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ON THE INTERNAL STRUCTURE OF THE MOTOR CELLS OF THE ANTERIOR HORNS AND ITS CHANGES IN POLIOMYELITIS

BY

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The neurological problems connected with poliomyelitis are to a considerable extent stamped by neurocytology. By this statement I mean that the study of the internal structure of the nerve cells and of its changes is of pertinent importance to our knowledge of the structural pathogenesis of this disease. Moreover, the changes produced by poliomyelitis imply some very interesting cyto-biological problems, which we are now able to tackle by a considerably more exact staining technique than previously (see *Einarson* 1947). Further, I do not think we ever can obtain a fuller understanding of the effect of the poliomyelitis virus without due attention to the internal structure of the nerve cells and its histochemistry, because here we are undoubtedly dealing with a very intimate interaction between the component parts.

1. ON THE NISSL SUBSTANCE AND THE CHANGES IN BASOPHILIA OF THE NERVE CELLS

An extremely important structural element of nerve cells is the so-called Nissl substance, which appears in the cytoplasm as strongly basophil, coarser or finer fragments or granular particles. Their distribution is very characteristic of the main structural cell types, but within each type it is profoundly influenced by the state of activity of the neuron. All motor cells, both in the spinal cord and in the entire brain

stem, which innervate striated musculature developed from the myotomes and the branchial arches, possess the so-called somatochrome, stichochrome distribution of the Nissl substance, and the anterior horn cells represent the prototype of this arrangement (see *Einarson* 1945). The progressive- selective staining of the Nissl substance and chromatin with gallocyanin-chromalum produces an extremely clear and characteristic cellular picture (the Nissl picture, see fig. 1), which is of fundamental importance in neurocytology, since it furnishes the most delicate and accurate criterion for the estimation of the state of neuronal activity as well as of every deviation from the normal (*Einarson,* 1945; *Einarson & Lorentzen* 1946).

During life the cytoplasm of the anterior horn cells must be regarded as a polyphasic colloidal system of a structure resembling a vesicular gel. Within it the Nissl substance constitutes an internal discontinous phase including several components of various degrees of viscosity; thus it is itself a complex colloidal state. The external continuous phase must also be of a complex nature, since it contains at least two components, one of a somewhat lower viscosity (neuroplasm) and another of a considerably higher viscosity (neurofibrillar substance). The fixatives essentially precipitate the colloidal state of the living nerve cells, and the pattern obtained is a fairly true stiffened or fixed picture of the polyphasic gel-system of the cells, as it was at the moment of fixation *(Einarson* 1935, 1945).

Through a series of studies I arrived at the result that the material from which the Nissl substance is elaborated is formed primarily round the nucleolus inside the nucleus, migrates towards the periphery of the nucleus, and then diffuses gradually through the nuclear membrane to form Nissl bodies in the cytoplasm (*Einarson* 1933, p. 162). Ten years later this has been confirmed by *Hydén* (1943) by the aid of ultraviolet microscopy and absorption measurements.

I have also shown (*Einarson* 1932-1935) that the Nissl substance contains at least three different components, nucleo-

proteins (chromatin substance), acid and basic proteins respectively. The most important result was expressed as follows: "*The natural conclusion is that the Nissl substance contains some nuclein compounds,*" (*Einarson* 1935, p. 117). With this statement nothing was predicted as to the exact nature of the nuclein compounds in question, nor that the nucleins of the Nissl substance necessarily had to be chemically identical with those of the nucleus, in spite of their nuclear origin. My results were later confirmed by *Landström, Caspersson & Wohlfart* (1941) and by *Hydén* (1943), who have shown that the nucleic acid of the Nissl substance is of the ribose type and thus is different from that of the nuclear chromatin; this explains the negative Feulgen reaction of the Nissl substance.

Further, I have shown that the specific staining of nerve cells with gallocyanin-chromalum (*Einarson* 1932) depends on a stable chemical binding of the stain to the Nissl substance, especially to its nucleoprotein (*Einarson* 1934). Continued studies on the theory of staining (*Einarson* 1936, 1945; *Einarson & Bentsen* 1939; *Einarson & Lorentzen* 1946) led to the conclusion (*Einarson* 1947, p. 13) that the pure specific staining of the Nissl substance, the nuclear chromatin and the nucleolus is due to a chemical binding of the gallocyanin-lake-ion+ to the nucleic acids of the structures and most probably to their phosphoric acid groups. When the pH of the staining solution lies on the acid side of the isoelectric point of the structures the binding of the lake-ion+ to the nucleic acids is markedly specific; with a pH lying on the basic side of the isoelectric point, the stain also becomes linked to the proteins of the structures. The form of the nucleic acid does not affect the staining, i.e. the reaction is positive for ribonucleic acid as well as for desoxypentose nucleic acid (*Einarson* 1947). These results have now been confirmed and elaborated in some valuable papers by *Lagerstedt* (1947, 1948).

The consumption and resynthesis as well as the histochemical composition and quantity of the Nissl substance varies greatly with the state of activity of the neuron. Emph-

asis must therefore be laid on the changes in the staining intensity of the cells as evidenced by the occurrence of various degrees of chromophily and chromophoby (*Einarson* 1932-1937; *Einarson & Ringsted* 1938 etc.). It has been definitely established that this variation in the staining intensity depends directly on the inherent capacity to bind the stain, possessed by the living cell at the moment of fixation (see *Einarson* 1945; *Einarson & Lorentzen,* 1946). Since the gallocyanin-lake-ion+ becomes linked selectively to the nucleic acids of the structural elements, the staining intensity gives a quantitative expression of their content of nucleic acid. Thus the degree of basophily of the different structures of the cell varies directly with their content of nucleic acid, and the staining can be used for its quantitative estimation on the basis of the principle of photometric determination, as I have already pointed out several times (see *Einarson* 1947, p. 14).

