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HISTOPATHOLOGIC STUDY ON TWO CASES OF "CENTRAL NEURITIS"; DEMONSTRATION OF A NEW GRANULE ("NUCLEOPROTEID-LIKE GRANULE") IN THE NEUROGLIA CELLS *

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INTRODUCTION

Since Dr. Adolf Meyer¹ described, in 1901, the symptom-complex "central neuritis," which he introduced as a short designation of the "parenchymatous systemic degeneration mainly in the central nervous system," a number of cases have been added to the literature by different authors, and there is no doubt that this condition can be recognized from its clinical symptoms and established anatomically. According to the descriptions of authors, this symptom-complex is found to occur in depressive psychoses at the time of involution, in alcoholic senile states and in alcoholic phthisical-cachectic states, and the clinical condition is characterized by diarrhea, emaciation, twitchings and rigidity of the extremities, some fever and some changes in the reflexes, and the mental condition is an anxious, perplexed agitation, delirium or stupor. Anatomic investigation revealed "changes somewhat similar to those found in the motor cells of the cord and the medulla, from which the nerve process has been cut off at the periphery" in the Betz cells and other cells, and systemic degenerations of the central nerve fibers. Beside the accurate description of Dr. Meyer, there are a number of excellent histologic studies on changes of the nerve cells; however, we find relatively few investigations on alteration of the neurofibrils of nerve cell as well as the neuroglia tissue. The material of the Phipps Clinic recently offered the opportunity to study two cases of "central neuritis." The alteration of the intracellular neurofibrils of nerve cells as the effect of cutting off the nerve process has been a topic of several experimental studies. In view of the clear relationship of the changes of the nerve cells in

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1. Meyer, Adolf: On the Parenchymatous Systemic Degeneration Mainly in the Central Nervous System, Brain, Lond. **24**: 1901.

"central neuritis" and the axonal alteration resulting from section of fiber, an investigation of the appearance of the neurofibrils may be of value, as "central neuritis" is, so to speak, an experiment of nature, although the nerve process is not really cut off. Thorough investigation of the changes of the neuroglia tissue led me to demonstration of a new granule in neuroglia cells, a finding which may throw a new light on the problems of the metabolism and catabolism process of the central nervous tissue.

Before taking up the description of my investigation, I beg to express my highest appreciation of the kindness of Dr. Adolf Meyer, who gave me the privilege of studying these cases and many other specimens of our laboratory, and also gave me many useful suggestions in this study.

ILLUSTRATIVE CASES

CASE 1.—Clinical History.—E. B., a housewife, aged 29, in January, 1918, presented the skin lesion of pellagra, constipation, epigastric distress, and restlessness. Admitted to the medical service May 25, she showed marked emaciation and peculiar very dark pigmentations over the entire body. She became delirious May 29, and was transferred to our clinic. She developed a stupor and a typical picture of "central neuritis," with tenseness, rigidity, tetanoid positions and jactitations, and a terminal temperature of 107 F.

Family History.—A sister has nervous headaches, the daughter has tantrums and night terrors; an aunt broke down after the death of her husband.

Personal History.—The patient had the childhood diseases, typhoid fever at 16 and rheumatic fever at 20. Her development was abnormal; she married at 19 against her parents' wishes. Her married life was uneventful except for a miscarriage at 20 and an abortion at 28. The patient's habits have been regular, but the diet consisted mostly of vegetables. She has two children.

Present Illness.—In November, 1916, she began to become irritable. A spontaneous abortion in March, 1917, with hemorrhage for three months increased this condition and weakened her. She recovered somewhat during the summer, but in September, 1917, restlessness, night sweats, and her old symptoms returned. By December, 1917, she was worse, easily upset and much worried when her younger child developed scarlet fever. In January, 1918, epigastric distress, giddiness, flashes of light, and roughening of the hands appeared, and grew gradually worse. She took to bed on March 13; two days later her temperature was 101 F. and she had difficulty in swallowing. The last two symptoms left her March 16, and on light diet and rest she gradually improved till May 8, being out of bed she showed thirst, tachycardia, sleeplessness and a return of the other symptoms. On May 25 she was admitted to the medical service of the Johns Hopkins Hospital, with her hands in the condition described. Her mouth and tongue were sore; she was emaciated, very weak, slightly anemic, and her skin was dry and darkly pigmented. She was mentally clear until May 29, when she had an episode of screaming and confusion. On May 30 she was at times disoriented, frightened and was incontinent. On May 31 she was completely disoriented, delirious and incontinent. Her temperature was 101.5 F., and the pulse 140.

Mental Status.—The patient lay in bed twisting her hands and face, picking at the bed clothes and moving her jaw frequently. She mutters unintelligibly. Her arms and legs are rigid in flexion. She is usually unresponsive. She is completely disoriented and in the medical service had imagined she was giving birth to a baby. Incontinence.

Physical Status.—The patient is extremely emaciated; her skin harsh and dry and diffusely pigmented. On the backs of her hands and front of wrists and on the knuckles there is a dry, cracked, raised, very dark thickening of the skin symmetrically distributed. She is pale, her tongue red with swollen papillae; pyorrhea and dental caries. Tenseness and rigidity of musculature, and limbs in flexion, very hyperactive deep reflexes.

Progress in Clinic.—After admission in our clinic the patient grew continually worse; she muttered to herself, became more rigid and more restless, slept none and appeared to have slight spasms with retraction of the head. Later she was unable to swallow. Her temperature rose to 107 F., on June 2. Her pulse rose to 180 and Biot's breathing appeared. She died June 3.

