contamination with surface water is excluded. As regards the further special treatment, a proved radical cure, through which the parasites could be killed, is not known to me. Glycerine was recommended by Zürn against the gregarinosis of fowls, and it appears worth while to try experiments with this, as well as with proper disinfectant media.

Meanwhile, the therapeutic treatment will remain more a symptomatic and tentative one, directed partly against the hæmorrhage (sesquichloride of iron, opium), partly against the intestinal inflammation (mucilage, astringents), at the same time taking care to provide an appropriately rich nourishment (eggs, milk, even wine).

In the case of an animal having to be killed on account of this disease the consumption of the flesh may be permitted.

ON CERTAIN CHARACTERS OF THE HISTOLOGICAL LESIONS OF RABIES.¹

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THE symptoms and lesions of canine rabies are well known; in their totality, when well pronounced, they leave no doubt as to the nature of the disease. However, it sometimes happens that we have not had the opportunity to note the symptoms shown by the animal that has caused the bite, and as a result the medical man hesitates to prescribe the antirabic treatment and to recommend the patient to submit himself to it. Even when the madness of the animal that has inflicted the wound is probable, a speedy and exact diagnosis is always desirable, so as to be able, should such a case occur, to calm the fears of the person bitten.

Lastly, cases arise in which the symptoms of rabies may be mistaken for those of a different disease—epilepsy, for example. There are others where the spinal cord of the animal that has caused the bite reaches us putrid or changed, or, it may be, preserved in alcohol, so that animals inoculated with this cord succumb to septicæmia, or remain well although the dog that inflicted the bite was rabid.

All these constantly recurring difficulties have urged me, from the very beginning of my researches on rabies, to seek for a diagnostic method more rapid and certain than the presence of foreign bodies in the congested stomachs of suspected animals, or other lesions even less constant. This disease, so well marked by a constant course of symptoms, ought to show characteristic lesions in the nerve centres, and especially in the ganglia which preside over the production of these symptoms.

I have therefore sought for the presence of lesions in the motor centres of the medulla oblongata, the spinal cord, and especially in the grey substance surrounding the lateral ventricles of the cerebrum.

Researches from this point of view have already been made by

¹ Annales de l'Institut Pasteur, April 1892.

different authors. Benedikt published in 1874 observations upon the lesions of rabies.¹ In 1875 the same writer commences a work² upon the lesions of rabies in these words:—"The reason why the pathological lesions of rabies have escaped us is because they must be sought for in the form of *miliary* pathological centres." However, this authority described as rabic lesions a condition which he identified with the granular disintegration of the English. In his opinion there occurred a perivascular exudation of a granular or hyaline substance, and he admits even the rupture and compression of the vessels at the level of the lesion. Several illustrations in his work show a diapedesis of red corpuscles and leucocytes forming centres around the blood vessels. At the same time there exist small hæmorrhages due to rupture of the walls of the vessels, masses of yellow pigment around the vessels, and an accumulation of leucocytes in certain lymphatics. As to the situation of this lesion, the author notes the frequency of minute lesions in the olfactory lobe, Sylvian fissure, and origin of the motor root of the 5th nerve, and, lastly, between the *ansa* and the *processus cerebelli ad pontem*.

As is well known, granular disintegration has since been recognised as being partially an artificial modification, and in reality the drawings which accompany the work show to us lesions which certainly did not exist during life. Again, in other drawings we see hyaline masses around vessels, hæmorrhages in the perivascular spaces, and small centres—hæmorrhagic, pigmentary, and inflammatory—around the vessels.

In the same year Kolesnikoff³ noticed almost identical lesions, in addition to hyaline masses producing obliteration of certain vessels.

Pfitz and Friedberger⁴ have also noticed hyaline masses in the vessels and in their walls, as well as a yellowish discoloration of the walls and tissues surrounding the vessels.

Dr Coats⁵ found extensive lesions in three cases of human rabies, for example, miliary centres, especially in the axial part but also in the convolutions. These centres are formed by lymphatic cells, accumulated not only around the vessels, but also around the nerve cells. He does not mention the hyaline masses described by Benedikt. Dr Coats therefore, without doubt, described the most characteristic lesions; but his description is faulty, and it does not specify the topography of the lesions.