Many years ago I showed (*Einarson* 1937, p. 12-13) that the stages of chromophily and chromophoby involve a shift in the isoelectric point of the Nissl substance (as to the technique and for further information see *Einarson* 1945 and *Einarson & Lorentzen* 1946). In extremely chromophil cells the isoelectric point lies approximately at pH 1.9, while in extremely chromophobe cells it lies at pH 3.5. The maximum difference in the position of the isoelectric point thus corresponds to an electromotive force of approximately the same order of magnitude as the current of action. In chromoneutral cells the isoelectric point lies approximately at pH 2.7. The various components of the Nissl substance possess different isoelectric points, and the shift mentioned is due to a change in the mutual quantitative ratio between these constituents, mainly between the quantity of polynucleotides on the one side and basic proteins on the other.

As to the functional meaning of the various stages of chromophily and chromophoby I have, through a series of investigations since 1932 (for reference see *Einarson* 1945 and *Einarson & Lorentzen* 1946), arrived at the following main conclusions: The chromoneutral condition represents cells

at rest or in the usual state of gentle activity (*the indifferent phase of activity*); slight chromophily (*hyperchromasia*) characterizes cells when their activity begins to increase (*chromophily of initial activity*); while slight or moderate chromophoby gradually becoming more marked characterizes of cells in increasing activity of longer duration (*chromophoby of prolonged activity*); the intense chromophoby is an expression of cells in fatigue (*chromophoby of fatigue*), and extreme chromophoby characterizes cells in exhaustion (*chromophoby of exhaustion; depression of fatigue*). On the other hand extreme chromophily characterizes either cells in a state of primary, active inhibition of prolonged duration (*chromophily of inhibition*), or cells, whose activity has been lowered or abolished for some time (*chromophily of depressed activity*).

Extreme chromophoby may be produced by prolonged irritation, by experimental fatigue (see *Einarson & Lorentzen* 1946), by experimental acute anoxia (see *Erik Krogh* 1945), as the initial effect of vitamin E deficiency (*Einarson & Ringsted* 1938) and by experimental insulin shock (*Lorentzen,* unpublished work); in human brains and spinal cords it also occurs in various diseases and toxic conditions, and it may reach such a degree as to resemble closely the most intense phase of chromatolysis. It may proceed to total dissolution of the cell.

Extreme chromophily may be produced experimentally by prolonged inhibition (*Einarson* 1933, 1945), by chronic sublethal anoxia (*Morrison* 1946), as the chronic effect of protracted vitamin E dificiency (*Einarson & Ringsted* 1938) and as the result of transneuronal degeneration (*Einarson & Lorentzen* 1946); it also occurs in various toxic conditions and diseases e.g. amyotrophic lateral sclerosis and chronic poliomyelitis, and it may gradually proceed to an irreparable cellular atrophy.

The classic example of chromatolysis is the so-called retrograde cellular change of *Nissl* which occurs after a lesion of the axis cylinder of the neuron (axon-reaction of the cell).

It is in fact a cellular reaction to the enormous demands for growth during the regeneration of the neuron. This regeneration from the condition of loss of substance involves a considerable increase in the consumption and resynthesis of cytoplasmic polynucleotides. Thus retrograde chromatolysis is a growth reaction, while chromophoby, due to prolonged hyperactivity, is a work reaction. Chromatolysis also occurs as the result of direct injury to the cell, as in various degenerative, toxic and infectious processes, e.g. in acute poliomyelitis; in such cases chromatolysis in an injury reaction (see *Einarson & Lorentzen* 1946).

Chromatolysis is in itself a reversible change, but it is not necessarily so. When restitution of the neuron does not take place, chromatolysis may either lead to acute total dissolution of the cell, e. g. in severe cases of acute poliomyelitis, or it may gradually proceed to extreme chromophily and finally to irreparable cellular atrophy, e.g. in chronic poliomyelitis (see below).

All the anterior horn cells shown in the figures of this paper (Figs. 1-12) were stained with a solution of gallocyanin-chromalum of pH 1.64 and photographed at a magnification of 600, under exactly identical conditions.

II. ON THE CELLULAR CHANGES IN POLIOMYELITIS

a) *Precursory remarks.*

The changes involved in the various phases of poliomyelitis largely illustrate the whole range of the most essential changes which the Nissl picture of the cells may display, and which may occur in other disorders or, as already mentioned, be produced experimentally in various ways. It is actually a question of structural stages in a general process of changes with which the cells respond to various noxious factors or experimental conditions.

On the basis of numerous investigations it must now be considered established that the cellular changes are primarily due to a direct, selective action of the virus on the nerve cells,

and that this is the real causative factor of the actual poliomyelitic paralyses; thus it is a question of a markedly neurocytotropic virus. The previously prevailing conception that the degeneration of the nerve cells was a secondary consequence of the inflammatory process must now be finally abandoned; a direct parallelism between the degeneration of the neurons and the inflammatory changes does not necessarily exist neither with regard to their intensity, localization, or distribution, nor with regard to the time at which they occur.

With the discovery of the selective neurotropism of the virus the study of the internal structure of the nerve cells and its histochemistry has become of far greater theoretical interest and importance in the research on poliomyelitis than one was previously inclined to believe. As an example of the intimate interaction between the nerve cells and their structural condition on the one hand and the poliomyelitis virus on the other, it is sufficient to mention the interesting observation made by *Howe & Bodian* (1941) that regenerating nerve cells show an increased resistance to the destructive action of the virus, i.e. that a nerve cell, which is in a chromatolytic state before the onset of the infection, is, to a high degree, protected against the virus.

b) *Acute changes.*

Numerous works on the changes of the anterior horn cells in poliomyelitis, based both on series of patients and on animal experiments, have been published, mainly on the acute changes and their rapid development.

The acute changes of the anterior horn cells are now well known, but by means of the selective staining reaction of the gallocyanin lake it is now possible to estimate their nature and importance more exactly than was previously the case.

