FROM THE DEPARTMENT OF ANATOMY, ÅRHUS UNIVERSITY, AND THE INSTITUTE OF BRAIN PATHOLOGY OF THE STATE MENTAL HOSPIAL AT ÅRHUS

NOTES ON THE HISTOCHEMICAL ASPECT OF THE CHANGES OF THE SPINAL MOTOR CELLS IN ANOXIA, VITAMIN E DEFICIENCY AND POLIOMYELITIS

BY

LÁRUS EINARSON

I. INTRODUCTION

The investigations on which the following notes are based have been carried out mainly by means of the progressive selective staining reaction of gallocyanin-chromalum (*Einarson,* 1932) supplemented by some other histochemical determinations; they will be published in detail in subsequent papers. It is sufficient to say that by means of photometric measurements staining with gallocyanin-chromalum allows a quantitative estimation of the basophilia of the cells. Since the stain becomes selectively bound to the nucleic acids of the cell structures, the staining intensity, as expressed by the states of chromophily, chromoneutrality and chromophoby respectively, gives a fairly accurate measure of the nucleic acid content of the cells (*Einarson,* 1934, 1945, 1947; *Einarson & Lorentzen* 1946; *Lagerstedt* 1947, 1948). This content is considerably altered in anoxia and vitamin E deficiency as well as in the changes following poliomyelitis. Thus the degree of the staining intensity depends directly on the inherent capacity of the cell to bind the stain; this depends on its content of nucleic acids, which is profoundly influenced by the state of functional activity of the neuron; the latter shows an intimate correlation with the structural stages of chromophily and chromophoby (for reference see *Einarson,* 1945 and

1949). It is sufficient to say that the various stages of chromophoby represent cells in increasing and prolonged activity, while extreme chromophily represents cells whose impulse activity has for some time been depressed or totally abolished. Since the Nissl substance is composed of nucleoproteins, acid and basic proteins respectively (*Einarson,* 1935), the stages of chromophily and chromophoby involve a shift in the isoelectric point of the Nissl substance (*Einarson,* 1937, 1945), due to a change in the mutual quantitative ratio between its nucleic acids (polynucleotides) on the one hand and its basic proteins on the other.

These intracellular and functional conditions of the neurons are profoundly altered in the structural changes following anoxia, vitamin E deficiency and poliomyelitis, and I think it is of some interest to investigate their mutual relationship in terms of their histochemical correlate.

II. ANOXIA AND VITAMIN E DEFICIENCY

Changes of the anterior horn cells similar to those described in poliomyelitis (see *Einarson,* Acta ortopaed. scand. 1949) may also be produced experimentally by anoxia (*Erik Krogh* 1945; *Morrison* 1946) and by vitamin E deficiency (*Einarson & Ringsted* 1938).

The initial effects of both anoxia and vitamin E deficiency are always swelling and chromophoby of the nerve cells, accompanied by hyperexcitability and spastic rigidity of the muscles. Many years ago it was established, and has since been confirmed several times (see *Einarson & Lorentzen,* 1946), that anoxia of an intensity sufficient to produce spasms and convulsions need not be either so pronounced or so prolonged as to produce irreparable cellular changes or necroses. A short acute anoxia will invariably produce an initial state of excitation, which may be more or less pronounced before the activity is paralysed. As a morphological expression the initial chromophoby corresponds to this state of excitation, i.e., a more or less pronounced breakdown and disappearance

of the polynucleotides of the cell take place (see fig. 1). A more prolonged, intermittent anoxia, which is not sufficient to produce disintegration and necrosis of the cells (sublethal anoxia), will, on the other hand, lead to extreme chromophily accompanied by paralysis of the impulse activity, and gradually later to cellular atrophy; the same is true of the prolonged effect of chronic vitamin E deficiency (see fig. 1). If the

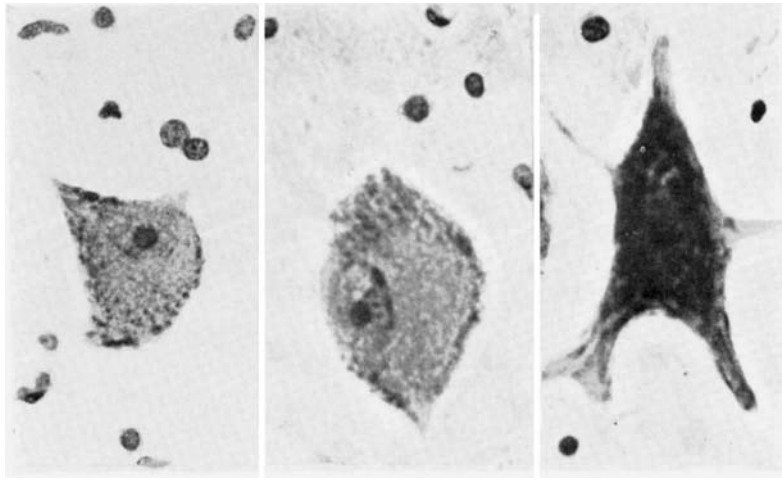

Fig. 1.

A) Motor cell in extreme chromophoby, representing the initial structural effects of vitamin E deficiency and acute anoxia respectively.

B) Motor cell showing the initial chromatolysis of acute poliomyelitis.

C) Motor cell in an early stage of extreme chromophily representing the late or retarded structural effects of chronic, sublethal anoxia, protracted vitamin E deficiency and chronic poliomyelitis respectively. Gallocyanin-chromalum, pH 1.64 × 600.

anoxia is initially either too intense or too prolonged, the nerve cells will, of course, be totally destroyed by a rapidly progressing degenerative process.

How are the structural effects of anoxia to be explained? There is no doubt that the necessary nucleic acid derivatives, which are so important in the metabolism of the neuron and for the formation of acetylcholine, originate from the de-

composition of the nucleic acids of the cytoplasm, which are in turn re-formed from the nucleus of the cell (*Einarson*, 1933, 1945). It appears that the presence of rather ample amounts of adenosine triphosphate (ATP) is required in the nervous system, presumably in connection with the great consumption and resynthesis of acetylcholine. ATP is constantly re-formed by liberation of adenylic acid from the stored polynucleotides of the cytoplasm (the Nissl substance) and subsequent phosphorylation of the liberated acid. This process will be particularly evident when there is a highly increased and prolonged impulse activity with its concomitant intense decomposition and resynthesis of acetylcholine, for then the stored polynucleotides, which may be demonstrated histochemically, are consumed (see the diagram fig. 2). The constant trophic maintenance of the structural integrity of the axon must presumably make heavy demands on the metabolic-chemical work of the nerve cell, but it must be assumed that during the normal resting phase the presence of phosphocreatine as a phosphate-donor reserve is sufficient to secure constant re-phosphorylation of ATP, as in muscles. During the enormous growth requirements in connection with regeneration of the neuron this is scarcely the case, for then the gray substance of the anterior horns loses about 40 % of its normal content of phosphocreatine. (*Bodian*, 1947, p. 172). Simultaneously the heaviest decomposition and consumption of the stored polynucleotides of the cell occurs, as evidenced by chromatolysis, and undoubtedly has a certain relation to the liberation of important nucleic acid derivatives such as adenylic acid. *In the adult organism, the cells of no other tissue contain such large amounts of cytoplasmic polynucleotides as the nerve cells, and this must be explained by the special metabolic conditions of the neurons, since these substances are being constantly decomposed and re-formed in close correlation with the functional and trophic activity of the neuron* (*Einarson*, 1933, 1945; *Einarson & Lorentzen*, 1946).

