon’s horn) (H. E.) Five foci, 4 in the white and 1 in the grey matter. The larger foci show a great many Gitterzellen. The smaller contain macrophages inside which are seen parasites. Infiltration of the lymph-spaces of the vessels only in the foci. Leptomeninges with no lesions.

Section VII. Brain [Ammon’s horn.] (H. E.) Four large foci with oedema and desinintegration of nervous tissue round them. They display a number of fatty-granular cells and plasma-cells, inside which the parasites are seen in abundance. In some of these cells 60 or 70 parasites in the shape of leishmaniform bodies could be seen. The foci appear to be at the stage of diffusion of the fatty-granular cells. In these large foci polymorphonuclears are not scarce.

Section VIII. Cerebellum (H. E.) Three foci in the molecular stratum. These foci are small and made up of macrophages containing many parasites.

Section IX. Cerebellum (Pappenheim-Unna’s method). Infiltration of leptomeningeal vessels by plasma cells.
Dog. n. 10.

Section I. Spinal cord at expansion (H. E.) Four foci in the white matter, 2 at the limits of the anterior horns with the white matter and one smaller in the gray matter. The lesions are clearly unilateral.

The most extensive focus in the white substance which attains the periphery shows an indistinct outline; there is evident desintegration of the nervous substance and the focus is essentially mad up of fatty-granular cells among which are seen bin arborescent neuroglia cells [?]. The other focus, which is also extensive, are made up of macrophages and plasma-cells. Cells with the appearance of \textit{Gitterzellen} are scarce. There is an evident connection between the foci and vessels: infiltration of the lymph-spaces in the vicinity of the foci. The vessels [capillary] in the most extensive focus are conspicuous owing to the infiltration of the lymph-spaces. Some of these vessels show a tunefaction of the covering endothelium with a partial obliteration of the lumen of the vessel. New formation by sprouting cannot be seen clearly. Plasma cells are easily found in the exudate, either in the lymph-spaces or in the foci. In the latter, after the macrophages which are clearly the dominant element, they are the most frequently seen type of cell. In the older foci, in which \textit{Gitterzellen} are prevalent, plasma-cells are rare.

There are no diffuse inflammatory lesions.

Leptomeninges and dura are not well shown up in the section. Facing the large peripheric focus in the white matter, there is a cellular infiltration of the leptomeninges. The lumen of the ependyma is free; there is no proliferation of cells.

The nervous cells, as far as is to be judged from this method, retain a normal structure.

No parasites were found in the foci [although 3 sections of the block were examined].

Section II. Cerebellum [H. --.]. No foci or perivascular infiltration. Leptomeninges congested in some points, their meshes infiltrated by mononuclears [macrophages and lymphocytes] and red blood-corpuscles. Lesions of PURKINJE's cells, as far as can be judged by this method, are to be found but are scarce.

Section III. Cerebral cortex [H. --.]. No foci. No perivascular infiltration; some praeCapillaries have their lumen stuffed with mononuclears so that they imitate the appearance of histologic sections of cases of leucemia. The lymphatic spaces of the same vessels are free. This leucemia appearance of the vessels is not uniformly seen in all vessels seen in the section some of them show mostly red blood-corpuscles in their lumen. The same leucemia appearance is shown by small vessels of the leptomeninges; in the meshes of these membranes the exudation is however very inconsiderable.

Dog. n. 11.

Killed.

Section I. Spinal cord at expansion [H. --.]. The foci are located on one half of the cord. There are seven of them, of which 4 are in the grey matter and the rest at the limits of the white matter with the grey but clearly in the white matter. The foci are very extensive and show an evident loss of nervous substance. The cells dominant in the lesions are fatty-granular cells \textit{Gitterzellen}. At the outskirts of the foci there are seen, mixed with the \textit{Gitterzellen}, macrophages and plasma-cells. The latter are never prevalent, however, in the foci. In the vessels [praeCapillaries] round the foci there is considerable infiltration of the lymphatic space. In these the cells are mostly plasma cells and lymphocytes.