They begin with swelling of the cell body and chromatolysis with dissolution and diffuse disappearance of the Nissl bodies (Figs. 2 and 3); the site of the nucleus in the cell may

become more or less eccentric, and the nuclear membrane often shows pronounced basophily with nuclear caps (Fig. 3). As early as 24 hours after the experimental infection the chromatolysis is often very distinct (*Bodian,* 1947); the same applies to patients who have died shortly or immediately after the onset of the meningeal reaction of the preparalytic stage. As soon as the climax of the initial chromatolysis is reached the impulse activity ceases, and there is paresis of the muscular

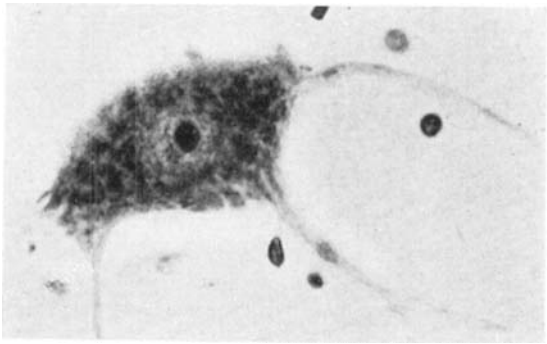

Fig. 1.
Normal, chromoneutral cell; somatochrom-stichochrom pattern of the Nissl substance.

fibres innervated by the affected neuron. In the typical severe cases the changes progress rapidly with marked vacuolar breakdown of the cytoplasm and degenerative shrinkage and disappearance of the nucleus and nuclear membrane (Fig. 4). The contour of the cell assumes an irregular ragged appearance, the cytoplasm becomes structureless, frequently with relatively increased acidophily before it disappears altogether as a cell shadow (einfacher Schwund, *Horányi-Hechst,* 1935) or is destroyed by neuronophages. It corresponds, clinically, to the paralytic stage, with its rapid development of paralyses, which often reach their maximum after 24-48-120 hours. At the same time the microglia is mobilized, most frequently with occurrence of marked neuronophagia, which leaves behind more or less definite microglial nodules round the

severely degenerated cell remnants, or in the place of the nerve cell which has completely disappeared (Fig. 5). As already mentioned, the nerve cell may also be destroyed with-

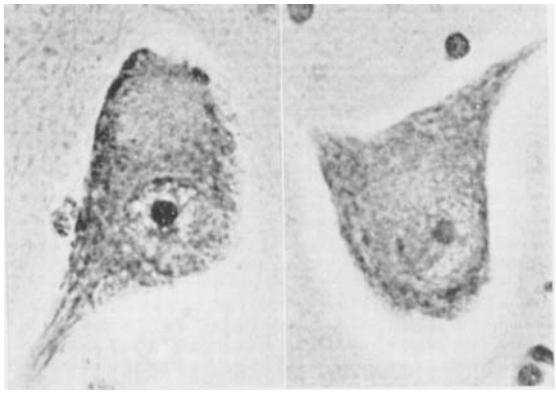

Fig. 2.
Poliomyelitis; death almost 3 days after the onset of the paralyses. Moderate chromatolysis.

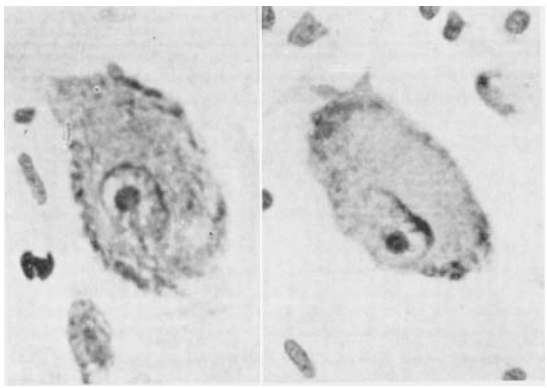

Fig. 3.
Same patient as in fig. 2. Very pronounced chromatolysis, increased basophilia of the nuclear membrane and formation of nuclear caps; nucleus more or less eccentric.

out any noteworthy neuronophagia, at most with increased satellitosis or pseudoneuronophagia. In all cases it is the primarily damaged nerve cell that is secondarily dissolved by

the neuronophages; the primary degeneration of the nerve cell is a conditio sine qua non for the ensuing neuronophagia. Such changes are, of course, absolutely irreparable, and the patients who survive an acute stage resulting in such severe changes will of course be permanently disabled as far as the affected neuromuscular units are concerned.

On the other hand, the initial chromatolysis (Figs. 2 and 3) is a reversible condition, and the process need not necessarily proceed to the acute, severe, cellular dissolution mentioned above (Figs. 4 and 5). Accordingly patients who survive the disease with such comparatively mild lesions may recover from their paralyses. Any physician who has treated poliomyelitis patients knows that it is by no means rare for a totally paralysed, and even considerably atrophied, muscle to recover its function, either completely or partially. A typical example of partial recovery in an experimentally infected monkey was described by *Hurst* (1929). The acutely developed motor symptoms consisted of bilateral ptosis, paralysis of the right arm, and paresis of both legs. The animal was killed on the 35th day after the onset of symptoms, at which time a considerable partial restoration of function in the affected parts had occurred. Histologically there was a distinct degenerative loss of nerve cells in the areas of innervation of the muscles affected, while it was evident that the remaining nerve cells had regenerated, and many of them had even become completely normal. It is, of course, impossible to say with certainty afterwards to what extent the preserved nerve cells had been affected, but at any rate a certain reversibility was beyond doubt. Thus, a not inconsiderable chance of reversibility exists, and the reversibility of the changes of the nerve cells is an indispensable condition for subsequent training and restoration of function of a totally paralysed muscle. This applies quite apart from the fact that when only some of the anterior horn cells of a muscle have degenerated irreparably or completely disappeared, while others are fully preserved, it is possible to train the unaffected muscule fibres to such an extent that they can compensate the loss of the

severely affected neuromuscular units, if this loss is not too extensive.

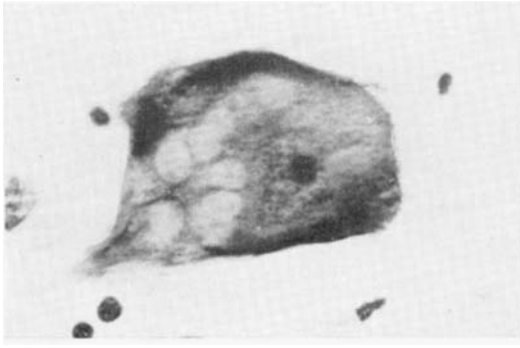

Fig. 4.