It is possible that nerve cells possess depots of chemically-

bound oxygen, which are utilized during the short initial state of excitation following anoxia, with its concomitant increased requirements on decomposition and resynthesis of acetylcholine (chromophoby). But it is equally plausible to assume that, during the brief period of excitation, the nerve cells are capable of mobilizing anaerobic energizing processes, and through these, if only for a very short time, are able to satisfy the acetylcholine requirements. The extreme chromophily of chronic ,sublethal anoxia is tantamount to a paralysis of the impulse activity of the neuron; here the energy reserves, whether they are depots of chemically-bound oxygen or due to anaerobic processes, must be exhausted, so that the nucleic acid derivatives necessary for the resynthesis of acetylcholine are not split off from the polynucleotides of the cytoplasm, and these consequently accumulate in the cell owing to the still active production of nucleotides from the nucleus (see the diagram fig. 2). Now, it has also been found (*Stone, Marshall & Nimms,* 1941; *Gurdjian, Webster & Stone,* 1944) that the content of phosphocreatine and ATP in the nervous system is considerably reduced during anoxia and after experimental head and brain injury.

How are the structural effects of vitamin E deficiency to be explained? It has now been established that vitamin E inhibits oxidation; thus it protects certain substances (fatty acids, vitamin A) against oxidation. In vitamin E deficiency the oxygen consumption of the tissues is enormously increased; so that vitamin E must either have some control over the tissue respiration, or diminish the oxygen consumption (*Victor,* 1934; *Houchin & Mattill,* 1942; *Houchin,* 1942). In vitamin E deficiency, administration of the relatively water-soluble α-tocopherol phosphate will rapidly reduce the oxygen consumption to the normal level (*Houchin & Mattill,* 1942), and it may be important that phosphorylation of the tocopherol, by which it is transformed into a water-soluble compound, takes place in the organism. The initial structural effect of vitamin E deficiency, the pronounced chromophoby of the nerve cells, may thus be explained by the removal of

a factor inhibiting oxidation; thus the tissue respiration cannot be kept at the level of the resting phase, and the polynucleotides stored in the cell must necessarily be decomposed under the increased requirements on the metabolism and the resynthesis of acetylcholine, as in acute anoxia. Similarly, it is possible to explain the chronic structural effect of prolonged vitamin E deficiency, the extreme chromophily of the nerve cells, by the fact that the highly increased oxygen consumption will lead to an exhaustion of the energy reserves of the cells and to the gradual development of a chronic sublethal anoxia with the ensuing accumulation of cytoplasmic polynucleotides and an arrest of the resynthesis of acetylcholine. Our own investigations, which I shall not mention here, show *that in prolonged vitamin E deficiency the phosphocreatine and the ATP content of the gray substance of the nervous system diminish considerably and finally disappear as the state of deficiency leads to irreparable cellular atrophy* (Einarson, unpublished work). Furthermore, it has been found that in rats on a low-fat, vitamin E-free diet, administration of tocopherol increased their resistance and prolonged their survival time under anoxia; increasing the fact content of the diet shortened the survival time under anoxia (*Howe, Hickman & Harris,* 1945).

Several investigators have pointed out that vitamin E probably also has some special tissue affinity and thus has a protective or antidystrophic action on tissues. On a diet low in vitamin E, or high in unsaturated acids, which bind or attack the vitamin, the tissues become dystrophic (see *Einarson & Ringsted,* 1938; *Einarson,* 1941) but only a very slight amount of vitamin E is necessary for their protection. *Eppstein & Morgulis* (1941 and 1942) found that the minimum antidystrophic daily dose in the rabbit was 200-400 γ α-tocopherol (ca. 0.32 mg) per kg body weight. *Einarson & Ringsted* (unpublished data) found that in a 30-day-old common white rat the minimum dose necessary for protection against neuromuscular disturbances is as low as 25-50 γ α-tocopherol per day. I have made certain observations which suggest *that,*

owing to its special tissue affinity, vitamin E is an instrumental factor in the liberation of adenylic acid from the cytoplasmic polynucleotides of the nerve cells (*Einarson*, unpublished work), and thus is more closely involved in the synthesis of acetylcholine than is generally assumed. If we accept that the decomposition of pyruvic acid occurs under the agency of H_3PO_4, by which acetylphosphate is formed, then the acetyl phosphate may be of central importance in the synthesis of acetylcholine. It may act partly as an acetyl donor and partly as a powerful phosphate donor, which in

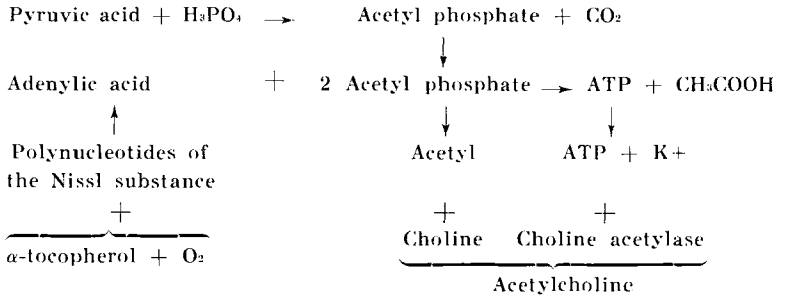

Fig. 2.