Necropsy.—No macroscopical lesion in the brain and in the spinal cord; the microscopic study revealed "central neuritis."

CASE 2.—Clinical History.—A. S., widow, aged 48. Dating from September, 1917, the patient was nervous and weak. Dilatation and curettement operation followed by delirious state. On admission (December, 1917) the patient showed amebic dysentery with suspicion, hallucinations of hearing, disorientation, delusions and fear. This mental state kept pace with her physical dilapidation, which rapidly led to exitus.

Family History.—Unimportant.

Personal History.—Negative. The patient felt "run down" and weak for the past year, without, however, any failure in business activities. In November, 1917, the patient appeared very weak; kept in bed for two weeks and on Nov. 17, 1917, a D. and C. operation was done with prolonged response to anesthetic. Following the operation the patient was agitated and suspicious of food. When she tried to jump out of the window she was transferred to a sanitarium. The patient developed diarrhea, six to seven stools a day, watery and bloody; she was very weak and during the last three days prior to admission she was tube-fed.

On admission the patient seemed confused and disoriented and profoundly exhausted. For a time she was restless, showing sudden paroxysms as though in pain. The knees were flexed and the abdominal muscles felt contracted. During the attacks she rolled from side to side in the bed; but between the attacks she seemed relaxed and quiet.

Physical Status.—The patient is a profoundly undernourished, emaciated anemic-looking woman. The neurologic examination showed definite exaggeration of deep reflexes. The abdomen is boat-shaped; the patient holds the muscles tense. Stool examination revealed the existence of *Ameba histolytica*. The patient began to run a temperature after three days in the hospital, ranging between 99 and 101 F.

Mental Status.—The patient thought her food was poisoned and she refused food, so that tube feeding was at times required. She also sometimes talked vaguely of being married to a Dr. Adams. She also had auditory hallucinations. The patient was usually oriented to time and place, but had difficulty in getting names straight. Retention and memory for remote past were good. She showed no definite affective disturbances.

Progress in the Clinic.—The diarrhea continued throughout the course in the hospital with from one to seventeen stools a day. The temperature ranged between 99 and 101 F. The above mentioned mental state continued till the end of January, 1918, interrupted now and then by remissions, in which she was fairly well oriented and showed no delusions. On January 31, marked twitchings of facial muscles and tremor of the lips were noticed.

From the beginning of February, 1918, the patient's condition appeared worse; the weakness increased and nausea and vomiting came on after meals. Defecation and micturition often occurred involuntarily. On February 13 the temperature rose from 101 to 104, and the pulse to 140. The patient became semicomatose and died.

Necropsy.—The brain showed superficially normal findings; microscopic study established typical "central neuritis."

MICROSCOPIC FINDINGS

(A) *Manifestations of the Nerve Cells.*—In both cases of "central neuritis" the paracentral lobule and the anterior central gyrus were subjected to a most extensive study. The spinal cord was examined only in the first case. To avoid repetition I shall describe below the findings of both cases together, and besides mention only points of difference between two cases.

We shall begin with the description of the findings in thionin preparations. So far as the cytoarchitecture of the cortex is concerned, both cases showed nothing abnormal. Almost all the Betz cells are in both cases in a more or less advanced stage of axonal reaction. The cell body is more or less swollen, and shows in a certain place an area of an almost homogeneous character due to a decay of the stainable substance into dust-like particles. In the extremely altered cell, the latter looks like a more or less deeply blue stained balloon. In the second case we find fairly well preserved stainable portions in the dendrites and also lumps of the Nissl bodies at the periphery of some cells, forming a rim around the margin. The "whirlpool" arrangement of the Nissl bodies, described by Dr. Meyer,^{1, 2} did not come to our observation in this case. In the first case the superimposed fever alteration complicated the characteristic features; even at the periphery of the cells and in the dendrites the Nissl bodies have disappeared and the protoplasm is stained diffusely blue. The nucleus is found more or less dislocated in every cell usually to the side or toward the neighborhood of apical dendrite, causing sometimes a projection of the cell surface. The nucleus itself is sometimes round, sometimes it looks elongated or distorted and flattened, as if pressed to the wall by the swollen portion of the cell. The nuclear membrane is usually sharply marked, the content is pale,

2. Meyer, Adolf: Demonstrations of Various Types of Changes in the Giant Cell of the Paracentral Lobule, *Am. J. Insan.* 54: 1897.

the nucleolus is round and generally deeply stained. Along the margin of the swollen cell or sometimes accumulated in one point of the cell there appears a heap of yellowish, greenish pigment. Figure 1 was taken from a Betz cell of the second case.

Fig. 1.—A Betz cell from the second case.*

In our first case some of the pyramidal cells of outer layers also show axonal reaction; however, most of them give an entirely different picture. They are mostly swollen; the dendrites are also enlarged. The Nissl bodies had largely disappeared from the cell body; no dislocation of the nucleus was noticed. The whole picture corresponds to that which Goldscheider and Flatau described as the effect of arti-

*Figures 1 to 6 are photomicrographs and Figures 7 to 9 were drawn with the use of the Abbe camera lucida with slight rotation of the micrometer screw.

Figures 1, 5, 6, 8 and 9 are thionin preparations.

ficial increase of body temperature. We also found some shrinkage cells (so designated by Hoch³) in the second and third layers.

In the second case the medium-sized and small pyramids also show a close resemblance to Hoch's shrinkage cells; the "alteration with rarefication around the nucleus" of Hoch was also found in some of the larger pyramids. The thionin preparations of alcohol material show practically the same picture as the above described preparation of formalin material.