M. Benedikt⁶ returned again in 1878 to rabic lesions. He noticed the lesions described in a horse dead from rabies; according to his view, the miliary centres are always found surrounding the vessels of the anterior horns of the cervical portion of the spinal cord and in the axis of the central nervous system; in the same places he noticed again minute dissecting aneurisms, produced frequently by a hyaline degeneration of the walls of the vessels.

Bollinger, Forel,⁷ and Fr. Schultze were unable to find any other lesions than a hyperæmia and a lymphstasis—lesions which, according to these authors, have no particular significance.

In 1887 Gowers,⁸ when examining the brains of four individuals dead of rabies, also noted excessive lymphatic accumulation around

¹ Wiener mediz. Presse, 1874, n. 27.

² Centralblatt f. med. Wiss., 1875, p. 873.

³ Lancet, 3rd Feb. 1877 (quoted by Benedikt).

⁷ D. Zeitsch. f. Thiermed. und. vergl. Path., 1876, t. 111.

² Virchow's Archiv., 1875, t. 64, p. 587.

⁴ Zeitschr. f. pract. Veterinarwiss., 1876, p. 59.

⁶ Archives de Virchow. t. 72, p. 425.

⁸ Pathol. Transactions

the small veins; these on transverse section corresponded to the lesions described by authors.

This authority, as well as Friedberger and Benedikt, described in the regions mentioned vascular thrombi—reticulated, granular, hyaline, often containing blood pigment, or enclosing leucocytes in way of dissolution. He believed, like Benedikt, that these lesions are characteristic of rabies, and that perivascular centres of embryonic cells are found also in other diseases. For instance, Benedikt mentions a case of progressive paralysis of the cerebral nerves (bulbar?) with like centres upon the floor of the fourth ventricle.

Weller,¹ like the majority of investigators, found centres of embryonic cells in the perivascular spaces, which also included uniform yellow masses, like drops of some fatty substance. A like appearance was noted around the neighbouring nerve cells.

Kolesnikoff,² relating the results of the examination of 20 dogs rendered mad and killed on the 4th or 5th day of the disease, found again the lesions described in previous researches, and insisted especially upon this point, that *the microscopic lesions were in every case the same*; they were localised in the corpus striatum, optic thalamus, medulla, and spinal cord. The most marked lesions exist near the blood vessels. They consist, in the first place, in a proliferation of endothelial cells; secondly, in a proliferation of the connective tissue of the external tunic, with infiltration by lymphoid cells; thirdly, in an accumulation of lymphoid cells between the middle and external coats; fourthly, in an extension of the infiltration into the middle coat and proliferation of the muscular elements. In this case, all the layers of the vascular wall have been infiltrated by lymphoid cells.

These lesions are primarily nodular, later on they become diffuse. They are complicated by hæmorrhages, due either to rupture or diapedesis. These hæmorrhages are mainly caused either by the lesion in the vessel wall or by obliteration of the vessels by thrombi formed of hyaline masses and leucocytes. The author then enters into an analysis of the hyaline substance found in the walls of the changed vessels; he finds that the masses give either an amyloid reaction or that obtained from colloid material and albuminoid substances. In his opinion these accumulations are due to a modification of the red blood corpuscles. Without turning his attention to the localisation of the lesions around the nerve cells, he insists upon the insular character of the rabic lesions, markedly noticeable in the grey substance of the brain and spinal cord; but on looking into the cases described by this author, we clearly see that the centres around the nerve cells are not so disseminated as the vascular centres. The first nodules exist especially in the grey substance in the neighbourhood of the ependyma of the ventricles, in the pons, in the nuclei of the hypoglossal, pneumogastric, and spinal accessory nerves, and, lastly, in the anterior horns of the cord.

In a short article which appeared in the Hungarian journal *Orvosi hetilap* of 1886, I gave the summary of a long series of examinations made upon the organs of men and animals dead from rabies, and in my exhaustive publication upon rabies (*Virchow's Archiv.*, t.

¹ Arch. f. Psychiatrie, 1879.

² Virchow's Archiv., 1888, p. 445.