One point, noughbouring infiltrated vessels and old foci give the impression, at first sight, of a diffuse inflammatory process, which does not, as a matter of fact, occur.

Leptomeninges with their meshes infiltrated. This infiltration is limited and occurs in the vicinity of foci located at the periphery of the white substance. The lesions of the meninges represent possibly propagation of the process of myelitis. Dura-mater normal.

In the extensive foci described, \textit{Schizotrypanum crusi} was not found, in spite of painstaking search.

Section II. Spinal cord (dorsal) [H. --.]. 17 foci spread indistinctly over white and grey matter. The foci are more plentiful but not as big as in section I. In none of them is there evident loss of nervous substance. The foci are made up mostly of macrophages; cells are seen with the appearance of \textit{Gitterzellen} but not in large numbers. In some of the foci \textit{Schizotrypanum crusi} is seen [Leishmaniform bodies] making agglomerations of a considerable size in some places. In the exudation seen in the lymph-spaces none of the parasites were found.

Section III. Spinal cord, dorsal region. [H. --.]. Only 2 foci are found; they are small, one in the white matter, one in the grey. No parasites are found within the foci.

Section IV. Spinal cord, dorsal region. [H. --.]. Seven foci are seen, some of them fair-sized. In one of them a few parasites [2 or 3 leishmaniform bodies] are seen in the plasma of the cells.

Section V. Cerebellum. [H. --.]. No inflammatory foci. In the white matter, pin points of hæmorrhage are seen. Capillaries and præCapillary vessels are entirely free of exudation in their lymph-spaces. In spite of the staining process used intense lesions of PURKINJE's cells are to be seen [pynosis of nucleus, vesicular appearance of nucleolus, homogenisation, globosity and eosinophilia of cytoplasm].

Section VI. Brain Cortex—H. --. Lesions much less marked than in spinal cord. In the white matter there is one extensive focus with loss of nervous substance, as well as a smaller focus. In another section of the white matter there is an area of diffuse infiltration which has not led up to focus formation. Infiltration of the lymphatic sheets only in the neighbourhood of the focus. In the lymphatic spaces elsewhere there is no infiltration. The largest focus appears to be undergoing organisation, round its edges there are seen ma-
ny arborescent cells (neuroglia? fibroblasts?). In the middle there are mononuclears. Fatty-granular cells are not plentiful as in the spinal cord. The parasite, which was looked for in three sections from this block, was not found.

Section VII. Brain, AMMON's horn. H. — e There is a small focus made up of macrophages and plasma cells, in the immediate neighbourhood of a capillary. In this focus Schizotrypanum crassus is found.

**Inoc. Dog. 19.**

Spinal cord at expansion H. — e. Seven foci in the grey matter, 3 in the white matter, 7 on the border line as well as other foci less clearly differentiated. The largest focus found in the white substance is formed by macrophages between which are seen large cells, with a conspicuous nucleus and a clearly arborescent protoplasm (neuroglia?, fibroblasts?). There are no clearly differentiated <i>Gitterzellen</i>.

Almost all the smaller foci are made up of arborescent cells, similar to the ones already pointed out before (cells of the fibrous neuroglia? fibroblasts?). These are in the large majority so that the inflammatory foci have the appearance of old foci undergoing cicatrization. There are some plasma-cells, but they are not predominant. There is no diffuse inflammatory process. Dura-mater and leptomeninges show no lesions, ependymal duct normal.

In one of the smaller foci, which appears to be made up of fibrous neuroglia or of fibroblasts, Schizotrypanum crassus 8 or 10 leishmaniform bodies are seen. The parasites are scarce in the foci. In another focus the parasites are within a macrophage, which is the type of cell predominant in that particular focus.

Section II. Cerebellum H. — e. No foci. No infiltration of the lymphatic spaces round the vessels. No diffuse inflammatory process. Discrete mononuclear infiltration of the leptomeninges. The vessels show a diffuse infiltration of the adventitia by plasma-cells.