A portion of the motor cells of the spinal cord and some of the cells of the Clark column show in our first case typical axonal reaction, although in less advanced stages, and some of them show, moreover, the fever alteration. Some other cells show merely fever alteration.

The manifestations of the intracellular neurofibrils of the nerve cells were studied in Bielschowsky preparations. Cotton and Southard⁴ found in their Cajal and Bielschowsky preparations a definite change in the fibrils within the chromatolyzed area. Lambert⁵ observed a disappearance of the fibrils exactly corresponding to the area of swelling in the Nissl equivalent. Figure 2 shows the alteration of the fibrils and the nerve cell; with the lower magnification the Betz cells appear as swollen bodies with sharply marked dendrites, and thus reminds us of the bed-bugs. In higher magnification we can follow different stages of the neurofibril alteration in different cells. The fibrils are in more or less advanced stages of fragmentation, and in the extreme state they are dissolved to fine dust-like pieces. In the central part of the cell body, where thionin preparations show an homogeneous area, we find also in the Bielschowsky stain a glossy area; however, at the periphery of the cell body and also in the dendrites, in which thionin stain disclosed well marked Nissl bodies, we find fairly well preserved neurofibrils, sometimes running from one dendrite to another. In the apical dendrite of the Betz cells the neurofibrils are especially clearly demonstrated and run isolated or in bundles toward the cell body. It is a striking feature that these neurofibrils end sharply at the base of the dendrite. These findings are very interesting, as they tend to indicate that the alteration of the neurofibrils keep pace with the dissolution of the Nissl bodies. As a

3. Hoch, August: Nerve Cell Changes in Somatic Diseases, *Am. J. Insan.* **55**: 1889.

4. Cotton, H. A., and Southard, E. E.: A Case of Central Neuritis with Autopsy, *Am. J. Insan.* **65**: 1908.

5. Lambert, C. I.: Notes on Pathological Material, *N. Y. State Hosp. Bull.* **1**: No. 3, 1908.

Fig. 2.—Bielschowsky stain; a Betz cell from the second case. The nucleus is found on the left-hand side of the cell; the pigment is found on the right-hand margin of the cell.

further evidence of this phenomenon may serve the Bielschowsky preparation of our first case; here we do not find any neurofibrils in most of the Betz cells even at the periphery of the cell body and the dendrites and thionin stain disclosed, as already remarked, dissolution of the Nissl bodies in the same. This corresponds to the statement of Lambert,⁵ who observed more or less apparent fragmentation and granulation of the fibrils in the fever change. Marinesco⁶ stated that the changes in the neurofibrils and the chromatophil elements as a rule occur and progress together. Dr. Meyer⁷ and Lambert consider the changes in the fibrils almost invariably coincident with the changes of the local protoplasm. The Bielschowsky preparation of the spinal cord in our first case brought out samples of axonal reaction in the anterior horn and the Clark column; but owing to the superimposed fever alteration the neurofibrils are marked only in a few cells at the cell periphery and in the dendrites.

(B) *Manifestations of the Nerve Fibers and the Axis-Cylinders.*—The Marchi preparations showed definite myelin-sheath degeneration in the marrow of the paracentral lobules and the anterior central convolutions; however, the degenerated myelin-sheaths are so scanty that only a few appear in one Marchi section in both of our cases (Fig. 3). The Marchi cross-section of the spinal cord of our first case shows a number of degenerated fibers in the anterior and lateral pyramidal (Fig. 4) bundles as well as the cerebellar afferent bundles; but the degeneration in the pyramidal bundles is much slighter than the findings in the paracentral lobule and the anterior central convolution would make one expect; Dr. Meyer¹ and others also noticed this incongruity.

The Alzheimer-Mann preparations of the anterior central convolutions disclosed a very interesting finding in both of our cases (Fig. 7). In this preparation normal axis-cylinders are brought out in a deeply blue stain; but among those we find axis-cylinders with red-stained parts of different length in their course. Some fibers show colorless parts instead of the red-stained part. Between the red-stained part and blue-stained part of the fiber we find sometimes a very short colorless part, and sometimes we observe that the red-stained part is interrupted by colorless parts in many segments. Some axis-cylinders have in their course more than one red-stained or colorless part, so that they look like strings of changing color. It admits no doubt

6. Marinesco: Lesions of the Neurofibrils in Certain Pathological Conditions, *Rev. Neurol and Psychiat.*, 1905.

7. Meyer, Adolf: Discussion of the Data Concerning Neurofibrils, with Illustrations, *Proceedings New York Neurol Soc.*, Feb. 6, 1906. *J. Nerv. & Ment. Dis.* **33**: 1906.

Fig. 3.—Marrow of the anterior central convolution (first case). Marchi's stain.

Fig. 4.—Cross-section of the lateral pyramidal bundle (first case). Marchi's stain.

that these red-stained and colorless segments of the fibers are degenerated, and that the red-stained parts are in a more advanced state of alteration than the colorless parts. Now it is worthy of note, that these degenerated axis-cylinders were found in the sections much more numerous than the degenerated myelin-sheaths demonstrated by the Marchi method. The Alzheimer-Mann stain of the longitudinal sections of the cord in our first case revealed many such degenerating axis-cylinders in the lateral pyramidal bundles and in the cerebellar afferent bundles.

On the basis of the fact, that in acute cases the cell change far outweighs the fiber degeneration, Cole⁸ claimed that the former are almost certainly primary, and the latter appear to be simply a secondary manifestation of the former. But considering the possibility that the degeneration of the myelin sheaths may be demonstrable by the Marchi method in different parts of their course in different fibers, as Turner⁹ suggested, and that the axis-cylinders are altered, even when the Marchi degeneration is absent, I am of the same opinion as Turner, that the degeneration of the fibers is sufficient to account for the state of the Betz cells.