Diagram illustrating the assumption that α-tocopherol acts by stimulating the liberation of adenylic acid from the polynucleotides of the Nissl substance, and its consequent importance in the formation of ATP and the synthesis of acetylcholine. See text.

the presence of free adenylic acid will form the ATP necessary for the synthesis of acetylcholine (see the diagram fig. 2). *Undoubtedly the free adenylic acid is to an essential degree formed by the direct decomposition of the cytoplasmic polynucleotides (the Nissl substance) of the nerve cells, and it is in this primary liberation of adenylic acid that vitamin E plays a role owing to its tissue affinity.*

As a working hypothesis this is in harmony with the essential structural effect of vitamin E deficiency, the tissue dystrophy, which is so characteristic in the gray substance of the nervous system, in the muscles, and in the seminiferous epithelium of the testicles. That vitamin E may be involved

in a special way in acetylcholine metabolism is suggested by the fact that in the neuromuscular dystrophy produced by vitamin E deficiency the content of cholinesterase of the atrophic muscles is very considerably reduced (*Stoerk & Morpeth,* 1944). It has also been maintained that α-tocopherol stimulates the synthesis of acetylcholine (*Torda & Wolff,* 1945), and in my opinion this is due to its action in promoting the liberation of adenylic acid for the production of ATP. *Whether the cronic structural effect of vitamin E deficiency is due to removal of a factor inhibiting oxidation or to a defective occupation of special tissue affinities, it will always histochemically manifest itself by a reduced decomposition of the polynucleotides of the nerve cell and by a defective liberation of adenylic acid; this is the gist of the matter.*

III. POLIOMYELITIS

How, then, are the structural effects of poliomyelitis to be explained? The initial chromatolysis and the subsequent acute cellular dissolution are due to a direct action of the virus (see *Einarson,* 1949). However, this can scarcely be the case in the chronic changes (extreme chromophily, lipodystrophy, cellular atrophy), since these do not occur until later, when the acute changes have passed, or in close relation to the regeneration of the nerve cells; thus the chronic changes are secondary, as compared with the acute ones. We might possibly speak of an indirect effect or an after-effect of the virus.

Which factors can be surmised to be instrumental in the development of the chronic changes of the anterior horn cells? In my opinion, three possibilities ought especially to be considered, namely:

1) The changes are due to a secondary chronic, sublethal anoxia,

or

2) There is a defective utilization of vitamin E (dysvitaminosis), in which a surplus of vitamin E must be

supplied to the nerve cells for the restoration of their normal activity,

or

3) We are dealing with transneuronal cellular changes (see *Einarson & Lorentzen*, 1946) due to a primary injury of spinal internuncial neurons and reflex pathways.

Chromophoby is a cytological expression of increased impulse activity, and must be associated with an increased decomposition and resynthesis of acetylcholine. In the same way, extreme chromophily and cellular atrophy are always, whether they are due to sublethal, chronic anoxia, vitamin E deficiency, or transneuronal degeneration, expressions of interrupted impulse activity and must be associated with reduced or arrested decomposition and resynthesis of acetylcholine. Irrespective of which of the three factors plays the most important rôle in the pathogenesis of the chronic changes, it seems to me to be worth while, as part of the after-treatment of poliomyelitis, to administer large doses of vitamin E simultaneously with the physiotherapeutic treatment and training of the muscles. *From a theoretical point of view it is not impossible that the administration of vitamin E might be an adjuvant to the physical training and orthopaedic support of the paretic muscles, and I would recommend a dose of 30-60 mg α-tocopherol by mouth daily over a long period (1 to 2 years).* New evidence, both clinical and experimental supports *the view that α-tocopherol should be given by mouth;* the parenteral administration of the vitamin is less effective, suggesting that α-tocopherol undergoes some important changes in the gastro-intestinal tract (see, e.g. *Milhorat & Bartels*, 1945). It will, of course, be difficult to evaluate the results, but nevertheless one must ask why vitamin E should not be given during the acute stage of the disease, or even as a prophylactic, especially to persons who have been in contact with poliomyelitis patients or live in their immediate surroundings. In this connection I want to cite the following statement of *Bicknell & Prescott* (1942, p. 553): "*Sabin &*

Duffy from experimental work on young mice believes that vitamin E plays a part in the resistance of the nervous system to virus infections. *Einarson & Ringsted*'s experimental work stresses that the lower motor neurons degenerate when there is a deficiency of vitamin E. These two observations suggest that vitamin E might be of value in increasing the resistance of children to infantile paralysis."

Incidentally, it is possible that the suggested vitamin E treatment should be combined with the administration of factors from the vitamin B group. In this connection I will mention the work of *Milhorat & Bartels* (1945), which indicates that the utilization of α-tocopherol in patients suffering from muscular dystrophy is increased by the simultaneous administration of inositol. The combination of both these factors was found to be many times as effective in reducing creatinuria as wheat germ oil alone, and both factors should be given by mouth.

It is possible that a tocopherol-inositol condensation product is formed in the gastrointestinal tract, and that the constitutional defect in muscular dystrophy is a defect in this process of condensation, and that the patients need the condensation product itself. *It might therefore be worth while to administer an extract of hog stomach and duodenum (ventriculin, pylorin) simultaneously with α-tocopherol and inositol, and this treatment should be tried not only in cases of muscular dystrophy and spinal atrophy, but also in poliomyelitis.*

Actually, this is in full agreement with the view, expressed 11 years ago by *Einarson & Ringsted,* that some components of the vitamin B complex might require the coexistence of vitamin E for their action, and that it would be conceivable that a certain balance between factors of the B group on one side and vitamin E on the other side (*Einarson & Ringsted,* 1938, p. 153) may be necessary.

Finally, I want to add a few remarks on the vulnerability of the motor cells in relation to their localization in the anterior horns. It was recognized long ago that the various

cell groups are not affected to the same degree, and attempts have been made to explain this fact by the distribution of the vessels within the anterior horns, as it was thought that the distribution and extent of the process was conditioned by the various architectonic vascular areas. This explanation is, however, untenable, for within the same vascular area some of the cells of the groups in question may be severely affected, while others remain normal. The explanation of the varying degree of vulnerability has therefore been sought in the hypothesis that the individual cells possess varying degrees of resistance or susceptibility at the onset of the disease, and that such conditions may play a certain rôle. Obviously, if the process is of sufficient intensity, all the cells may be affected and destroyed almost simultaneously. Hence the varying vulnerability of the cells will be most apparent in cases where the process is less intense and less widespread.