110, 1887) I tabulated the result of my own researches upon rabic lesions, which I may state as follows:

1st. In animals dead from street rabies, there are found usually a hyperæmia and an acute generalised œdema of the cerebral and spinal meninges, acute hæmorrhages localised around certain vessels, as well as inflammatory lesions. On microscopic examination we find an increase of the plasma cells, augmentation of the reticular substance, fibrinous in character, between the several layers of the meninges.

2nd. The epithelium of the cerebro-spinal central canal has proliferated. In the grey matter which surrounds the canal, and especially in that of the floor, hæmorrhages, sometimes symmetrical, are often found. Microscopically, we often find an obliteration or thrombosis of a vessel by a reticulated, hyaline, pigmented material, or by leucocytes or hyaline globules, and sometimes a hyaline degeneration or even an inflammation of the vascular tunics. The extravasated blood also contains much of the hyaline material. The hæmorrhages are often limited by the lymphatic sheath of the vessels. At the same time the epithelium of the ventricles and central canal may be partially lost. This last occasionally is filled with blood or plugs, either granular or hyaline in character.

3rd. With the naked eye small centres of degeneration may sometimes be noted in the grey matter, but often they may be sought for in vain.

4th. The most constant lesions are microscopic in character; they are found more especially in the grey matter surrounding the cerebro-spinal canal, and in the motor centres of the medulla and spinal cord. These lesions consist at first in hyperæmia and accumulations of embryonic cells around the small vessels, perithelial or migratory in origin, often showing indirect division; finally there are also found lesions of nerve cells.

5th. The lesion of the nervous elements of the parts indicated is quite characteristic; it consists in signs of proliferation, namely, in the presence of several small cells in place of one large one, or in a uniform degeneration, and often in the appearance of vacuoles with a reduction in size or disappearance of the nucleus, or again, its chromatic network disappears. These cells frequently contain pigment. Round uninuclear, more rarely multinuclear, elements of a lymphatic origin often invade the protoplasm even of the cell, and fill out the dilated pericellular lymphatic spaces by a multiplication of small nuclei.

6th. The lesion of the medullary substance is less pronounced; it consists chiefly in an œdema of the medullary sheath of the nerve fibres.

7th. In certain plasma cells, in the interior of and around vessels, sometimes in leucocytes, in lymphatic spaces, in the altered parts of certain nerve cells, and in the dilated sheath of nerve fibres, may be seen rounded or amœboid granules about $1\ \mu$ in diameter, pigmented or stainable by aniline dyes, and which in part seem to possess the power of movement.

The same lesions have been described later on by different authorities, who have also added controversial details. Thus Schaffer believes that he is able to note certain protoplasmic or pigmented granules of nerve cells and of nuclei, and also more pronounced protoplasmic streaks, as if this were something out of the common; and he contends that the lesions which he regards as an acute myelitis, are always

more pronounced at the point corresponding to the entry of the nerve from the region bitten into the spinal cord or brain. This author insists also upon the accumulation of lymphatic cells around the nerve cells, especially in the anterior horns. He attaches some importance to the lesions of nerve fibres and to the pigmentary atrophy found in rabies, and insists upon the localisation of lesions at the level of the motor nuclei. However, Popoff and myself have been unable to confirm the statements of this authority concerning the constant localisation indicative of myelitis, and the specific nature of pigment or certain granules; further, I have noted in several other acute lesions of the cord the lesions of the ganglion cells described by Schaffer.

In a recent work along with M. Mihailescu, I have noted, besides the lesions mentioned, that the alteration of the nerve cells is usually accompanied by a modification of their protoplasmic network, as shown by methyl blue in sections hardened in alcohol. This network and its modifications were demonstrated by me at the International Congress held in Berlin in 1890.

The modified cells are surrounded by a zone of embryonic cells. Between these cells may be distinguished a dilated lymphatic network and a small quantity of a rigid material, horny in appearance, forming fragments of a delicate meshwork. Often this lesion encloses also a little black pigment between and within the cells.¹

As the embryonic condition of the small vessels, especially of the motor tracts, as well as the other lesions indicated, may be found in many other acute affections of the central nervous system, these lesions, although very constant, do not suffice to furnish us with a diagnostic sign of the disease; whereas the small very pronounced embryonic centres around a single nerve cell of a motor root, or of a very small group of cells probably derived from a proliferation of a single cell, give a very characteristic combination, which I have not noticed in other diseases of the nervous system.