The longitudinal section of the spinal cord, stained with thionin showed another interesting picture in some of the fibers of the lateral pyramidal bundles and the cerebellar afferent bundles. As Figure 6 indicates, those fibers show in their course variously long stretches of a deeply blue stain, inserted between colorless parts of the fibers. These blue-stained segments of the fibers hold their stain even if the differentiation by alcohol is pushed further, and to my mind they correspond to the red-stained segments of the fibers in the Alzheimer-Mann preparation described above, and are to be interpreted as degenerated portions of the fibers.

(C) *Manifestations of the Neuroglia Tissue.*—In cases of “central neuritis” an increase of satellite cells as well as of free nuclei was described by Dr. Meyer,¹ Lambert,^{5, 10} Sims¹¹ and others. Our first case showed only moderate increase of satellite cells around the Betz cells and some of the pyramidal cells, but the second case did not give the picture of satellitosis. Lambert¹⁰ observed nests of neuroglia

8. Cole, S. J.: On Changes in the Central Nervous System in the Neuritic Disorders of Chronic Alcoholism, Brain, Lond. **25**: 1902.

9. Turner, J.: An Account of the Nerve Cells in Thirty-Three Cases of Insanity, Brain, Lond. **26**: 1903.

10. Lambert, C. I.: Demonstrations of Pathological Anatomical Cases, N. Y. State Hosp. Bull. **2**: No. 3, 1909.

11. Sims, R.: Anatomical Findings in Two Cases of Korsakow's Symptom-Complex, J. Nerv. & Ment. Dis. **32**: 1905.

Fig. 5.—Longitudinal section of the cerebellar afferent bundle of the spinal cord (first case).

cells, in which from twenty-five to fifty were closely crowded together, and he said that they multiply by direct division. Similar nests of from three to fifteen neuroglia cells were found in both of our cases (Fig. 8a). As Lambert¹⁰ suggested, they rarely show mitosis, and I also think they divide in the way of amitosis, partly at least.

Fig. 6.—A Betz cell after pancreatin digestion.

Special attention was paid to the so-called catabolism process and the catabolism products. In both cases we observed the production of a number of ameboid glia cells, carrying different kinds of granules, which will be described later. Young ameboid glia cells show relatively small amounts of protoplasm, while larger and older ones have

much protoplasm and also protoplasm processes. Keeping pace with the appearance of these ameboid glia cells we find regressive changes of the neuroglia nuclei; these are: homogeneous stain of the nuclei, dilapidation of the nuclei into small spherules and pale stain with evidence of swelling. It is worthy of special note that in our second case we found a large number of neuroglia cells, the nuclei of which are quite small and round and deeply and diffusely blue stained with thionin, and containing a large deeply blue-stained nucleolus (Fig. 8 *a, b*). These nuclei are mostly surrounded by a certain amount of greenish, yellowish pigment, which evidently belongs to the cell body of the neuroglia cells. I think that these nuclei are also in a state of regressive alterations.

Prior to the description of ameboid glia cells, I should like to call attention to an interesting appearance of the neuroglia cells in the marrow of the paracentral lobule. As Figure 8 *d* shows, thionin preparation disclosed many spindle-shaped cells, the long axis of which runs parallel with the nerve fibers in the marrow. The nuclei of these cells are oval or oblongate and deeply blue stained, and have a deeply blue stained large nucleolus; the cell body is also diffusely blue stained or sometimes it shows a honey-comb appearance and tapers off at both ends. From each end of this spindle cell there extends a varicose more or less dark blue-stained fiber. The whole picture gives the impression that the glia cell is lying on an altered nerve fiber. This impression is strengthened, when we study the Mallory, Mann and Alzheimer preparations. In all these preparations we find in the marrow of the paracentral lobule many a spindle-shaped cell with a homogeneously stained oblong or oval nucleus containing a large nucleolus. The relation of the cell and the underlying nerve fiber is clearer in these preparations, because the latter is demonstrated clearly. The cell protoplasm of this spindle cell carries different kinds of granules, which will be described later. It will be noted here that in the spinal cord section of our first case the same spindle-shaped glia cells lying on the nerve fiber were observed in the pyramidal bundle and the cerebellar afferent bundle. I think that this finding is identical with the picture shown in Alzheimer's¹² Text Figure 3 A. Ameboid glia cells were found in both of our cases in the cortex as well as in the marrow, often forming a group independently or in the neighborhood of the blood vessels. Also in the spinal cord of the first case I found a large number of ameboid glia cells.

12. Alzheimer, A.: Beiträge zur Kenntnis der pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgängen im Nervengewebe, *Histolog. u. Histopatholog. Arbeiten* 3, 1910.

The ameboid glia cells with large protoplasm show one or multiple vacuoles of different sizes, which may be well explained as fat cysts; the Herxheimer stain revealed red stained, variably large fat drops around the neuroglia nuclei. The Mallory and the Mann preparations showed in a relatively small number of the ameboid glia cells and the above described spindle-shaped glia cells the Alzheimer methyl blue granules. The Alzheimer fuchsin light green preparations, however, demonstrated in a large number of both kinds of the cells the fuchsinophil granules. The Alzheimer light green granules never did come to our observation; nor did the fibrinoid granules come to observation in our Weigert's neuroglia preparations. Besides the methyl blue and fuchsinophil granules the thionin preparations brought to light in a large number of the ameboid glia cells another kind of granule, to which we wish to devote the next chapter.