Section III. Brain, Ammon's horn. H. — e. Ten different foci, mostly superficial, are seen. The foci show a similar structure to those of the spinal cord. They have the appearance of foci undergoing cicatrization; in all of them cells of the fibrous neuroglia are predominant. Infiltration of the lymph-spaces round the vessels in the neighbourhood of the foci. Plasma-cells present. Discontinuous infiltration of the leptomeninges, at times, but not always, in the neighbourhood of the foci.

**Inoc. Dog. n. 25.**

Section I. Spinal cord. H. — e. Three foci in the white matter of one side. On the other side a small focus in the white matter of the anterior funiculi and one in the grey matter. The foci are made up mostly of macrophages. No parasites found. No clear perivascular infiltration. In the grey matter, on both sides, punctiform haemorrhages.

Section II. Brain, Cortex. H. — e. Ten foci in the grey matter. They are small foci made up mostly of macrophages, evidently in connection with the vessels, which show an infiltration of the adventitia and of the lymphatic sheath. In the foci there are no haemorrhages. Congestion. In some foci, it is not uncommon to see macrophages joined together to form a giant cell. Near one of the foci, there is intense proliferation of the endothelium of a capillary with partial obliteration of its lumen. The leptomeninges show, in their meshes, red blood-corpuscles and mononuclears, as well as plasma-cells; this exudation is more abundant round the vessels. No parasites were found in the foci or in the meningeal exudation. These are small foci and appear to be of recent formation.

Section II. Brain, Cortex. Nissl's toluidin-blue method. The large and small pyramidal cells show the lesions described by Nissl in their first stages. These are seen in isolated cases. Neuronophagism is also seen discretely.

Section III. Cerebellum. Hæmatoyxin-Van Gieson. A focus is seen in the white substance, with no parasites. Lesions of the cells of PURKINJE's, as far as is to be judged from sections with this stain, are considerable.

**Inoc. Dog. n. 27.**

Section I. Spinal cord (Nissl's toluidin-blue method). More than 10 foci were counted in the white substance and 3 or 4 in the grey. The cells show a normal appearance as regards chromophile granules. A large cell of the anterior horn, found right in a focus of myelitis, has retained normal Nissl bodies. The foci are made up of macrophages and some plasma-cells; there are no Gitterzellen. With this method no parasites were seen, which, as we were able to ascertain, is due to total unstaining of Schizotrypanum in the differentiational stage of Nissl's method. Sections of the roots of the spinal nerves close to the cord, show inflammatory foci made up of macrophages in whose protoplasm is found Schizotrypanum. The vessels of the intrafascicular connective tissue shows perivascular infiltration.

Section II. Cerebral cortex (Nissl's method). The zone caught in the section is that of the large pyramidal cells; lesions of the cells are not marked. Parasites are not shown up.

Section III. Cerebral cortex. (H — e). Fourteen foci in the white substance, located near the vessels, some of which show perivascular infiltration and infiltration of their lymphatic sheaths.

The foci are mostly small. One however is more extensive and shows desintegration of the nervous substance. The predominant cells are fatty-granular and macrophages. There are plasma-cells as well. Schizotrypanum is found abundantly in the foci, sometimes in the plasma of fatty-granular cells.

1 There is a more or less diffuse haemorrhagic eptomeningitis. The exudate is mononuclear.
Section IV. Cerebellum (H.—e.). Contains no foci. Hemorrhagic leptomeningitis with mononuclear exudate.

Dog. n. 44.

Killed 47 days after inoculation.

Section I. Spinal cord. (H.—e.). On one side there are plenty of foci both in the white and the grey matter. On the other side there are three foci, located 2 in the grey matter and one at the outskirts of the grey matter and white. Leptomeninges distended with a diffuse mononuclear exudate.