On reviewing the litterature I found certain common features with regard to the localization of the most vulnerable cells in the anterior horns, which are in full agreement with my own observations. Thus *Schwalbe* (1902, p. 492-494) reported that when the process was of a moderate extent, it was the cells localized in the central parts of the anterior horns which were first affected, and that there was a marked tendency for the process to spread upwards and downwards in the central parts of the anterior columns. On the other hand, the cells localized along the outer periphery of the anterior horns were much better preserved; incidentally, *Schwalbe* found that the medial cell groups were slightly more vulnerable than the lateral groups. Practically the same findings have later been reported, e.g. by *Horányi-Hechst* (1935, p. 34-35). *Warburg* (1931, p. 1208-1211) described exactly the same findings in her material of experimental, chronic poliomyelitis and concludes by saying: "*There was also a variable number of cells approximating the normal, generally near the periphery of the anterior horns*". Finally, *Elliot* (1945) found that the dorsomedial cell group was the first to be affected, and that the process spread from here, via the central part

of the anterior horn, in the direction of its ventrolateral periphery, so that the cells along the periphery were preserved for the longest time. *In my own material I have found ample evidence of the fact that the nerve cells in the central parts of the anterior horns are most vulnerable to the poliomyelitic process, and therefore they are attacked first, whereas the cells along the periphery are more resistant and accordingly the last to be attacked.*

How is this very important fact to be explained? Here it is of importance that the same picture, i.e., breakdown and atrophy of the central cells with preservation of the peripheral cells, may be produced experimentally with considerable accuracy. Thus *Einarson & Ringsted* (1938) produced this picture of the anterior horns in adult rats by experimental chronic vitamin E deficiency. The cells localized at the periphery of the anterior horn, which are more resistant to this deficiency, innervate principally the extensors (the antigravity muscles), while the more vulnerable central cells supply the adductors and flexors, and it is just these muscles which are attacked first and most severely (see *Einarson & Ringsted,* 1938, pp. 72-77 and 117). The same picture has also been produced very clearly by *Erik Krogh* (1944, 1945) by experimental acute anoxia of the lumbar portion of the spinal cord of the rabbit. The anoxia was produced by a specially designed clamp (*Häggqvist,* 1938) by which the aorta was compressed against the ventral surface of the spinal column. By injection of radioactive Na *Krogh* established the fact that a very slow circulation took place in the capillaries in the parts thus occluded, so that it would take an hour or more before the blood in the capillaries would be completely renewed; under normal conditions this renewal lasts less than a minute. Furthermore, injection preparations with India inkgelatin showed that there was no difference between the density of the capillary network in the centre and in the periphery of the anterior horn respectively. The difference in the vulnerability of the peripheral and the central cells of the anterior horn to anoxia is thus not due to a denser or sparser vascu-

larization respectively, but to the position of the cells in relation to the capillary. *Krogh* made the very important observation *that the arteries, even those passing through the centre of the anterior horn, split up into capillaries in the periphery of the anterior horn, while the veins are found in and radiate from the centre. Krogh*'s explanation of the difference in the vulnerability of the cells is based on this fact. He says, "*The result of the examination of cleared preparations is therefore that the cells in the periphery of the anterior horn are in the main close to the arterial ends of the capillaries, while the central cells lie close to the venous ends. By the very slow circulation produced by the occlusion the peripheral cells will have an opportunity to use up most or all of the oxygen available, and this is the reason why they better resist the lack of oxygen*". (*Erik Krogh*, 1945, p. 280).

Finally, *Krogh* discussed the possibility that the greater resistance of the peripheral cells to vitamin E deficiency compared with the central ones, as shown by *Einarson & Ringsted*, may be due to the same cause. That there may arise a difference in the vitamin E concentration in the arterial and venous ends of the capillaries respectively in spite of the normal rapid circulation, is due to the fact, that vitamin E like all other fat-soluble substances diffuses very easily and quickly through the capillary walls in the central nervous system. It is thus possible that, although the quantities of vitamin E are actually insufficient, the cells in the periphery of the anterior horn may take up any small amounts of vitamin E which may be present in the "vitamin E-free diet", or may still be mobilized from the depots of the animal, and may, therefore, resist the vitamin deficiency better and longer than the cells in the central part of the anterior horn.

It is not only possible, but even reasonable, to assume that the explanation of the varying degree of vulnerability of the anterior horn cells in poliomyelitis is to be found in the conditions discovered by *Erik Krogh*. Thus it may be surmised that the increased need for oxygen and vitamin E arising during the poliomyelitic process may manifest itself to such

an extent that these substances, so vital to the cells, are almost or entirely consumed in the arterial ends of the capillaries, and accordingly little or nothing is left for the central cells, lying at the venous ends of the capillaries. *In these cases the quality of the blood with regard to its content of oxygen and vitamin E within the capillaries is of decisive importance to the resistance of the cells.*

IV. ON THE ISOELECTRIC POINT OF THE NISSL SUBSTANCE AND ITS IMPORTANCE TO THE STAINING REACTIONS

As already mentioned in the introduction, the structural stages of chromophily and chromophoby as well as chromatolysis involve a shift in the isoelectric point of the Nissl substance as I showed 12 years ago (*Einarson,* 1937) and later discussed further (*Einarson,* 1945; *Einarson & Lorentzen,* 1946). This shift occurs independently of the conditions under which the structural changes mentioned have developed (vitamin E-deficiency, anoxia, poliomyelitis).

For this special purpose toluidin blue and cyanol were used as basic and acid stain respectively, and the pH of the dye solutions was controlled between 1.40 to 4.64 by adding certain amounts of *Sørensen*'s citrate—n/10 HCl buffers whereupon the staining intensity was measured photometrically. The tissue sections were washed both before and after staining and were finally embedded under the cover glass in a buffer solution of exactly the same pH as the staining solution concerned. The calculation and the preparation of these buffer solutions is of vital importance to the investigation, as their use as embedding medium is the only absolutely safe procedure to avoid any even slight artificial weakening of the staining intensity before the photometric measurement is performed. Thus any trace of differentiation or extraction of the stain is eliminated. The staining intensity is expressed in mm, as the oscillation of a beam of light reflected upon a mm scale by a mirror galvanometer (see fig. 3). Thus at a certain pH of the staining solution the intensity of the light

passing through a given cell will be a measure of the inherent capacity of that cell to bind the stain.

By entering the pH values of the staining solutions along the abscissa, and the staining intensity in mm along the ordinate, it is possible to draw the toluidin blue and cyanol curves for chromoneutrality, chromophily and chromophoby. The point of intersection between the toluidin blue and cyanol curves respectively represents the approximate location of the isoelectric point of the Nissl substance; fig. 3 shows the curves for chromoneutrality.

In chromoneutral cells the isoelectric point of the Nissl substance lies at approximately pH 2.7 (see fig. 3); with increasing chromophoby the isoelectric point shifts towards the alkaline side, and in an extremely chromophobe cell it may reach pH 3.5, while in extreme chromophily it has shifted toward the acid side as far as pH 1.9. The maximum difference in the position of the isoelectric point corresponds to an electromotive force of approximately the same order of magnitude as the current of action.