Poliomyelitis; patient died from respiratory paralysis 5 days after the onset of the paralytic stage, with severe paralyses in all four extremities. Chromatolysis, swelling and acute vacuolar dissolution of the cell.

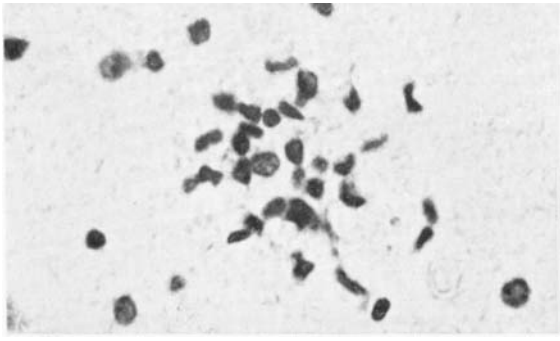

Fig. 5.

Poliomyelitis; death approximately 1 month after the onset of paralysis. Glial nodule at the site of a motor cell which has entirely disappeared by acute disintegration and neuronophagia.

The chromatolysis is a structural expression of a radical change in the nucleic acid metabolism of the nerve cell, and the dissolution of the Nissl substance is tantamount to a breakdown and disappearance of the nucleotides of the cell. Using the staining technique employed here the quantitative

diminution of the Nissl substance is an indicator of the depletion of the nucleotides of the cell, for the gallocyanin-lake-ion is bound selectively to the nucleic acid component of the cellular structures, and the staining intensity gives a quantitative measures of the content of nucleic acid of the cell. The compact nucleolus, the intensely stained nuclear membrane with the accumulations of perinuclear substances and nuclear caps with a great content of nucleic acid are an expression of the nuclear resynthesis of polynucleotides and the persisting tendency to regeneration of the cytoplasmic nucleotides and proteins of the cells. In poliomyelitis it is the virus and its multiplication in the cell which produces the changes in the nucleic acid metabolism with the quantitative reduction in the content of nucleotides; the breakdown and depletion of the nucleotides of the cell may proceed to the point where total degeneration and death of the cell must necessarily occur.

The multiplication of the poliomyelitis virus must, at least to some extent, depend on an increase in virus nucleoprotein, and it is possible that this increase takes place partly by a transformation of the nucleoproteins of the nerve cell. As far as I know we have so far no detailed knowledge of the relation between the growth and multiplication of the virus and the nucleic acid metabolism of the nerve cell.

During chromatolysis the content of phosphatase in the cytoplasm is considerably increased simultaneously with the breakdown of the polynucleotides. It is possible that the phosphatase has a dissolving effect on the virus and thus counteracts its multiplication, i.e., that there is an antagonistic interaction between the phosphatase system of the cell and the virus, upon which the outcome of the acute phase depends, i.e., whether it results in the destruction of the cell or of the virus while the cell is still viable; in the latter case it may be possible for the cell to regenerate. The fact that a previously chromatolytic cell exhibits an increased resistance to the action of the virus (*Howe & Bodian*) may possibly be explained by the increased content of phosphatase of the cell, which affects the penetration or the growth of the virus in

the cell. Whether this is true or not must be the subject of subsequent investigations.

c) *Chronic Changes.*

Although the acute attack of the disease may be limited to milder lesions with the possibility of later regeneration of the nerve cells, the final outcome of the process is not necessarily determined, *for during the regeneration, or immediately after, the cell may undergo a chronic, slowly progressive change, sometimes ending in totally irreparable cellular atrophy.* It is particularly these chronic changes I have made the subject of my studies.

As far as I can see from a review of the literature *Schwalbe* (1902) was the first to pay attention to the chronic stages of the disease and to subject the late changes to a more thorough and surveyable study. Apart from the total cellular dissolution, which is invariably a consequence of the acute severe changes (Figs. 4 and 5) *Schwalbe* classified the late cellular changes in the following main categories: *1)* Pale cells with still visible nuclei or nuclear remnants, indistinct or atrophied processes, and absence of the Nissl substance, *2)* Shrunken, intensely stained cells, sometimes with granular cytoplasm, otherwise as compact stained bodies, in which neither nuclei, nor processes are discernible, *3)* Cells in all stages between the categories mentioned and normal cells, generally localized in the pheriphery of the lesion, *4)* Cells which he described as "calcified". Furthermore, *Schwalbe* surveyed up to his own time the most important publications of histological examinations of the spinal cord from poliomyelitis patients who had died either in the acute stage, or subsequently from an intercurrent disease. In this connection we are especially interested in the cases with pronounced paralyses reported by *Leyden, v. Kahlden,* and *Kawka.* On their cases autopsy was performed from 5 months to 17 years after the first onset of paralyses, and the late changes described by *Schwalbe* were strongly represented.

It is, of course, very difficult to estimate the exact value of these older investigations owing to the very varied techniques in use at that time. As late as 1902 *Nissl*'s technique was not commonly used in the laboratories, and even *Schwalbe* himself did not use it in his studies on poliomyelitis. In spite of their shortcomings the older investigations have shown *that lasting pareses and muscular atrophies may be the consequences of chronic cellular changes, and need not necessarily be due to an acute loss and neuronophagia of the cells.*

A very important contribution to the study of the chronic, persistent histological changes in experimental poliomyelitis in monkeys has been given by *Warburg* (1931). It is a study of the nervous system of 15 monkeys (macacus rhesus) with typical paralyses and atrophies. They lived for a period varying from 19 to 309 days after the first onset of symptoms. *Warburg* gives a detailed account of the changes in the connective tissue, vessels and glia in addition to the neuronal changes in the various regions of the nervous system. Here, however, we are interested only in the changes of the nerve cells, particularly the anterior horn cells. In 9 monkeys which survived the disease for periods varying from 40-84 days (7 were killed and only 2 died of the disease) the numerical loss of anterior horn cells varied, both from case to case and within the various segmental regions of the same spinal cord; the majority of the cells was, however, as a rule present in the tissue, although changed to a greater or lesser extent. The dominant cellular change was an intense hyperchromasia of both cytoplasm and nucleus with increased staining and distinctness of the dendrites. The Nissl substance could sometimes be seen as granular particles or small flakes, but most frequently its pattern was not discernible in the compact, intensely stained, and occasionally spindle-shaped cell body. *Warburg describes these cells as distinctly affected, but probably still viable, and they correspond closely to the cellular change which I have designated as extreme chromophily* (Figs. 8 and 9), *and they are closely related to, or even identical with the cells which Hurst described as "recoverable".* Furthermore,