The foci are made up of branching cells (fibroblasts? fibrous neuroglia?) and contain a good many cells brought together by the exudation process (plasma-cells, lymphocytes and macrophages); there are no fatty-granular cells. One of the connective-tissue septa running out from the leptomeninges and conveying the vessels shows at one place a fibrous thickening, the branching cells which are to be seen here shows a morphology similar to those of the foci. No parasites were found in the foci.

Section II. Cerebral cortex. (H.—e.). Two extensive foci are seen in the white matter; one of them shows the structure as the foci described in the spinal cord. In the other one the vessels show progressive lesion (new-formation of vessels), the cells are ramified or branching (fibroblasts? fibrous neuroglia?). In the sheaths of the vessels, infiltration with plenty of plasma-cells.

No parasites were found in the foci.

Dog. n. 158.

Killed on the 49th day of disease.

Pathology. Leptomeninges show a diffuse plasma-cell infiltration, the plasma-cells being found round the vessels and in their immediate neighbourhood.

This lesion occurs independently of the lesions of the nervous tissue. Thus it is quite conspicuous in sections of the brain, which in this animal shows very few inflammatory foci, and in sections of the cerebellum, in which there are no inflammatory foci. The small arteries and praeacapillaries running in the nervous tissue and away from the foci show no perivascular infiltration.

Focal lesions are found with ease in the spinal cord where they are seen only in the white substance. In several sections it was possible to locate them in the white substance of the antero-lateral funiculi, about the crossed pyramidal bundle. In other sections they occupied the direct cerebellar funiculus, in others the direct pyramidal tract.

In all foci it is possible to find out a vessel in most cases a small artery in others a praeacapillary vessel. The vessel displays proliferation of the endothelial cells, which partly obliterate its lumen. In other foci obliteration of the lumen of the vessel appears to be complete.

Some polymorphonuclears are seen in the immediate neighbourhood of the vessel, but are never plentiful.

The most numerous cells in the central part of the focus are macrophages, some containing the parasite. Between the macrophages and round the perivascular mononuclear infiltration there are plentiful fatty-granular cells; this is the type of cell occupying the greatest extension in the focus, as was to be seen in SCHARLA CH-ROT preparations. CAJAL's gold-sublimed method shows up fibrous astrocytes, in small numbers, in the zone surrounding the focus of myelitis.

In the brain few foci are found.

In the spinal cord, the foci show varying sizes.

The extension of the focus is probably connected with the date of its formation and agreeing with a greater or lesser dissemination of the granular bodies. UNNA-PAPPENHEIM methyl-green-pyronin method. (Sections of brain and spinal cord).

There is an infiltration of plasma-cells which may be considerable, of the leptomeninges, which is to be seen even in sections which show no foci in the nervous substance.

NISSL-method. (Sections of spinal cord and brain). In the spinal cord a few nervous cells show lesions corresponding mostly to the first stages of alteration (toxic lesions) of NISSL's description. In the cerebral cortex on the other hand, the cells show frequent lesions and it is possible to count 8 or more cells in each microscopic field with a small magnification ( Oc. 2, Obj. AA).

Inoc. Dog. 178.

Section I. Spinal cord (H. e.). On one side 11 foci in the white matter on the other, 4 foci in the white matter and one extensive focus in continuity with the posterior horn. Foci are small, made up principally of macrophages, inside which SCHIIOTRYPSIN crass is plentiful (Leishmanian bodies). The parasite is present in all foci. Perivascular infiltration and infiltration of the lymph sheats of the vessels near the foci. In the grey matter, there are discrete punctiform haemorrhages. Leptomeninges normal.

Section II. Cerebral cortex (H.—e.). Foci very numerous and limited to the grey matter. Foci are small, contain many parasites and are made up mostly of macrophages. There are no haemorrhages in the nervous substance. Haemorrhagic leptomeningitis, with mononuclear exudation (plasma-cells not plentiful). There are no parasites in the meningeal exudation.

Section III. Cerebellum. (H.—VAN GIESEN). A small focus in the plexiform zone, with no parasites. In the granular stratum, there are punctiform haemorrhages in several points. Infiltration of meninges moderate and irregular.