In chromoneutrality the steep fall of the toluidin blue curve begins at approximately pH 3.25, at pH 2.7 the staining has reached its minimum; it disappears entirely on the acid side of the isoelectric point (fig. 3). In the cyanol curve the steep fall begins at pH 2.25; at pH 2.7 the staining reaches its minimum and it disappears entirely on the alkaline side of the isoelectric point (fig. 3). When the quantity of components with acid properties (nucleoproteins) increases in the Nissl substance, the latter must assume a greater negative charge and thus it will acquire an increased capacity for binding basic stains; the result is chromophily of the cell. On the other hand, when there is a quantitative decrease of the acid components with a simultaneous proportional increase in components with basic properties (basic proteins), the Nissl substance must acquire relatively more positive charges (decreased negativity), and it will therefore show a diminished capacity for binding basic stains, and an increased capasity for binding acid stains; the result is chromophoby of the cell.

By far the most accurate method of evaluating quantitatively these cellular conditions is, however, staining with gallocyanin-chromalum, since this stain enters into a very selective and stable compound with the polynucleotides of the cells. The photometric estimation of the staining intensity of gallocyanin-chromalum thus gives a measure of the cellular

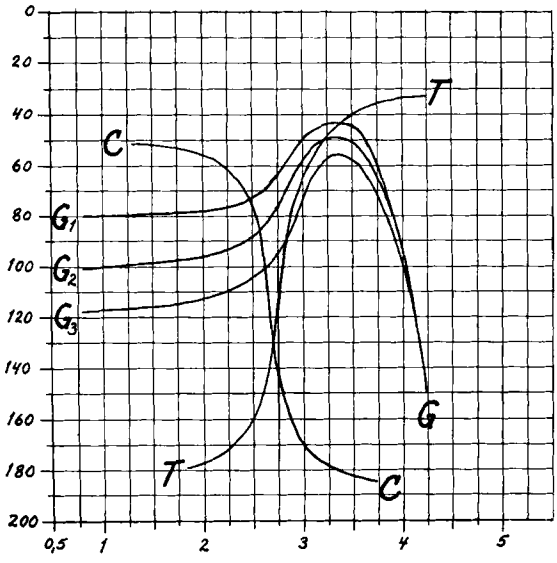

Fig. 3.

T-T and C-C showing the toluidin blue and the cyarol curves for chromoneutrality of the nerve cells; the isoelectric point of the Nissl substance lies at pH 2.7. G-G_1, G-G_2 and G-G_3 representing the gallocyanin curves for extreme chromophily, chromoneutrality and extreme chromophoby respectively. See text.

content of nucleic acids, as it may vary, e.g. in vitamin E-deficiency, anoxia and poliomyelitis.

The selectivity of gallocyanin-chromalum staining and its different nature from that of the usual basic blues, can be seen from the intensity curves of the staining; fig. 3 shows also the gallocyanin curves for extreme chromophily, chromoneutrality and extreme chromophoby respectively. The slight ascent of the curves from pH 0.8 to pH 2.0, which becomes

steeper from pH 2.0 to pH 2.7, is due to the increasing, adsorption of the stain, i.e. the unspecific co-staining of the tissue. This would mean a source of error in the photometric estimation of the intensity of the specific staining if the adsorption were not so slight; at pH 1.64, which is the acidity of the staining solution we usually use, the adsorption is rather negligible and practically unimportant. The intense specific staining in the whole range of pH on the acid side of the isoelectric point *is due to the selective binding of the stain to the polynucleotides of the Nissl substance and the nucleolus, to be explained by the strongly acid properties of the nucleic acids;* here no staining of the proteins can take place (see *Einarson,* 1947, p. 7-9). On the other hand the steep rise of the curves on the alkaline side of the isoelectric point, reaching a peak at pH 3.42, is due to a binding of the stain to the proteins of the Nissl substance in addition to the staining of its polynucleotides (see *Einarson,* 1947, p. 10). The curves fall steeply toward zero from approximately pH 3.6, i.e. the staining fades away; at pH 4.25 it is just perceptible. *The conclusion is that the staining intensity obtained by means of a gallocyanin-chromalum solution, the pH of which lies on the acid side of the isoelectric point of the Nissl substance (e.g. pH 1.64 or less), gives a fairly accurate measure of its content of nucleic acids; most probably the gallocyanin lake-ion+ become attached to the phosphoric acid groups of the polynucleotides.* (*Einarson,* 1947, p. 13).

The toluidin blue and cyanol curves are typical of staining by electrostatic adsorption. The proteins being amphoteric electrolytes, only combine with cations and positively-charged stains on the alkaline side of the isoelectric point, and with anions and negatively-charged stains only on the acid side. At their isoelectric point the proteins combine with only small amounts of both basic and acid stains.

On the acid side of the isoelectric point of the Nissl substance the gallocyanin lake-ion+ only combines with the nucleic acids of the cell structures; in the range of pH 0.83-2.7 the binding of the positively-charged stain is independent

of the ionisation of the proteins. *Thus the gallocyanin lake-ion+ possesses a selective affinity for nucleic acids and it may be characterized and evaluated quantitatively as a histochemical staining reaction.*

V. EXTREME CHROMOPHILY AND LIPODYSTROPHY OF THE NERVE CELLS

I have emphasized repeatedly that the state of extreme chromophily of a nerve cell, characterized by the great content of substance stainable with gallocyanin-chromalum, gradually proceeds to irreparable cell atrophy, the final outcome of which is a pale cell shadow from which the Nissl substance has disappeared (e.g. *Einarson,* 1949). Frequently this process is associated with lipodystrophy and vacuolication of the cell (see *Einarson & Lorentzen,* 1946, p. 62-64). As the substance stainable with gallocyanin-chromalum gradually disappears it is replaced by a peculiar, diffuse or densely packed, fatty substance which has a rather dark, greyish-yellow appearance, and sometimes shows a more distinct granular or corpuscular distribution. Undoubtedly we are dealing with a mixture of lipoids, proteins and some lipoproteins, which do not contain nucleic acids; these degeneration products increase simultaneously with the disappearance of the nucleic acids of the Nissl substance.

Finally, the nerve cells may become filled with these lipoid and protein substances, which stain intensely with toluidin blue and often with hematoxylin as well, but which are left entirely unstained by gallocyanin-chromalum (see figs. 4 and 5). This is important *since the staining with toluidin blue will simulate extreme chromophily, while gallocyanin-chromalum reveals that this is actually a lipodystrophy of the cell, and very little or nothing of the selectively stainable substance remains* (figs. 4 and 5).