THE "NUCLEOPROTEID-LIKE GRANULE" IN THE NEUROGLIA CELLS

The granule referred to was demonstrated in the protoplasm of ameboid glia cells in the marrow and also (a little less) in the cortex. Our second case of "central neuritis" showed much more numerous cells with this granule. Besides the ameboid glia cells with large cell body, we found the same granule also in those glia cells, which have a more or less large cell body, but not the characteristic shape of an ameba (preameboid glia cell Rosenthal¹³). The grouping of the granules is quite different due to the different shapes of the cell body; sometimes we find granules all around the nucleus, and sometimes we find them either on one side or on both sides of the nucleus. Here and there we meet groups of granules without showing the neuroglia nuclei, especially often in the neighborhood of blood vessels; they are evidently sections of cell body or protoplasm processes of the glia cells, carrying the granules; according to my observation the latter never lie freely in the tissue or in the perivascular spaces.

The granule is round, or has the shape of an irregular lump; it is rather small and of almost uniform size. It appears in the thionin preparation of both formalin and alcohol fixed materials in a blue stain or in an exquisite metachromatic color, and between the blue stained and the metachromatic basophil stained ones there appear granules with many transition colors (Fig. 8 *a, c*). In the illumination by electric light they are especially brilliantly visible, and even the blue-stained granules glisten with a more or less metachromatic tinge.

13. Rosenthal, S.: Experimentelle Studien über amöboide Umwandlung der Neuroglia, Referat. Ztschr. f. d. gesamt. Neurol. u. Psychiat. Referat. u. Ergebnisse **8**: 1914.

Moreover, the electric illumination disclosed the same granules even in the spindle-shaped glia cells described above, the protoplasm of which appeared in a diffusely blue stain in the daylight. The whole picture of the granules is thus far similar to the granules which I¹⁴ observed in the ameboid glia cells in the synapse of the Mauthner cell in fatigue. As I was not able at that time to study the solubility of that granule, I identified it with the Alzheimer basophil metachromatic granule, which Alzheimer himself identified with Reich's granule on the basis of its morphologic character. The Reich granule is characterized as soluble in warm alcohol at 45 C., ether and in warm xylol. The question now arises whether our granule is identical with the Reich granule, because our specimen showed the granule despite the fact that it had gone through warm xylol in process of embedding. The present cases furnish sufficient material to investigate the microchemical nature of our granule. The fact that we find the granule in the embedded specimens speaks from the first rather against the probability of its being a fat or lipoid substance. Paraffin sections from 2 to 10 μ thick were placed in alcohol (80 per cent., 95 per cent., and absolute), ether, equal mixture of ether and absolute alcohol, chloroform xylol and turpentine oil, and kept in a water bath at 70 C. for from one to three hours, and then were stained with thionin in the same way as the control sections from the same block. Microscopic examination showed no definite decrease in the amount of granules.

Acetic acid (diluted as well as concentrated), concentrated hydrochloric acid, nitric acid and sulfuric acid were applied to sections for two to three hours; however, they did not affect the characteristic stainability of our granule. On the other hand, our granule is quite easily soluble in diluted as well as concentrated alkaline solution; 0.5 per cent. aqueous solution of sodium hydrate dissolves the granule in a short time.

A very interesting picture for the microchemical characterization of our granule was given by the digestion experiment. Paraffin sections from 2 to 6 μ thick were digested by pepsin hydrochloric acid solution at 37 C. for from twelve to eighteen hours. The ground substance of the protoplasm of ameboid glia cells was dissolved, whereas our granule remained undigested around the nuclei, and much more distinctly visible after the digestion. Moreover, it is interesting to note that the granule showed the metachromatic color much more beautifully after the digestion, and it is noteworthy that the granules,

14. Marui, K.: The Effect of Overactivity on the Morphological Structure of the Synapse, *J. Comp. Neurol.* **31**: 1919.

which appeared in a more or less blue tinge before the digestion, showed an exquisite metachromatic color after the experiment.

Extremely interesting is the fact that in the experiments thus far described our granules behave microhistochemically in quite the same manner as the Nissl bodies of the nerve cells; as Held¹⁵ described in 1895, the Nissl substance was dissolved only in alkaline solution as in our experiments, but not in different kinds of acid, in fat-solving mediums, and by pepsin. The only difference consists in the fact that our granules are demonstrated in a more or less brilliant metachromatic color, whereas the Nissl bodies appear in a more blue violet color.

Now, as Held¹⁵ observed, the Nissl bodies show an exquisitely granular structure, if we look at very thin sections (2μ thick). The granules are round and do not look much larger than our granules in the neuroglia cells, and show a more or less metachromatic tinge, especially in electric illumination. After pepsin digestion they appear in a very characteristic metachromatic color in the thionin stain, just as some of our granules in the neuroglia cells. I cannot give a definite explanation to this interesting phenomenon; the fact does, however, indicate further evidence that both granules resemble each other. For the further characterization of our granules I undertook pancreatin digestion of sections. Paraffin sections, from 2 to 6μ thick, were placed in a neutral pancreatin solution for several hours at 38 C., and then they were stained in the ordinary way. Contrary to the result of pepsin digestion the granules of the neuroglia cells in the cortex as well as in the marrow disappeared entirely. The cortex gave a peculiar picture after this procedure; as Figure 6 shows, the Nissl bodies are entirely gone, and we can locate the nerve cells only by the existence of the yellowish pigment and also by the appearance of the nuclei. After five hours' digestion the nuclei of the nerve cells and the glia cells were stained rather distinctly and the nucleoli of the nerve cells were stained pale. Hence, in this point too, our granule reacts microchemically in the same way as the Nissl substance. The microhistochemical reaction of phosphoric acid was done following the direction of Lilienfeld and Monti;¹⁶ our granule and the Nissl substance showed a slightly positive reaction, as they appeared in a brownish, yellowish color.