Warburg describes other cells with less intensely stained or pale cytoplasm and occasionally with a hyperchromatic nucleus, as well as cells which appear as extremely pale cell shadows with irregular contours. *Warburg* is decidedly of opinion *that these are cells which have definitively succumbed to the morbid process, and they are identical with what I have designated as progressive irreparable cellular atrophy* (Figs. 10, 11 and 12). In the monkeys which survived for periods varying from 242 to 309 days, and which were all killed, the two categories of cellular changes just mentioned were strongly represented, but the number of pale cell shadows had become relatively larger. In some places the numerical loss of cells was somewhat larger, while the preserved cells only presented "few deviations from the normal other than slight distortions of contour and of the fibrillar network, which was sometimes knotted and clumped" (*Warburg*, 1931, p. 1210). It is also noteworthy that in contrast to the animals which died early with acute severe changes and loss of cells, these late-surviving cases with chronic cellular changes showed mostly simple satellitosis and not actual neuronophagia. The same chronic changes were also observed by Horányi-Hechst (1935) in patients who died 27 and 30 days, respectively, after the first onset of the paralyses; he did not enter into the details of the problem, but just designated them as "fortschreitende Nervenzelldegenerationen im chronischen Stadium der Poliomyelitis".

The results of my own examinations of material from patients are in complete agreement with those of *Warburg*'s examinations of the experimental material from monkeys. I shall merely add that the staining with gallocyanin-chromalum allows of a much more exact estimation of the cellular changes than the staining technique employed by *Warburg*. In the first place, I want to emphasize that I have made observations which decidedly favour the view that the extreme chromophily (Figs. 8 and 9) and the irreparable cellular atrophy (Figs. 10, 11, and 12) do not represent isolated, independent cellular changes, but that the chromophily gradually passes into in-

creasing cellular atrophy which ends in the formation of cell shadows. The same applies to the initial chromatolysis (Figs. 2 and 3). This also is not an isolated phenomenon, as there are cell forms (Figs. 6 and 7), which decidedly represent transitional stages from chromatolysis to extreme chromophily; it is thus possible to speak of a postchromatolytic chromophily. In the stages shown in Figs. 7-10 the cell may,

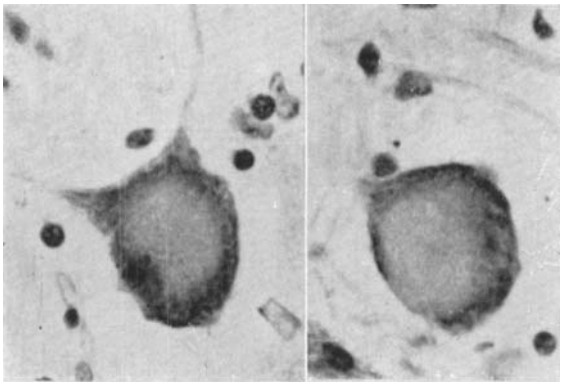

Fig. 6.
Poliomyelitis; death 14 days after the onset of paralyses. Chromatolysis has culminated and the cells are on their way to chronic irreparable atrophy.

however, undergo a diffuse fatty degeneration, which leads to irreparable cellular lipodystrophy in the same way as in amyotrophic lateral sclerosis and experimental vitamin E deficiency (see *Einarson & Lorentzen*, 1946, p. 62-63). *If the initial chromatolysis does not lead to acute, severe, cellular change with neuronophagia and total cellular dissolution, or the cell does not regenerate completely in the course of some months, the chromatolysis may change into a state of gradually increasing chromophily, which may become extremely intence, and in which the cell appears as a very intensely stained, compact body, which then undergoes irreparable atrophy and ends as a more or less pale, atrophic, or structureless cell shadow.* Thus it is here a question of stages in a chronic

progressive cellular change (see Figs. 2-3 and 6-12), the most characteristic structural feature of which is the varying staining intensity of the cell, i.e., its variable capacity of binding the gallocyanin-lake-ion, which is tantamount to a quantitative change of the nucleic acid content of the cell. *After the initial vigorous breakdown of the nucleotides of the cell, as evidenced by the chromatolysis, an active resynthesis and*

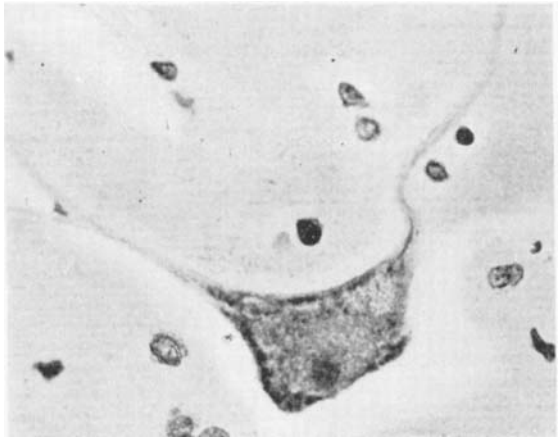

Fig. 7.

Poliomyelitis; death from intercurrent disease 40 days after the onset of rather mild paralyses showing marked tendency toward recovery. Postchromatolytic restitution, the cell is becoming decidedly more chromophilic.

gradual accumulation of nucleotides take place in the cell, the process, being manifested structurally by the increasing chromophily; the nucleotides then again gradually diminish and at last disappear completely, and the irreparable cellular atrophy is an expression of this stage.