The inflammatory lesion occupies, in certain of these affections, more extensive tracts—the vessels and usually at the same time the medullary substance. The lesion of the motor centres in affections other than rabic is more extensive, more generalised, and rarely so acute; in a word, there is not found the localisation seen in the lesions of rabies.

To assure myself of the specific nature of the lesions found in rabies, I have examined a number of acute nervous diseases complicating the motor tracts, both in dog and man. In the dog some cases of distemper show nervous lesions more or less simulating those of rabies. However, we may clearly note the following differences: (1) in this last disease the perivascular lesion (diapedesis, hyaline masses) is much more intense, and the centres especially are more extensive than in rabies; (2) we do not find there a localisation of the inflammatory points around the nerve cells; (3) there is usually noticed a manifest participation of the white substance; (4) the medulla and the grey substance around the cerebro-spinal canal are in this disease less altered, and do not present the small characteristic spots.

In diseases produced experimentally, whether toxic or traumatic,

¹ I need not here enter into the details of other microscopic lesions of rabies, as for instance those of the nerves, glands, and ganglia mentioned by Wagner, Kolesnikoff, Polakillon, Nepveu, Botkin, Klebs, Heschl, etc., and others that I have described in part in my "Researches on Rabies" published in the *Annales de l'Institut de pathologie et de bactériologie*, Bucharest 1888-89, t. 1, vol. 2.

notably those described by Popoff (*Beitr. zur Kenntn. d. acuten tox. Myelitis* 1882) and my own (*Experim. Beitr. etc., Arch. für Dermat.*, 1878), there is observed a lesion belonging rather to the parenchyma, with hyperæmia, exudation of hyaline masses, degeneration of cells and especially of nerve fibres, whilst the inflammatory lesions of the vessels are less pronounced; and in these cases I have not seen groups of embryonic cells localised around the nerve cells of the motor roots.

If we kill a dog by asphyxia, we may sometimes observe, along with the congestion, the presence of some lymphatic cells around certain of the ganglion cells; but the lymphatic cells are less numerous, the ganglion corpuscles are intact, and the lesion does not present the topography indicated.

In man, it is especially bulbar paralysis, progressive muscular atrophy, and infantile paralysis that may produce somewhat similar lesions to those of rabies. But, in bulbar paralysis the medulla is generally intact; the lesion commences in all these diseases by a proliferation of the neuroglia, which induces an atrophy of cell groups, and I have been unable to find in my preparations obtained from subjects affected with this disease the zone of migratory embryonic cells which is so characteristic of rabies. Again, the nodules found in rabies, always acute, and less confined to the anterior horns, cannot be confounded with the sclerotic and atrophic lesions of muscular atrophy.

Acute myelitis of man is, as is well known, either systematic, insular or diffuse, transverse, ascending or descending. It shows, even in acute cases, parenchymatous lesions, and especially œdema, proliferation of the neuroglia, and the appearance of cells showing fat granules, whilst the vessels are usually less changed. Further, its lesions are rarely limited to the grey and motor portions of the central nervous system. The lesions of this disease, so marked and evident to the naked eye, can never be confounded with the histological lesions of rabies.

Although acute myelitis shows from the commencement a well pronounced degeneration, with the appearance of globules containing fat granules in great numbers around the vessels of the parenchyma, with lesions well marked and limited to the parts which one recognises generally with the naked eye as those most readily attacked by the disease, there are nevertheless cases of acute myelitis where only microscopic nodular lesions are found. But in these cases also, we do not describe the points of inflammation as limited to the motor tracts of the grey matter, and especially those which are found in rabies around certain nerve cells.

It was, above all, in a case of tetanus in man and in another of puerperal eclampsia (nephritic), that I found lesions of the central nervous system which most resembled the lesions of rabies. The cellular aggregations, however, although often miliary, exist especially around the small vessels; the regions are less limited, and even the small nodules seen in the case of eclampsia, and whose connection with the small vessels is not evident, are not situated around the nerve cells.