Judging from all the results thus far described, I came to the conclusion that our granule in the neuroglia cells has chemically a close relation to the Nissl substance. Since the thorough microchemical

15. Held, H.: Beiträge zur Struktur der Nervenzellen und ihrer Fortsätze. Arch. f. Anat. u. Physiol. Anat., Abt., 1895.

16. Lilienfeld and Monti: Ueber die mikrochemische Lokalisation des phosphors in den Geweben, Ztschr. f. physiol. Chem. **17**: 1893.

Fig. 7.—Marrow of the anterior central convolution (second case); Alzheimer-Mann's stain.

Fig. 8.—Different manifestations of neuroglia cells in “central neuritis” cases.

Fig. 9.—Granule cells and blood vessels in a small fresh hemorrhage.

study of Held on the Nissl substance there is no doubt that the latter belongs to the category of nucleoproteid; so I would like to designate our granule as "nucleoproteid-like granule" in the neuroglia cell. There is no doubt then that our granule is chemically quite different from the Reich π -granule; I therefore made a mistake when I identified the granule in the ameboid glia cells found in the synapse of the Mauthner cell in fatigue with the Alzheimer basophil metachromatic granule (Reich's π -granule). In what relation our granule stands with the so-called "albumin-like granule," which was found in the granule cells in the brains of new-borns, and on which there was a controversy between Virchow^{17, 18} and Jastrowitz,¹⁹ I do not know. As far as I can see, the only fact known about that granule is that it is soluble in alkaline solution; a further study is required to decide this question. It is also doubtful now whether the Alzheimer basophil metachromatic granule is really identical with the Reich granule. He described his granule as stained in a metachromatic color, and as especially visible in electric illumination; but these properties are not specific for the Alzheimer granule nor for the Reich granule, as our granule has the same qualities. Alzheimer admitted, moreover, that the morphology of his granule is somewhat different from that of the Reich granule, and also that the solubility condition appeared to him a little different. He stated that his granule decreased distinctly by putting the section in alcohol. So this question also must be reserved for further investigation. At least, we can state that a metachromatic stain and electric illumination do not by any means suffice for the characterization of his granule.

Under the heading of "simple basophil catabolism substances," Alzheimer¹² described several basophil substances, which show no, or very little, tendency to metachromatic stain. His description is somewhat vague. He characterized one of them through the fact that (1) it is demonstrated only in alcohol sections, not in formalin material; (2) it is found in nerve cells, in neuroglia cells and also in groups in the perivascular spaces, and (3) it is demonstrated much more in the cortex than in the marrow. This granule is quite different from our granule in many respects. Another kind of basophil products of Alzheimer (Table 33, Fig. 3) is found, according to him, in the neuroglia cells, especially in the marrow, and demonstrated in the shape of lumps. To my mind, this granule resembles somewhat our

17. Virchow, R.: *Encephalitis Congenita*, Berl. klin. Wchnschr., No. 46, 1883.

18. Virchow, R.: *Verhandlungen d. Berlin med. Gesellschaft. Sitzung. v. 14 Oktober, 1883*, Berl. klin. Wchnschr., No. 46, 1883.

19. Jastrowitz: *Diskussion zu den Vorträgen von Jacusiel u. Virchow in den Sitzungen der medizinischen Gesellschaft*, Berl. klin. Wchnschr., No. 46, 1883.

granule, but I am far from being able to say that they are both the same thing. As already remarked, sometimes our granule is demonstrated in a basophil stain, showing little tendency to metachromasia. So I must declare that whenever we speak of the latter kind of the Alzheimer "basophil catabolism product," we have to exclude first in a microchemical way our "nucleoproteid-like" granule. As far as I know, our granule is quite new, at least in so far as its chemical nature is determined.

Then the question comes, whether our granule is connected with any specific morbid condition of the central nervous system and how frequently we meet this kind of granule in the neuroglia cells. Through the kindness of Dr. Meyer, I was given the privilege of studying many specimens in our laboratory; in all, I went over thionin preparations of seventy cases, mostly taken from the paracentral lobule. The cases belonged to both insane and general hospital cases, and the age of the patients ranged from 2 weeks to 68 years. To my surprise, I found the same granule very easily in twenty cases, and with some effort in twenty-five cases and none in the rest. Generally speaking, the granule was demonstrated in typical ameboid glia cells or in glia cells, the protoplasm of which is more or less larger than usual (preameboid glia cells Rosenthal?), in the cortex as well as in the marrow. So far as my observation went, I could not make out any specific cause for the appearance of the granule. As a most interesting finding I emphasize here that in a case of aplastic anemia, I found in and around a small fresh hemorrhage in the corpus callosum a large number of granule cells loaded fully with the "nucleoproteid-like granules." Newly produced granule cells have round or more or less simple shapes, and do not show any basket-like structure of their cell body; the latter is filled with the granules. Those young granule cells, which have a more or less irregular shape, have a close resemblance with ameboid glia cells (Fig. 9, *a*). On the other hand, the granule cells show an exquisite tendency to form basket-like structures, and I observed all the transition forms between the young granule cells and the typical basket-like granule cells; in other words, we found many cells with both basket-like structure and a more or less large protoplasm rest. Extremely interesting was the finding of the nucleoproteid-like granules in this protoplasm rest and in the ribs between the basket meshes (Fig. 9, *a, b, c*). There were also found many phagocytic cells in the hemorrhage, which contained many red blood corpuscles in their cell body. Especially noteworthy was here as well the finding of the same granules between those red blood corpuscles. These findings are very interesting in so far as a new chemically well-defined granules were observed in granule cells. The