As far as these cellular changes are concerned, the question of their possible reversibility is of very great importance. It is a fact that the atrophic cell, the pale cell shadow, represents a stage of irreversible degeneration; it is a cell which has been finally destroyed, and this is also indicated by the designation *irreparable cellular atrophy* (Figs. 10, 11, and 12). However,

it is equally certain that the increasing intense chromophily represents stages which to a considerable extent are still reversible (Figs. 8 and 9). It cannot be determined at what stage the reversibility sets in, for we are as yet unable to draw a welldefined limit between extreme chromophily, cellular sclerosis, and cellular atrophy. However, numerous observations favour the view *that the extreme chromophily, which is*

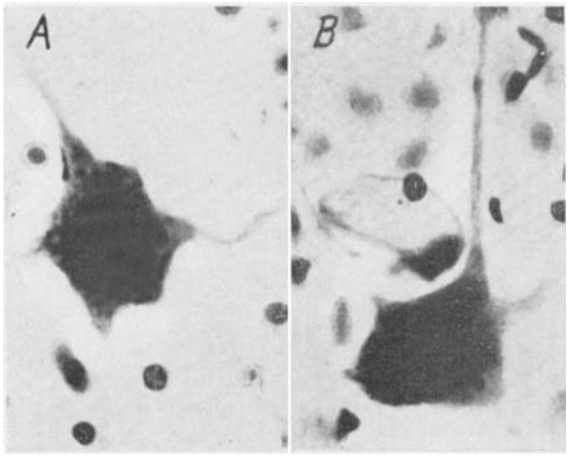

Fig. 8.
Poliomyelitis; A) Patient died 3 months after the onset of paralyses which were mostly moderate. B) From another patient, who died 8 month after the onset of rather severe and widespread paralyses. The cells are in a still reversible state of extreme chromophily.

always an expression of an accumulation of nucleotides in the cell, may persist for a long time as a still reversible state of the cell, and in my opinion this is not without importance in the aftertreatment of poliomyelitis.

It must, of course, be realized that extreme chromophily also plays a rôle in other morbid processes, such as spinal muscular atrophy and amyotrophic lateral sclerosis, and here shows considerably less reversibility than in poliomyelitis. Chromophily may also be produced experimentally, e.g. by prolonged reflex inhibition (*Einarson,* 1933), where it is al-

ways reversible; by chronic vitamin E deficiency (*Einarson & Ringsted,* 1938), in which it is less reversible; by chronic intermittent anoxia (*Morrison,* 1946; *Erik Krogh,* 1948), where the reversibility is considerable; and finally as a transneuronal cellular change (*Einarson & Lorentzen,* 1946), in which it is more difficult to appreciate the degree of reversibility. The reversibility of the chromophily as a structural state thus

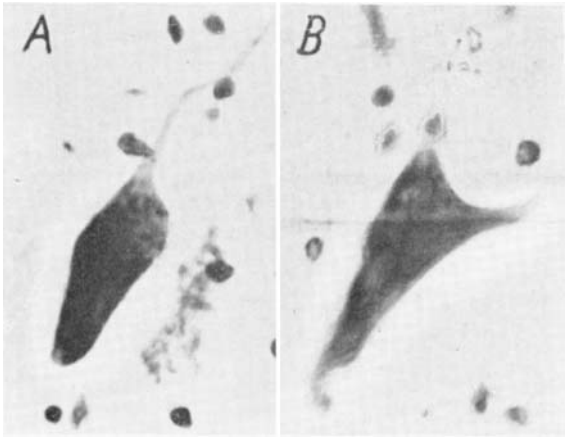

Fig. 9.
Same patient as in fig. 8 B. The most intense degree of extreme chromophily or cell sclerosis, a state possibly still reversible, at least the cell represented in A).

depends on the conditions under which it has arisen, and its tendency to develop into cellular sclerosis, cellular lipodystrophy, or irreparable cellular atrophy varies accordingly. *As a cellular reaction in poliomyelitis the reversibility of the chromophily is, however, not very unfavourable, and extreme chromophily always denotes suppression or loss of physiological impulse activity, a state of inhibition.*

When the cell enters the chronic state of extreme chromophily, it means that the impulse activity of the neuron is not initiated, and an important factor in the explanation of the chromophily must be that the formation of nucleotides con-

tinues in spite of the loss of impulse activity. This leads to an accumulation of these substances both in the perikaryon, the dendrites, and in the nucleus, as the increased dissimilative phase of the nucleotide metabolism connected with the impulse activity has been abolished, and the consumption of nucleotides is thus greatly reduced, i.e., the production is larger than the consumption. The result is *that the nucleic acid derivatives*

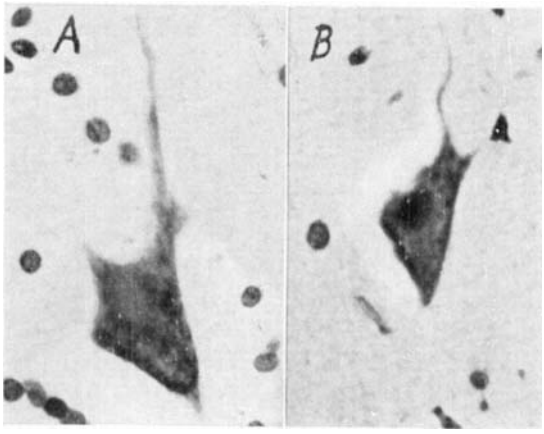

Fig. 10.
A) From the same patient as in fig. 8 B. and 9. B) From a patient, who died 11 months after the onset of the disease, with severe paralyses. Definitely irreversible cellular atrophy.

so important to the impulse transmission and the metabolism of the neuron are not liberated in sufficient amounts, and that basophilic substance, for which the gallocyanin-lake-ion has such a strong affinity, accumulates in the cell. By degrees the new formation of nucleotides will cease, the already accumulated substance will disappear, while the cell proceeds to irreparable cellular atrophy. This assumption is strongly supported by the observation that when a neuron neither emits nor receives impulses, its cell body gradually enters into a state of extreme chromophily.

d. *Discussion.*

My studies of the spinal cord from poliomyelitis patients who died after periods varying from some months to about 3 years after the acute stage of the disease, have shown that not nearly all the motor cells in the area of innervation of the paretic or atrophied muscles had disappeared or been