Section IV. Cerebellum. (NISSL's toluidin-blue method). The following lesions are seen: PURKINJE Cell A. General outline of cell retained but seeming to indicate a slight increase in volume; NISSL bodies diminished, especially round the nucleolus.
be the more plentiful, corresponding to cells having arisen from the microglia and are therefore microglial macrophages. It is also found in the protoplasm of fatty-granular cells, and less often in cells of the protoplasmic neuroglia. It has never been found in the nervous cell itself.

The presence of *Schizotrypanum* in the foci of encephalo-myelitis is so marked in some dogs, that one might assert that every macrophage of the exudate contained some.

In other animals, which are the less numerous, displaying foci made up in the same way, with the same term of infection and infected with the same strain of *Schizotrypanum*, for some unknown reason the parasites are few and may even be difficult to demonstrate.

*Schizotrypanum* may well be looked for in histologic sections stained by the usual Haematoxylin-eosin process. Excellent results are obtained in material fixed in Schaudinn’s sublimate-alcohol and stained with GIErMA’s stain or with HEIDENHAIN’s iron-haematoxylin.

NISSL’s stain is useless, as the optimum differentiation for the disclosure of chromophile granules, entails a complete unstaining of the parasites, which can then not be seen. A systematic search or a search for negative conclusions must never be tried by this method. Of course now and then a satisfactory stain is obtained of the parasite (cf. Fig. 34, Plate 51) by this method.

A comparative study of the material leads us to interpret the process of encephalo-myelitis by *Schizotrypanum* in the following way.

Figure 33, Plate 51 seems to show the first stage in the invasion of the nervous system by *Schizotrypanum cruzi* and throws light on the pathogeny of the process. It is, as a matter of fact,
with the morphology of a leishmaniform body and so to say passively that *Schizotrypanum* is carried to the nervous tissue itself. Of great importance in this spreading of the germ or this infection of the nervous centres, are the cells of the adventitia of the capillaries and praecapillaries or perhaps some special neuroglia cell (peri-vascular neuroglia of HELD). It is probable that *Schizotrypanum* pierces actively the walls of capillaries within the brain, which may be already more permeable by some peculiar conditions of structure, and is then taken up by some adventitial, neuroglial or connective-tissue cell, which may next remove itself from the vessel and emigrate in the nervous substance (see Figs. 33, 34 and 35, Plate 51).

These cells embodying the parasite in their cytoplasm become a centre of attraction for other cells, which arise, some from neighbouring vessels, which would explain the close connection between the foci and vessels infiltrated, others from the nervous substance itself (amoeboid neuroglia cells and microglia evolving towards fatty-granular cells). In this manner foci are built up in the nervous tissue itself. Disintegration of the nervous substance, formation of fatty-granular cells derived from those two orders of cells, their posterior dispersal, and cicatricial fibrosis are successive stages in a general process well known in pathology and which does not require reference here.

It would seem opportune to remember here an ingenious supposition of PINFIELD’s (1) which will explain how the parasite after entering into active proliferation in the cytoplasm of the macrophages of the foci, and after acquiring flagella, would return to the circulation.

CONCLUSIONS

1.—In 39 adult dogs inoculated with *Schizotrypanum cruzi* (strain isolated from *Tatus novencinctus* L., and exalted in virulence through successive animal inoculation) a process of encephalo-myelitis was seen, revealed clinically by astasia, paralyses, convulsions and other nervous disturbances.

2.—The process shows great regularity in its term of evolution, clinical symptoms and histo-pathologic lesions.