question whether these granule cells have something to do with those granule cells in the brains of new-borns is to be decided by further investigation. The cells of blood vessels show in and around this hemorrhage very distinct progressive manifestations. We observed new production of cells of blood vessels, which show a tendency to form mesodermal granule cells and also to form new capillaries. These cells all carry in their protoplasm the same "nucleoproteid-like granules" (Fig. 9 *d*). In two cases of "central neuritis" we observed the granules only here and there in the protoplasm of swollen adventitia cells of blood vessels, around which many ameboid cells with different granules were found crowded. Herxheimer's stain showed variously large fat drops in the ameboid glia cells, as well as in cells of the adventitia. The question, whether the "nucleoproteid-like granule" appears only in certain morbid conditions of the central nervous tissue, or whether it is a normal constituent of neuroglia cells, which is found increased in quantity in morbid conditions, I cannot answer definitely at present. Although I found this granule in brains, which showed no definite histopathologic manifestation, except for the fact that the neuroglia cells had a more or less larger protoplasm body, we can never be sure in our necropsy material that we are dealing with absolutely normal brains. It would be necessary to add here that in two "normal" sheep brains I did not observe any neuroglia cell with this granule.

BIOLOGIC SIGNIFICANCE OF THE "NUCLEOPROTEID-LIKE GRANULE" IN THE NEUROGLIA CELLS

Since the thorough study of Alzheimer^{12, 20} on the pathologic neuroglia, we know that in certain pathologic conditions the neuroglia tissue produces ameboid glia cells, which work as scavengers of catabolism products of the central nervous tissue. Alzheimer¹² described several catabolism products with different morphologic character and staining reaction. His methyl blue granule is produced according to him from the protoplasm of cells, which are bound to rapid decay, and dissolved and transformed into fat substance by mesodermal cells and carried away. Rosenthal,¹³ who wanted to explain the production of the methyl blue granule as a sign of necrobiosis of neuroglia tissue, regarded the appearance of the fuchsinophil granule as that of an increased scavenger activity. So there is no doubt, that in our cases of "central neuritis" ameboid glia cells are

20. Alzheimer, A.: Ueber die Abbauvorgänge im Nervensystem. Referat auf d. VII Jahresversammlung der Gesellschaft Deut. Nervenärzte un Breslau, Sept., 1913, Deutsch. Ztschr. f. Nervenhe. **50**: 1914.

working as scavenger of catabolism products. The "simple basophil catabolism product" was interpreted by Alzheimer as a manifestation of a characteristic dissolution process of cell protoplasm. The so-called basophil metachromatic granule has, according to Alzheimer, something to do with myelin, although he was in doubt whether the granule comes from decay or nutrition disturbance of myelin fibers. But the biologic significance of these granules is to my mind to be considered after their nature becomes clearer by further investigation. What then is the biologic significance of our "nucleoproteid-like granule"? Could it also be attributed to catabolism products in the same sense as that of Alzheimer? This is by no means an easy question to answer definitely. That our granule has microchemically a close resemblance to the Nissl granule, I described conclusively above. Now many investigations indicate a close relation between the Nissl substance and nuclear substance with regard to their histological character. Holmgren,²¹ Sjöval²² and I¹⁴ also expressed the opinion that on the occasion of tigrolysis the Nissl substance is regenerated from the nucleus; according to Scott, the Nissl substance comes out of the nucleus during the development of the nerve cell. On the other hand, it is known that in reparative activity of root ganglion cells (after section of the nerve cell process) and in specific nervous activity (fatigue) we always observe a change of the chromophil substance. Heidenhain²³ said that this substance is accumulated in the rest state, whereas in activity it undergoes specific metamorphosis and produces specific energy. It is then highly improbable that our granule, which is chemically closely related to such an important substance, should be given to ameboid glia cells and other cells as a catabolism product of the nervous tissue. Also the fact that our granule is found in larger quantity in the marrow than in the cortex, speaks rather against this view. The postmortem appearance of ameboid and preameboid glia cells was studied by Buscaino,²⁴ Rosenthal¹³ and Wohlwill,²⁵ but it is hard to conceive a postmortem production of our granule. Then, we must appeal to another possibility — whether our granule is given to neuroglia cells in an afferent

21. Holmgren, E.: Studien in der feineren Anatomie der Nervenzellen, Anatomische Hefte **15**: 1900.

22. Sjövall, E.: Die Nervenzellenveränderungen bei Tetanus und ihre Bedeutung, Jahrbuch. f. Psychiat. u. Neurol. **23**: 1903.

23. Heidenhain, M.: Plasma und Zelle, I., 1911.

24. Buscaino, V. M.: Sulle genesi e Sul significato delle cellule amoeboidei. Referat. Ztschr. f. d. gesamt. Neurol. u. Psychiat. Referate u. Ergebnisse, **8**: 1914.

25. Wohlwill: Ueber amoeboide Glia, Virchow's Arch. **216**: 1914.

direction; in other words, from blood, and is eventually related to a reparative or reconstructive and also nutritive process of the nerve tissue. The finding of the same granule in cells of blood vessels may afford a point of support to this assumption, although we do not know in what chemical form it is given and in what chemical process it is produced in the cells.