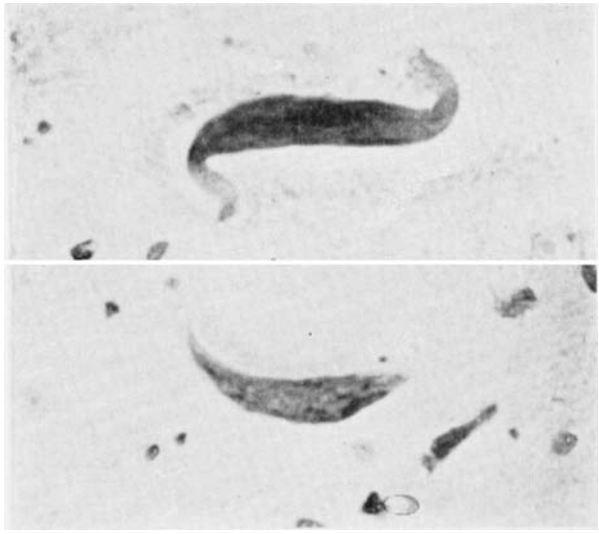

Fig. 11.
Poliomyelitis; patient died more than 2 years after the onset of the disease, with widespread paralyses in the lower extremities. Chronic, totally irreparable cellular atrophy.

absolutely irreparably changed. A surprisingly large number of cells was still present in a state of extreme chromophily, with the isoelectrical point of the Nissl substance lying below or around pH 2.0 (see *Einarson & Lorentzen,* 1946, p. 64). On the basis of our experimental studies it seems very plausible to conclude that functionally it is also here a question of cells, the impulse activity of which had been totally abolished, but which might have been activated again before they proceeded to irreparable cellular atrophy. In my opinion a motor

cell of the anterior horns may remain for a very long time (up to 12-20 months) in such a torpid state of extreme chromophily without being irreparably changed, and without the trophic control of the axon (the peripheral nerve) suffering any observable damage.

In many of the chronic cases the cells have, of course, died

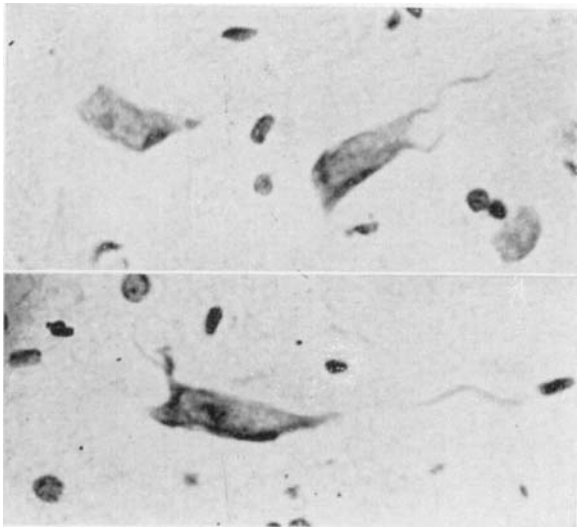

Fig. 12.
Same patient as in fig. 11. Chronic irreparable cellular atrophy and cell shadows.

or disappeared or passed into an irreparable state of atrophy, but it need not necessarily always be the case in spite of paralyses and muscular atrophies. Excellent results are now often achieved in orthopedic hospitals and sanatoria by physiotherapeutic treatment during the second stage of poliomyelitis, i.e., in the period between the acute stage and the occurence of irreparable damage, and here it is very important to secure active physical and psychological co-operation on the part of the patient. *The results achieved in such institutions cannot exclusively be due to training and compensatory hypertrophy*

of such muscular fibres, whose area of innervation (the anterior horn cells) has escaped the acute breakdown, but must also to an essential degree be due to a reversibility of neuronal changes. In this connection I want to emphasize that in any training an important part is played by central-neuronal exercise or adaptation, i.e., the overcoming or lowering of synaptic resistances in the central nervous system. In my opinion the good results achieved with the aid of modern active physiotherapy are to no slight degree due to the circumstance *that the systematically produced proprioceptive (stretch reflexes) and cerebrofugal impulses which impinge on the neurons of the anterior horns will gradually release the motor cells from their torpid state of chromophily and thus again evoke their impulse activity.* The synaptic resistance is overcome, the synapse is re-canalized for the impulses impinging on the cell, and its own impulse actvity is re-evoked. The ensuing increase of the dissimilative phase in the nucleotide metabolism involves a consumption of the accumulated substances, and the state of intense chromophily is gradually abolished; concomitant with this process the demands for resynthesis of the cytoplasmic nucleotides increase. In other words, *as soon as the impulse activity of the neuron is re-established, its nucleotide metabolism is activated.* Thus the neuromuscular units whose anterior horn cells were extremely chromophilic, have a chance of recovery, while those whose cells were irreparably changed or have completely disappeared, will, of course, be beyond any possibility of recovery.

On the basis of numerous investigations it has been estimated that anterior horn cells with reversible changes may be restored to function in the course of from 1-20 months. Some authors, indeed, maintain (see e.g. *Hansson,* 1939, p. 32) that nerve cells which have been attacked by the virus and damaged without being destroyed, may recover in from 3 to 5 years; and cases of poliomyelitis have been encountered, in which completely paralysed and even markedly atrophied muscles have recovered after such a long time. I do not want to say anything as to whether the restoration of the muscular

function in such cases is connected with peripheral axon regeneration. I shall merely emphasize the remarkable primary resistance to the poliomyelitic process which myelin sheaths and especially axons possess. When the cells degenerate in the acute phase, the axons will, of course, rapidly be subjected to secondary degeneration; but if the cells are restituted after the acute chromatolysis, such axon changes do not occur. I shall also emphasize the disproportion existing between the intensity of the muscular changes on one hand and the peripheral nerve changes on the other, which is so often encountered in poliomyelitis and amyotrophic lateral sclerosis. Several years after the acute stage of the disease severe diffuse muscular atrophies concomitant with surprisingly slight changes in the peripheral nerves have been encountered in the chronic stage of poliomyelitis (see *Kopits,* 1929; *Horányi-Hechst,* 1935). This condition often coincides with the presence of ample amounts of extremely chromophilic and sclerotic cells in the anterior horns,. Several authors believe that if the acute inflammatory process can evoke secondary slight changes in the nerve cells, the cells may recover in from 2 to 60 days (*Hansson,* 1939), but it is doubtful whether such cellular changes are of any importance at all.