3.—As canine distemper also displays a paralytic form, great care was taken to ascertain whether there was any associated infection by canine distemper and *Schizotrypanum*. In the experimental field (see Fig. 9) in which the dogs were kept in individual kennels, no paralyses were ever seen in dogs not inoculated with *Schizotrypanum*, although kept under the same circumstances of food, lodging and exposure to the different epizootics as inoculated animals. The virus of distemper in paralysed dogs was not to be demonstrated either by inoculation to sensitive animals (puppies of a few days of age and rabbits) of material from the nervous system, of blood passed through BERKEFELD filters, or by direct rubbing of nasal mucus of the paralysed dogs on the nose and mouth of sensitive animals. Lastly histo-pathologic lesions of distemper were not seen in paralysed animals.

4.—Encephalo-myelitis in paralytic
dogs inoculated with *Schizotrypanum* is made up of small foci, found always in the neighbourhood of capillaries and præ-capillary vessels within the brain and occurring indifferently in white and grey matter of the brain (cortex, AMMON’s horn) and of the spinal cord (in all segments) without any evident systematisation or preference. In the cerebellum foci are more rarely found, but here also no preference is shown for the different strata.

The foci are made up of macrophages, some evidently migrated from the vessels (endothelial leucocytes), others, in greater number, arising probably from the microglia of the region (microglial macrophages). The desintegration of nervous substance, the formation of many fatty-granular cells, their subsequent dispersal and consecutive cicatricial fibrosis, are successive stages in the evolution of the foci. *Schizotrypanum cruzi* with the appearance of a leishmania (leishmaniform body) is often found in the cytoplasm of the cells in the foci.

5.—The vessels of the nervous substance show an infiltration of adventitia and lymphatic sheath by macrophages, lymphocytes and plasma-cells. This infiltration is discontinuous and is seen in the neighbourhood of foci of encephalo-myelitis. It does not show the uniformity of distribution seen in other affections (General Paralysis, Sleeping sickness, Encephalitis Epidemica). In some places, the infiltration, more extensive, is evidently perivascular. In the small arteries and præcapillary vessels, mostly inside or

near a focus of encephalo-myelitis, there is to be seen a proliferation of the endothelial cells with partial or total obliteration of the lumen of the vessel.

6.—The plasma-cells are a constant element of the inflammatory exudate of the meningo-encephalo-myelitis caused by *Schizotrypanum cruzi*. They are never the dominant type of cell as in Sleeping Sickness according to SPIELMEYER.

7.—Lesions of the nervous cell are present but not conspicuous. The most affected cells are the PURKINJE’s cells of the cerebellum and the lesions are swelling of the cellular body, perinuclear chromatolysis and necrosis. Other cells constantly affected are the large pyramidal cells of the cortex, which often show, besides the above-mentioned lesions, ‘neuronophagism’.

The multipolar cells of the spinal cord are less often affected. In some cases we were able to see these cells located in the immediate neighbourhood or even within the focus of myelitis itself and in a perfectly normal condition as regards their NISSL’s bodies.

8.—Dura-mater is normal. The leptomeninges show a discontinuous inflammation. The inflammatory exudate, which is not abundant, is of mononuclear nature, made up of macrophages, which are prevalent, and of lymphocytes and plasma-cells. Often leptomeningitis is of a haemorrhagic type, specially round the cerebellum. The vessels of the leptomeninges show along great extensions infiltration by macrophages and plasma-cells.

9.—*Schizotrypanum cruzi* with its
trypanosome shape, as it is found in the blood, once passing through the adventitia of vessels, is embodied by or penetrates actively into cells surrounding the vessels (perivascular microglia? adventitia cells?). These cells, acting as macrophages, disseminate the germ throughout the nervous system. One might say, in a general way that the *Schizotrypanum* usually penetrates in the nervous tissue passively.

10.—Special methods used permit us to assert that the cells which usually contain *Schizotrypanum* in the nervous central system are macrophages. These cells do not stain by the specific methods, method IV of Alzheimer and sublimate-gold of Cajal.

The morphologic characters and known histologic methods are not able to make clear the precise origin of these macrophages, for it is a well-known fact that they may have a multiple origin. The evolution of these cells into fatty-granular cells, which is a constant phenomenon in the experimental paralysis studied, indicates that these macrophages arise mostly from the microglia. They are thus «microglial macrophages». Vascular lesions indicate however, that endothelial leucocytes also concur towards the formation of the macrophages. Besides the macrophages and fatty granular cells, Alzheimer's method IV shows that the parasite is also embodied by cells of the protoplasmic neuroglia (cf. Fig. 27, Plate 50).