Extremely interesting is the finding of the "nucleoproteid-like granule" in granule cells. Merzbacher²⁶ studied the morphology and biology of granule cells, to which he gave the designation "Abräumzellen"; among the granule cells, which are found in embryonic brains, he distinguished two categories: "physiological granule cell" ("embryologische Auffauzellen") and "Abräumzellen" in a narrower sense ("granulare metamorphosierte Gliazellen"). He said in his conclusion: "There is no decided difference between the "Abräumzellen" and "Auffauzellen"; they both have the property to take material and work it out and to pass it on. The distinction between the two comes into consideration when we follow the fate of the material, which was given to the cells. In the one case it is carried away, and at another time it is used for the production of a new substance and structure. The study of Wlassak²⁷ on embryonic material strongly suggests the activity of neuroglia during the evolution of the nerve tissue. De Montet²⁸ regarded the myelin as the expression of import and construction as well as of catabolism process. It is, then, perhaps not going too far to assume that in certain pathologic conditions and nutrition disturbances of nerve tissue other chemical substances besides fat substance may be given to the latter by the neuroglia tissue. Moreover, it is easy to understand that the nerve tissue needs some protein substance besides fat as its constructive and nutritive substance. To my mind our granule belongs to this kind of substance. The further question, whether this substance is given as such or if it is also given, after it is changed into some other substances, I shall leave undecided. According to Wlassak the origin of myelin is to be sought in the blood. While fat production from protein substance is decidedly denied by several physiologic chemists (Schenck, Gürber), others (Kunze) spoke for this possibility up to quite recently. Arnold

26. Merzbacher, L.: Untersuchungen über die Morphologie u. Biologie der Abräumzellen im Zentralnervensystem, *Histolog. u. Histopatholog. Arbeiten* **3**: 1909.

27. Wlassak: Die Herkunft des Myelins, *Arch. f. Entwicklungsmechn. d. Organ.* **4**: 1898.

28. De Montet: Ueber Wanderungen lipoider Substanzen im Centralnervensystem, *Inaugural Dissertation z. Erlangung d. med. Doktorwürde d. hohen med. tak. d. Universität Bern*, 1906.

and Dietrich considered that in certain pathologic conditions fat production from protein substance is possible but not proved. Jakob²⁹ also admitted the possibility of production of lipid catabolism products from axis-cylinders, which go to decay. Our findings also seem to indicate the same possibility, but we cannot consider this is proved. As already remarked, we found in granule cells and ameboid glia cells fat cysts beside the "nucleoproteid-like granule"; but it is always possible that they give one substance on the one hand and take the other on the other hand. In some glia cells we also found yellowish, greenish pigment beside the granule; but I do not know whether this pigment was present before or newly produced, because Obersteiner^{30, 31} showed the existence of this pigment in neuroglia cells of normal brains increasing in quantity with age. Marinesco and Sander assumed the production of pigment of nerve cells from the Nissl substance; Obersteiner regarded it as a residuum of metabolism processes of the nerve cell. In what relation our finding stands with the assumption of these authors I do not know.

So I came to the conclusion that the neuroglia tissue not only has the function of scavenger through production of granule cells and ameboid glia cells in certain pathologic conditions and nutrition disturbances, but also the function of "Auffau" in the sense of Merzbacher.

SUMMARY

The results of a thorough histopathologic study in two cases of "central neuritis" and many other cases can be summarized as follows:

1. Almost all the Betz cells in both cases and some cells of the spinal cord of the first case showed the typical axonal reaction; in the first case the fever alteration was superimposed upon this picture.

2. Fragmentation of the intracellular neurofibrils was found in the glassy area; the alteration of neurofibrils keeps pace with the dissolution of the Nissl bodies.

3. Besides Marchi degeneration of myelin sheaths, a very interesting picture of degenerated axis-cylinders was disclosed.

4. Ameboid glia cells showed the Alzheimer fuchsinophil granule, a finding which indicates an increased scavenger activity of the neuroglia tissue.

29. Jakob, A.: Ueber die feinere Histologie der sekundären Faserdegeneration in der weissen Substanz des Rückenmarks (mit besonderer Berücksichtigung der Abbauvorgänge), *Histolog. u. Histopatholog. Arbeiten* 5, 1912.

30. Obersteiner, H.: Ueber das hellgelbe pigment in den Nervenzellen und das Vorkommen weiterer fettähnlicher Körper im Centralnervensystem, *Arbeiten a. d. Neurol. Institut.* 10: 1903.

31. Obersteiner, H.: Weitere Bemerkungen über die Fettpigmentkörnerchen im Centralnervensystem, *Arbeiten a. d. Neurol. Institut.* 11: 1904.

5. In two cases of central neuritis and many other cases in ameboid and preameboid glia cells on the one hand, and in a case of hemorrhage in granule cells on the other hand, a new "nucleoproteid-like granule" was demonstrated and a conclusion was drawn that neuroglia has a constructive function besides a scavenger function, and that this granule is given the neuroglia cells in an afferent direction.³²

32. In addition to the references already given, the following will be found of interest:

Reich, F.: Ueber eine Granulation in den Nervenzellen, *Arch. f. Anat. u. Physiol., Physiolog. Abt.*, 1903.

Reich, F.: Ueber die feinere Struktur der peripheren Nerven, *Ztschr. f. Psychiät.* **62**: 1905.

Reich, F.: Ueber den zelligen Aufbau der Nervenfasern auf Grund mikrohistiochemischer Untersuchungen, *J. f. Psychol. u. Neurol.* **8**: 1906-1907.

Somers, E. M.: Central Neuritis, *New York State Hosp. Bull.* **1**: No. 3, 1908.