Whilst admitting that the lesions of rabies are not absolutely characteristic, and that it may be that in a case of diffuse very acute

myelitis similar lesions may be found, it is necessary all the same to state, that neither in writings, nor in my own personal experience, have I ever met with a similar case, so that at present we may consider the lesions of rabies as characteristic. In other infectious diseases there have also been found histological lesions characteristic as a whole, although composed of elements not absolutely specific.

Thus the lesions of tuberculosis, before the discovery of the bacillus which causes it, were recognised and regarded as specific, and yet this disease does not offer any element that is absolutely specific.

Perhaps also in rabies, in which the brain and cord are the seats of the virus, the virulence is connected with the elements which form the peculiar nodules; it is less probable that these nodules are the expression of a remote toxic action, as for example, are the nervous lesions in tetanus, in which disease the cord is not virulent. Further we are convinced that sterilised spinal cord from a rabid animal will produce neither rabies nor the nodules characteristic of this disease.

In fact, when comparing the rabic cord with that from animals dead from tetanus, or from an intoxication, we often see, in these last cases, the same hyperæmia and the same cellular infiltration and hyaline masses in the vessel walls: but one looks in vain for a miliary localisation of these lesions in the parts indicated, and one never finds the pericellular inflammatory islands of rabies. As a result of these comparative examinations, as well as from the authenticated reports of investigators upon the lesions of rabies, it follows that in this disease we have to do with miliary centres, especially in the grey substance, and by preference in the motor tracts, which by their form and their situation present peculiarities, amongst which the embryonic nuclei around certain modified nerve cells are, in my opinion, the most characteristic.

To satisfy myself as to the diagnostic value of the totality of the rabic lesions, and especially of the small pericellular centres, I examined for several months the specimens—spinal cord and brain, which were sent to me along with the bitten persons. The following is the method I employed: sections of cord or of brain, 5-10 mm. square, 3 mm. thick, are hardened in absolute alcohol, whilst a portion of the cord is inoculated into a guinea-pig or rabbit by means of the trephine. The next day thin sections may easily be made, and these are stained either by methyl-blue or Löffler's fuchsin. In a few minutes the sections are coloured: wash them in distilled water, and after dehydrating, clarify in oil of cloves and mount in Canada balsam. By this method, the medullary substance is not well preserved, but, as my researches have demonstrated, the white substance is little altered in rabies, and, moreover, these alterations are only slightly characteristic, so one may ignore them in this examination. The grey substance and the products of proliferation and of inflammation, on the other hand, are very well preserved and coloured by this rapid method. One may readily make out, by the aid of slight magnification, the groups of motor cells of the regions involved: among these cells are some which generally are pale, vacuolated, with ruptured poles, a modified nucleus (in the state of mitosis sometimes), invaded by small round uninucleated cells and forming the centre of a small nodule, itself composed of uninucleated migratory elements, which we have always noticed. There may also be seen at the same time pro-

liferated vessels, often dilated, containing many leucocytes, surrounded by dilated lymphatic spaces and filled with embryonic elements, sometimes pigmented.

The lesion is especially marked by the presence of these pericellular nodules.

As examples of our procedure, the following are several cases in which this examination gives us useful results.

I. Petrache Grigoriu, aged 37 years, of Băleni (Covurliu) was bitten on the 15th October by his dog, on his return home after an absence of 3 days. The same day the animal was destroyed, and the medulla and cerebrum preserved in glycerine were forwarded to the Institute. The autopsy was neglected.

The specimens were received on the 17th; they were hardened in alcohol, after being fixed in Flemming's solution, and on the 20th October sections were made; they were stained by methyl-blue, and we noticed abundant rabic nodules around the nerve cells of the medulla, especially in the nuclei of the pneumogastric and hypoglossal nerves.

The rabbit inoculated died of rabies.

II. A peasant, Gavril Prepelita, aged 40 years, of Ciumulesti (Suceava), was bitten on 22nd October by a strange dog, suspected of rabies, of which no history was forthcoming.