11. Presumption was obtained that the return to the circulation of the *Schizotrypanum* found in the foci of encephalo-myelitis is in part at least, associated with the migration of neuroglia cells to the outer surface of blood vessels.
EXPLANATION OF PLATES 38—51.

Figs. 1 to 17, Plates 38 to 47 are photographs.

Fig. 1. Inoc. Dog 62.—Astasia and dysbasia. Cerebellar titubation with a tendency to circular movement and to falling towards the left. Paresis of the 4 limbs.

Fig. 2. Inoc. Dog 29.—Onset of a convulsive attack (tonic convolution).

Fig. 3. Inoc. Dog 26.—Paralysis of the 4 limbs. Onset of a convulsive attack (tonic period).

Fig. 4. Inoc. Dog 44.—Paresis of the hind limbs, especially of the left, which is trailed behind in walking. Titubation (staggering).

Fig. 5. Inoc. Dog 158.—Paresis of fore-limbs.

Fig. 6. Inoc. Dog 158.—Keratitis with ulceration.

Fig. 7. Inoc. Dog 25.—Astasia and paralysis of the 4 limbs.

Fig. 8. Inoc. Dog 27.—Complete paralysis of the 4 limbs. Ulceration at buttocks.

Fig. 9.—Kennel in which experiments were made.

Fig. 10. Inoc. Dog 178.—Section of spinal cord showing the multiple nature of inflammatory foci (nine different foci are to be counted).

Fig. 11. Dog n. 23.—Section of spinal cord (anterior horn, outskirt of the white matter). Confluent foci giving the impression of a diffuse infiltration. Perivascular infiltration and infiltration of the lymph-spaces of the local vessels.

Fig. 12. Inoc. Dog 23.—Part of the preceding section seen under higher magnification. Foci are made up principally of fatty-granular cells and macrophages.

Fig. 13. Inoc. Dog 178.—Section of spinal cord. Three inflammatory foci and perivascular infiltration with infiltration of lymph-sheaths of blood vessels are to be seen. Some normal-looking cells are seen.

Fig. 14. Inoc. Dog 158.—Section of spinal cord. Proliferation of the endothelium and obliteration of the lumen of a vessel in the neighbourhood of a focus of inflammation of the spinal cord.


Fig. 17. Inoc. Dog Section of spinal cord. NISSL's stain using UNNA's polychromatic blue. Great numbers of plasma-cells in the perivascular exudate and in a focus of inflammation of the spinal cord.

Fig. 18. Inoc. Dog 158.—Section of spinal cord. Scharlachrot-hæmatoxylin. Oc. 5, obj. AA at height of table.

Confluence of different foci showing infiltration of the lymph-sheaths of blood-vessels and round them. Fatty-granular cells outlining the edges of each of the confluent foci («diffusion» of fatty-granular cells).

Fig. 19. Inoc. Dog 158.—Oc. 4. Imm. lens 1/12».

Fatty-granular cells seen under high magnification (same section of Fig. 18.)

Fig. 20. Inoc. Dog 178.—Oc. 1. Imm. lens 1/12. Height of table. Section of spinal cord. PAP-
PENHEIM—UNNA method.
Focus of inflammation in the spinal cord, high magnification, showing the predominant fatty-granular cells. Plasma-cells are seen with their specific stain. There is a proliferation of endothelium in the field, with partial obliteration of the lumen of a blood-vessel.

Fig. 21. Inoc. Dog 178.—Section of spinal cord. PAPPENHEIM—UNNA's method. Oc. 1. Obj. S. S. At height of table.
Small blood-vessel with obliteration of its lumen and infiltration of the lymphatic-sheath by plasma-cells.