The lipodystrophic change of the nerve cells occurs in the final stage of chronic vitamin E deficiency (fig. 4), in chronic poliomyelitis (fig. 5), as a chronic after effect of partial or

sublethal anoxia and in amyotrophic lateral sclerosis (see *Einarson & Ringsted,* 1938, *Einarson & Lorentzen,* 1946 and *Einarson,* 1949), and like true extreme chromophily is most frequently seen in the human cerebral cortex from various pathological conditions and mental disorders.

In a recent work *Hochberg & Hydén* (1949) studied by ultraviolet microphotography and absorption measurements

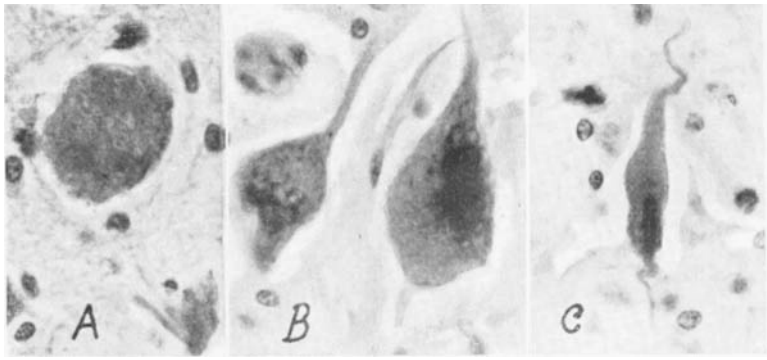

Fig. 4.

Lipodystrophy and irreparable atrophy of spinal motor cells from an adult rat in the final stage of chronic vitamin E deficiency, with severe paralyses and muscular atrophy. A) The cell is filled with dark lipoid-protein substances; B) cells with still visible remnants of stainable substance; C) a cell showing irreparable atrophy with corkscrew-shaped dendrites.

Gallocyanin-chromalum, pH 1.64. × 550.

the motor nerve cells to from rabbits with spastic paralysis produced by occluding the abdominal aorta according to *Häggqvist*'s method. They claim that they examined nerve cells, which showed extreme chromophily, and found that they contained no measureable quantities of nucleic acids. On the other hand the physical condition of the cell substance was altered and the intense ultraviolet absorption was caused by the considerable unspecific losses of light due to scattering in the specimen. The authors consider that this result contradicts my interpretation that nerve cells showing extreme

chromophily contain large concentrations of nucleic acids, as evidenced by their intense stainability with gallocyanin-chromalum.

However, there is no doubt that the cells studied by *Hochberg & Hydén* were not in the state of true extreme chromophily, as described by me after staining with gallocyanin-chromalum, *because in pure chromophily of the kind there is*

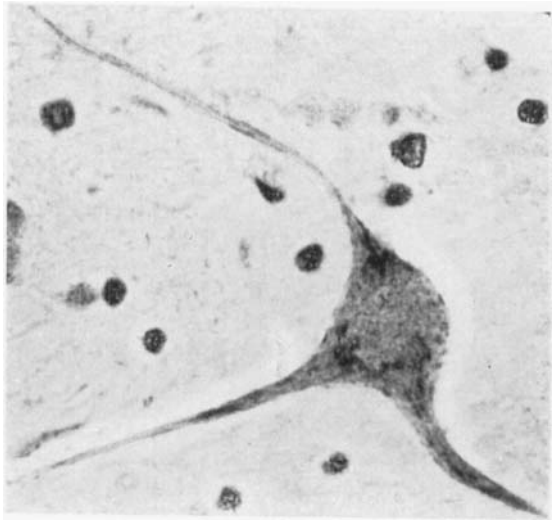

Fig. 5.
Lipodystrophy of a spinal motor cell in chronic poliomyelitis. Some remnants of stainable substance are still visible. Patient died 8 months after the onset of rather severe and widespread paralyses. Gallocyanin-chromalum. pH 1.64. × 600.

never any sign of vacuolization, and the cells of Hochberg & Hydén showed pronounced vacuolization throughout their entire cytoplasm. Vacuolization, lipodystrophy and granular protein degeneration, when following extreme chromophily as secondary changes, first set in at the subsidence or gradual disappearance of true chromophily. On the other hand vacuolization and lipodystrophy are frequently met with as primary changes in or after anoxia.

Further, *Erik Krogh* (Acta Jutlandica, 1945) has con-

clusively demonstrated *that true extreme chromophily (gallocyanin stain) never occurs as the primary result of acute anoxia as produced by means of the Häggqvist aorta clamp.* This particular paper by *Krogh* is quoted in the list of references of *Hochberg & Hydén*'s paper, but it is not mentioned in their text, although *Krogh* uses exactly the same occlusion technique as *Hochberg & Hydén* and *Krogh*'s paper was published 3½ years earlier; otherwise *Hochberg & Hydén*'s paper is very interesting and important. Probably the intense ultraviolet absorption in these dark cells of *Hochberg & Hydén*, caused by the unspecific losses of light, has been produced by the lipoid-protein degeneration substances of the cells, but this condition must not be confused with true extreme chromophily as it appears after staining with gallocyanin-chrom-alum.

According to *Alsterberg* (1948) the lipoids and lipoproteins of the nerve cells which can be stained by his own special JCN-AgC10$_3$ method are different from the Nissl substance, and are located between the Nissl bodies. After treatment with cold pyridin the Nissl bodies disappear, while the lipoid-protein substances are still sharply stained. This is in accordance with my own experience that pyridin, NH_4OH, NaOH and KOH partly dissolve the Nissl bodies and the nuclear substance, while the surrounding cytoplasm and the karyoplasm stain diffusely with gallamin blue (*Einarson*, 1935, p. 108-113).

The conclusion is that I must maintain my interpretation of the state of true extreme chromophily as being tantamount to large concentrations of nucleic acids in the nerve cells. All our work on experimental inhibition, stimulation, anoxia, vitamin E deficiency etc. and observations from human material strongly support the view *that the accumulation of nucleic acids in the nerve cells of extreme chromophily is due to a reduced consumption and decomposition of the polynucleotides of the cells and to a defective liberation of adenylic acid.* Then ATP is not formed and the ensuing arrest of the synthesis of acetylcholine means an interruption of the impulse transmission of the neurons concerned. *Thus the struct-*

ural state of extreme chromophily of a nerve cell signifies a total cessation of its impulse activity, which may be either temporary or persistent, whether it is produced by chronic, sublethal anoxia, chronic vitamin E deficiency, chronic poliomyelitis or other noxious conditions.

Important evidence supports the assumption that α-tocopherol acts by stimulating the liberation of adenylic acid from the polynucleotides of the Nissl substance, and thus α-tocopherol might possibly help in releasing the nerve cells from their torpid state of extreme chromophily, if it is given before the condition has become irreversible.