We received the brain of the dog preserved in glycerine on 3rd November; it was hardened in absolute alcohol, and on the 5th we made sections, in which the microscope showed numerous rabic nodules in the ependyma of the lateral ventricle.

III. M. Anton Sichni, 43 years of age, of Roman, was bitten on 31st October by a suspicious dog, which was killed.

In the sections which we obtained from the specimens sent, after hardening in alcohol, we observed the characteristic rabic nodules in great numbers, especially around the nerve cells of the walls of the lateral ventricle beneath the ependyma.

A rabbit inoculated at the same time died rabid on 24th of November.

IV. A dog showing suspicious symptoms died of rabies in the Institute. The anatomical lesions of rabies were noticed. We hardened the brain medulla and spinal cord of the animal in absolute alcohol, and two days afterwards one could see in the sections stained by fuchsin, numerous nodules around the motor cells in the nuclei of the floor of the 4th ventricle, especially that of the hypoglossal nerve, and in the anterior horns of the cervical portion of the spinal cord.

A rabbit inoculated with this cord died rabid 18 days after trephining.

V. On the same day a suspected dog was killed, the bulb and spinal cord were hardened in alcohol, and two days afterwards, sections were made in which we could not find the pericellular nodules.

The rabbit inoculated with the cord of this dog remained healthy.

VI. Maria Jonica Feraru, of Slatina, a child of eight years was bitten on 7th November by a dog showing the symptoms of rabies.

On 12th November we received several pieces of the medulla and cerebellum preserved in glycerine. After hardening in absolute alcohol we made sections in which we found, in the nuclei which are present in the 4th ventricle, a considerable dilatation of the vessels, with hæmorrhages, as well as several small rabic nodules.

The inoculated rabbit died of rabies.

VII. Petru J. Stefanescu, artisan of Turmi-Severin, was bitten on Nov. 18th by a suspicious dog, which was killed the same day.

On the 25th we received the brain of the animal; in the sections which

were obtained from it after hardening in absolute alcohol, we noted the presence of several small rabic nodules around the cells of the posterior pillar of the fornix.

The rabbit inoculated the same day died of rabies.

VIII. Jon Draghicescu, aged 21 years, soldier from Ramnicu-Valcei, was bitten on 16th Dec. by a suspected dog, which was killed.

We received on the 10th the medulla of the animal, and in the sections obtained by the method before mentioned, we noticed dilated vessels and hæmorrhages at the base of the 4th ventricle. Certain nerve cells are surrounded by embryonic elements, especially along the raphe. These cells are also pale and some without nuclei.

In the case under notice the lesion is more diffuse.

We thus see that if we possess the brain and especially the medulla or spinal cord of the suspected animal, preserved in glycerine, the histological examination of these specimens would complete the result of the observation and autopsy of the dog, as well as facilitate the rapid diagnosis of rabies.

In these cases sections including the ventricles ought to be made, in the medulla or the cervical part of the spinal cord. In a case of rabies, after hardening the specimens for two days, we should find there, besides hyperæmia and embryonic perivascular centres, less characteristic small nodules formed by a zone of embryonic cells surrounding a nerve cell degenerated or proliferated.

I am nevertheless very far from regarding this verification as an absolute proof, or even from wishing to suppress, by the introduction of this rapid process, the other methods of diagnosis, among which there are some, notably the symptoms and experimenting on animals, which give absolute proof. But there are some cases, as those which I have given at the beginning of this communication, where I believe the verification of the nodules described would be of real value.

From a practical point of view we may then conclude that *along with observation and autopsy of the dog that has inflicted the bite, histological examination of the central nervous system ought not to be neglected.*

We must only give up this examination if the brain of the animal that has inflicted the bite is entirely macerated, or so injured that one can no longer be certain what one is working with.

If we do not find on minute examination of the parts mentioned any perivascular or pericellular nodule, we may conjecture with great probability that the biting animal was not mad; on the contrary, the presence of the above-mentioned nodules and, especially, the pericellular nodules support the opinion that the animal was rabid.

These nodules are less pronounced in animals killed in the first period of madness than in those which have succumbed to the disease; in prolonged cases more especially, the cellular infiltration is very marked and may become very diffuse.