Fig. 22. Inoc. Dog 158.—Section of brain. Limited leptomeningitis showing plasma-cells in the inflammatory exudate. Oc. O. Imm.-lens 1/12 at height of table. PAPPENHEIM-UNNA's method.

Fig. 23. Inoc. 158.—Oc. 2, Imm.-lens 1/12 Height of table. PAPPENHEIM—UNNA's stain.
Infiltration by plasma-cells along the connective-tissue septa which run in from the leptomeninges and hold the blood-vessels. Section of spinal cord.

Fig. 24. Inoc. Dog 158.—Section of spinal cord. ALZHEIMER's neuroglia method IV.
Proliferation of cells of the protoplasmic neuroglia at the limits of a focus of myelitis.

Fig. 25.—Part of the same focus, further inside, seen with high magnification. (Oc. O. Imm.-lens 1/12, at height of table). Oc. 2, Imm.-lens 1/12. At height of table.
Cells of the protoplasmic neuroglia with lesions. Between them, fatty-granular cells.

Fig. 26. Cell of the amoeboid neuroglia seen at the edge of a focus of inflammation in the spinal cord. Same focus as in fig. 25.
Oc. 2, Imm.-lens 1/12. At height of table.

Fig. 27. Inoc. Dog 149.—Section of spinal cord.
Oc. O, Imm.-lens 1/12. Drawn at height of table. ALZHEIMER's method IV.
Cell of the protoplasmic neuroglia containing Schizotrypanum cruzi as a leishmania form body. In the neighbourhood a capillary vessel.

Fig. 28. Inoc. Dog 23.—Oc. O, Imm.-lens 1/12, at height of table. PAPPENHEIM—UNNA's method.
Macrophages found in foci of encephalo-myelitis are the cells in which Schizotrypanum is most often found (microglial macrophages ?—endothelial leucocytes ?).

Fig. 29. Inoc. Dog 149.—Section of cerebellum. Oc. O, Imm.-lens 1/12. At height of the microscope table. NISSL's method with UNNA's blue.
PURKINJE's cell with chromatolysis in the neighbourhood of a focus. The focus is made up mostly of macrophages.

Fig. 30. Inoc. Dog 158.—Section of cerebrum. Oc. O, Imm.-lens 1/12. At height of table. NISSL's method with UNNA's blue. Lesions of NISSL in the cells of the cortex of the brain.

Fig. 31. Inoc. Dog 11.—Section of cerebellum. Oc. 1, Obj. DD. At height of table. Haem.
eosin method. Lesions of PURKINJE's cells.

Fig. 32. Inoc. Dog 23.—Oc. O. Imm.-lens 1/12, UNNA—PAPPE. NHEIM's method. Multipolar cells of the anterior horn of the spinal cord with chromatolysis and nuclear lesions.

Fig. 33. Inoc. Dog 152.—Section of cerebellum. Oc. O, Imm.-lens 1/12. At height of table. NISSL's method using UNNA's blue. Cell in the adventitia of a capillary vessel containing Schizotrypanum cruzi with the morphology of a leishmaniform body.

Fig. 34. Inoc. Dog 152.—Section of cerebellum. Comp. oc. 6, Imm.-lens 1/12, at height of table. NISSL's method using UNNA's blue. Cells arising from the adventitia of a capillary vessel and containing Schizotrypanum cruzi. These cells are not to be distinguished morphologically from endothelial leucocytes. The figure shows the probable mode of dissemination of the parasite in the central nervous system.

Fig. 35. Inoc. Dog 23.—Section of brain. Oc. 2, Obj. 1/12, at height of table. Haematoxylin-eosin method. Parasites in the cells of the wall of a capillary vessel.

Fig. 36. Inoc. Dog 23.—Section of brain (AMMON's horn). Oc. O, Imm.-lens 1/12, at height of table of microscope. Haematoxylin-eosin. A zone in a large inflammatory focus in the brain showing numerous cells containing Schizotrypanum cruzi.
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