SUMMARY

The Nissl substance of the nerve cells is composed of polynucleotides, acid and basic proteins respectively.

The occurence of extreme chromophoby of the nerve cells (gallocyanin stain) is due to a reduction or depletion of the polynucleotides of the cells; it corresponds to a state of increased and prolonged physiological activity of the cells (a state of excitation) and represents the initial structural effect of acute anoxia and vitamin E deficiency respectively. It signifies an increased decomposition of the polynucleotides of the cells with an ample liberation of adenylic acid, which is necessary for the constant reformation of ATP, for which there is a great need in the central nervous system owing to the ample consumption and resynthesis of acetylcholine. Structurally, extreme chromophoby closely resembles the initial chromatolysis of acute poliomyelitis.

Extreme chromophily of the nerve cells (gallocyanin stain) is due to an increase or accumulation of polynucleotides in the cells; it signifies a cessation of the physiological impulse activity of the cells (a state of inhibition or totally depressed activity) and represents the late or retarded structural effect of chronic, sublethal anoxia, protracted vitamin E deficiency or chronic poliomyelitis. It is due to a reduced consumption and decomposition of the polynucleotides of the

cells with a consequent defective liberation of adenylic acid; and the ensuing lack of ATP means an arrest of the formation of acetylcholine.

Within a nerve cell the essential source of adenylic acid is the Nissl substance, of which ribonucleic acid (polynucleotides) is a most import component. Thus the liberation of adenylic acid takes place from the cytoplasmic polynucleotides of the nerve cells. Important evidence suggests that vitamin E stimulates this liberation of adenylic acid, which subsequently becomes phosphorylated to form ATP. This phosphorylation may possible be carried out by acetylphosphate, which at the same time may act as acetyl donor in the synthesis of acetylcholine.

In poliomyelitis the chronic changes of the nerve cells (extreme chromophily, lipodystrophy, irreparable atrophy) may be due to a secondary chronic, sublethal anoxia, to a defective utilization of vitamin E (dysvitaminosis) or to transneuronal degeneration. Irrespective of which of the three possibilities plays the most important rôle, and whether vitamin E acts by regulating oxidation or by the occupation of special tissue affinities, it is recommended that the administration of large doses of α-tocopherol (30-60 mg by mouth daily) to poliomyelitis patients should be tried as a supplementary treatment to the physical training and orthopaedic support of the paretic muscles; α-tocopherol should perhaps be given in combination with inositol; parenteral administration is less effective.

The greater vulnerability in poliomyelitis of the motor cells located in the central part of the anterior horn as compared with the greater resistance of the cells situated in its periphery, may be due to the circumstance that the peripheral cells lie close to the arterial end of the capillaries, while the central cells lie close to the venous end. If in poliomyelitis the need for oxygen and vitamin E is raised, the amounts available may be consumed at the arterial ends of the capillaries, so that little or none is left for the central cells lying at the venous ends.

In chromoneutral cells the isoelectric point of the Nissl substance lies at pH 2.7; the figure is important for staining. On the acid side of the isoelectric point the gallocyanin lake-ion+ combines only with the nucleic acids of the cell structures; in the range of pH 0.83-2.7 the binding of the positively-charged stain is independent of the ionisation of the proteins. At an appropriate pH the intensity of the staining with gallocyanin-chromalum gives an accurate measure of the content of nucleic acids in the cell structures; most probably the gallocyanin lake-ion+ becomes attached to the phosphoric acid groups of the polynucleotides.

Extreme chromophily of a nerve cell may proceed to irreparable atrophy and lipodystrophy, in which the gallocyanin-stainable substance is replaced by lipoid-protein degeneration products. The latter stain intensely with toluidin blue and hematoxylin, but are left entirely unstained by gallocyanin-chromalum, which differentiates sharply between the state of true extreme chromophily and the state of lipoid-protein dystrophy of a nerve cell. Most probably these lipoid-protein substances cause the intense ultraviolet absorption due to scattering and unspecific losses of light in the preparation, but in such cases the dark ultraviolet cell pictures or the toluidin blue pictures must not be confused with true extreme chromophily. At an appropriate pH (1.64 or less) gallocyanin-chromalum gives no unspecific co-staining.

RESUME

La substance Nissle des cellules nerveuses se compose respectivement de polynucléotides respectivement, de protéines acides et basiques.

Lorsqu'on se trouve en présence d'une chromophobie extrême des cellules nerveuses (coloration à la gallocyanine), c'est qu'il y a réduction ou disparition des polynucléotides des cellules. Ceci correspond à un état d'activité physiologique accrue ou prolongée des cellules (un état d'excitation) et représente l'effet structural initial d'une part de l'anoxie aiguë

et d'autre part d'une déficience en vitamine E. Ceci signifie par ailleurs qu'il y a décomposition accrue des polynucléotides des cellules avec libération appropriée d'acide adénylique nécessaire à la régénération constante de ATP dont il y a un fort besoin dans le système nerveux central par suite de la forte consommation et de la resynthèse de l'acétylcholine. La chromophobie extrême ressemble beaucoup au point de vue structural à la chromatolyse initiale dans la poliomyélite aiguë.

La chromophilie extrême des cellules nerveuses (coloration à la gallocyanine) est due à une augmentation ou une accumulation des polynucléotides dans les cellules; elle indique qu'il y a cessation de l'activité impulsive physiologique des cellules (un état d'inhibition ou d'activité entièrement supprimée) et représente l'effet structural tardif ou retardé soit d'une anoxie chronique sublethale avec déficience de vitamine E, soit de poliomyélite chronique. Elle est due à une consommation réduite et à la décomposition des polynucléotides des cellules avec libération défectueuse d'acide adénylique; le manque d'ATP qui s'ensuit entraîne la cessation de la production d'acétylcholine.

Dans les cellules nerveuses, la substance Nissle est la principale source de production d'acide adénylique et l'acide ribonucléique provient donc des polynucléotides cytoplasmique des cellules nerveuses. Un fait important indique que la vitamine E stimule cette libération d'acide adénylique qui produit ensuite de l'ATP par phosphorylisation. Cette phosphorylisation est peut-être produite par des acétylphosphate qui peuvent en même temps jouer le rôle de donneur d'acétyle dans la synthèse d'acétylcholine.