SUMMARY

The structural changes of the nerve cells have been studied by the aid of the progressive, selective staining with gallocyanin-chromalum, which allows a quantitative estimation of the basophilia of the cells. Since the stain becomes selectively bound to the nucleic acids of the structures, its intensity (chromophily, chromoneutrality, chromophoby) gives a fairly accurate expression of the cellular content of nucleic acids, which varies markedly in poliomyelitis as well as under experimental conditions (anoxia, vitamin E deficiency etc.).

In poliomyelitis the acute changes of the cells (swelling, chromatolysis, vacuolar dissolution, neuronophagia) are distinguished from the chronic or late changes (extreme chromo-

phily, cell sclerosis, lipodystrophy and irreparable cell atrophy), and both are estimated in relation to the varying cellular content of nucleic acids. In fact lasting pareses and muscular atrophies may be the consequenses of these chronic cellular changes, and need not necessarily be due to an acute loss of the cells and neuronophagia.

Several observations support the view that the motor cells may remain for a long time in the torpid but still reversible state of extreme chromophily, without the trophic control of the axon suffering any noteworthy damage. The good results of the physiotherapeutic aftertreatment of poliomyelitis (the systematic training of paretic and atrophic muscles) often achieved in orthopaedic hospitals, are thought to be due, in no slight degree, to a release of the motor cells from their torpid state of extreme chromophily and a gradual recovery of their impulse activity. This applies quite apart from the fact, that it is possible to train the unaffected muscle fibers to such an extent that they may compensate the acute loss of the severely affected neuromuscular units, if this loss is not too extensive.

RESUME

Les modifications structurales des cellules nerveuses ont été étudiées à l'aide de la coloration progressive, sélective à la gallocyanine-chromalum, qui permet d'effecteur une estimation quantitative de la basophilie des cellules. Depuis que la coloration est devenue sélective des acides nucléiques de ces structures, son intensité (chromophilie, chromoneutralité, chromophobie) donne une indication relativement exacte de la teneur des cellules en acides nucléiques, qui diffère notoirement dans la poliomyélite ainsi que dans les recherches expérimentales (anoxie, déficience en vitamine E, etc.).

Dans la poliomyélite, les altérations aiguës des cellules (enflure, chromatolyse, dissolution vacuolaire, neuronophagie) se distinguent des modifications chroniques ou tardives (chromophilie extrême, sclérose des cellules, lipodystrophie et atro-

phie irréparable) et il est possible de déterminer le genre de modification devant lequel on se trouve par l'estimation de la teneur différente des cellules en acides nucléiques. En fait les parésies durables et les atrophies musculaires peuvent être la conséquence de ces altérations cellulaires chroniques et ne sont pas nécessairement dues à une destruction aiguë des cellules et à une neuronophagie.

Plusieurs observations appuient l'hypothèse que les cellules motrices peuvent rester très longtemps dans un état torpide d'extrême chromopilie encore réversible, sans que le contrôle trophique de l'axone en ait souffert sensiblement. Les bons résultats des traitements complémentaires physiothérapeutiques de la poliomyélite (l'entraînement systématique de muscles parétiques et atrophiés) sont souvent obtenus dans les hôpitaux orthopédiques et ils sont probablement dus en grande partie au fait que les cellules motrices ont été arrachées à leur état torpide d'extrême chromophilie et qu'elles ont retrouvé graduellement leur activité d'impulsion. Il est d'ailleurs entièrement fait abstraction du fait qu'il est possible d'entraîner des fibres musculaires non atteintes à tel point qu'elles sont capables de compenser la destruction aiguë d'unités neuromusculaires sévèrement malades, à condition que cette destruction ne soit pas trop extensive.

ZUSAMMENFASSUNG

Die strukturellen Veränderungen der Nervenzellen wurden mit Hilfe der progressiven, selektiven Färbung mit Gallocyanin-Chromalum untersucht, die eine quantitative Schätzung der basophilen Substanzen der Zellen gestattet. Da der Farbkörper sich ausschliesslich mit den Nucleinsäuren der Strukturen verbindet, ist ihre Intensität (Chromophilie, Chromoneutralität, Chromophobie) ein ziemlich genauer Ausdruck des zellulären Gehalts an Nucleinsäuren, der sowohl bei Poliomyelitis wie unter experimentellen Bedingungen (Anoxie, Vitamin E-Mangel usw.) beträchtlichen Schwankungen unterworfen ist.

Bei der Poliomyelitis werden die akuten Veränderungen der Zellen (Aufquellen, Chromatolyse, vakuolare Auflösung, Neuronophagie) von den chronischen oder Spätveränderungen (extreme Chromophilie, Zellensklerose, Lipodystrophie und irreparable Zellenatrophie) unterschieden, und beide werden im Verhältnis zu den Schwankungen des Zellengehalts an Nucleinsäuren geschätzt. Tatsächlich können dauernde Paresen und Muskelatrophien die Folgen dieser chronischen Zellveränderungen sein und brauchen nicht notwendig auf einem akuten Verlust der Zellen und Neuronophagie zu beruhen.

Mehrere Beobachtungen stützen die Ansicht, dass die motorischen Zellen längere Zeit in einem starren, aber immer noch reversiblen Zustande extremer Chromophilie verbleiben können, ohne dass die trophische Kontrolle des Axons irgendwelchen nennenswerten Schaden erleidet. Die guten Resultate der physiotherapeutischen Nachbehandlung der Poliomyelitis (die systematische Uebung der paretischen und atrophischen Muskeln), die in den orthopädischen Krankenhäusern oft erzielt werden, müssen wohl in nicht geringem Grade auf einer Auflockerung des starren Verweilens der Zellen in extremer Chromophilie beruhen und auf einer gradweisen Wiedererlangung ihrer Reiztätigkeit. Dies gilt ganz abgesehen von der Tatsache, dass es möglich ist, die nicht angegriffenen Muskelfasern soweit zu trainieren, dass sie den akuten Verlust der schwerer angegriffenen neuromuskulären Einheiten kompensieren können, wenn dieser Verlust nicht zu ausgedehnt ist.

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