Dans la poliomyélite les modifications chronique des cellules nerveuses (extrême chromophilie, lipodystrophie, atrophie irrémédiable) sont dues à une anoxie chronique sublethale secondaire, une utilisation défectueuse des vitamines E (dysvitaminose) ou une dégénération transneurale. Quelle que soit celle de ces trois possibilités qui joue le plus grand rôle et si la vitamine E agit comme régulateur d'oxydation ou possède

certaines affinités de tissu, il est recommandé de donner de fortes doses de α-tocophérol (30—60 mg par jour et par la voie buccale) aux malades souffrant de poliomyélite comme un traitement complémentaire au traitement physique et orthopédique des muscles paralysés. Il faut peut-être administrer le α-tocophérol simultanément avec de l'inositol; l'administration parentérale est moins efficace.

La plus grande vulnérabilité dans la poliomyélite des cellules motrices situées dans la partie centrale de la corne antérieure, comparée à la plus grande résistance des cellules de la périphérie est peut-être due au fait que les cellules périphériques sont rapprochées de l'extrémité arteérielle des capillaires tandis que les cellules centrales sont rapprochées des extrémités veineuses. Si le besoin d'oxygène et de vitamine E est accru dans la poliomyélite, il est possible que la quantité qui est disponible soit consumée dans l'extrémité artérielle des capillaires et qu'il n'en reste que peu ou point pour les cellules centrales situées autour des extrémités veineuses.

Dans les cellules chromoneutrales le point isoélectrique de la substance Nissle est à pH 2,7; sa situation est importante pour la coloration. Du côte acide du point isoélectrique le ion-laque de la gallocyanine + n'est combiné qu'avec les acides nucléiques des structures cellulaires; dans pH = 0,83—2,3 la fixation de la couleur positive est indépendante de la ionisation des protéines. Avec un pH approprié, l'intensité de la coloration au chromalum-gallocyanine donne une indication exacte de la teneur en acides nucléiques de la structure cellulaire; l'ion-laque de la gallocyanine + est plus vraisemblablement fixé aux groupes de l'acide phosphorique des polynucléotides.

La chromophilie extrême d'une cellule nerveuse peut devenir de l'atrophie irrémédiable et de la lipodystrophie dans laquelle la substance colorable à la gallocyanine est remplacée par des produits de dégénération des protéines lipoïdiques. Ces derniers sont fortement colorés au bleu toluidine et à l'hématoxyline mais sont absolument incolorables au chromalum-gallocyanine, ce qui différentie nettement l'état d'extrême

chromophilie véritable et l'état de dystrophie des protéines lipoïdiques d'une cellule nerveuse. Il est très probable que ces substances de protéines lipoïdiques provoquent une absorption ultraviolette intense par suite d'une perte disséminée et non spécifique de la lumière dans la préparation, mais dans ces cas le dessin de la cellule ultraviolette foncée ou celui de la toluidine bleue ne doit pas être confondu avec la chromophilie extrême réelle. Avec un pH approprié (1,64 ou moins), le chromalum-gallocyanine ne donne pas de coloration auxiliaire non spécifique.

ZUSAMMENFASSUNG

Die Nissl'schen Körperchen der Nervenzellen bestehen aus Polynucleotiden, aus sauren, bezw. basischen Proteinen.

Das Auftreten extremer Chromophobie der Nervenzellen (Gallocyanin-Färbung) ist auf eine Reduktion oder auf den völligen Verlust der Polynucleotide der Zellen zurückzuführen; es entspricht einem Zustande erhöhter oder verlängerter physiologischer Aktivität der Zellen (einem Zustande der Erregung) und stellt die beginnende strukturelle Wirkung einer akuten Anoxie, bezw. eines Vitamin E-Mangels dar. Es ist ein Zeichen vermehrter Zersetzung der Polynucleotide der Zellen mit reichlicher Freisetzung von Adenylsäure, die für die konstante Neubildung des ATP erforderlich ist, für das im Zentralnervensystem infolge starken Verbrauchs und Wiederaufbaus von Acethylcholin ein grosses Bedürfnis vorhanden ist. Die extreme Chromophobie hat strukturell eine grosse Aehnlichkeit mit der anfänglichen Chromatolyse bei akuter Poliomyelitis.

Die extreme Chromophilie der Nervenzellen (Gallozyanin-Färbung) beruht auf einer Vermehrung oder Akkumulation der Polynucleotiden der Zellen; sie ist ein Zeichen dafür, dass die physiologische Impulsaktivität der Zellen aufgehört hat (ein Zustand behinderter oder vollständig unterdrückter Aktivität), und sie stellt die späte oder verzögerte strukturelle Auswirkung einer chronischen, subletalen Anoxie, eines pro-

trahierten E-Vitaminmangels oder einer chronischen Poliomyelitis dar. Sie hat ihre Ursache in reduziertem Verbrauch und Zersetzung der Polynucleotiden der Zellen mit einer defekten Freisetzung von Adenylsäure; und der daraus folgende Mangel an ATP bedeutet ein Aufhören der Acetylcholinbildung.

Die wichtigste Quelle der Adenylsäure in den Nervenzellen sind die Nissl'schen Körperchen, von denen Ribonucleinsäure eine sehr wichtige Komponente ist; die Freisetzung der Adenylsäure geht also von den cytoplasmatischen Polynucleotiden der Nervenzellen aus. Wichtige Tatsachen sprechen dafür, dass das E-Vitamin diese Freisetzung von Adenylsäure stimuliert, die dann durch Phosphorylisierung das ATP bildet. Diese Phosphorylisierung geht vielleicht mit Hilfe von Acetylphosphat vor sich, das gleichzeitig in der Synthese des Acetylcholins als Acetyldonor auftreten kann.

Bei Poliomyelitis können die chronischen Veränderungen in den Nervenzellen (extreme Chromophilie, Lipodystrophie, irreparable Atrophie) auf einer sekundären chronischen, subletalen Anoxie, auf einer defekten Ausnutzung des E-Vitamins (Dysvitaminose) oder auf einer transneuralen Degeneration beruhen. Ohne Rücksicht darauf, welche der drei Möglichkeiten die wichtigste Rolle spielt, und ob die Wirkung des Vitamin E auf einer Regulierung der Oxydation oder auf einer besonderen Gewebsaffinität beruht, empfiehlt es sich, bei Poliomyelitis-Patienten eine Zufuhr von grossen Dosen von α-Tocopherol (30—60 mg täglich oral) als unterstützende Behandlung der physikalischen Behandlung und orthopädischen Stützbehandlung der gelähmten Muskeln zu versuchen; α-Tocopherol sollte vielleicht mit Inositol zusammen gegeben werden; eine parenterale Zufuhr ist weniger wirksam.

Bei der Poliomyelitis ist die grössere Empfindlichkeit der motorischen Zellen im zentralen Teil des Vorderhorns im Vergleich mit der grösseren Widerstandskraft der Zellen seiner Peripherie vielleicht auf den Umstand zurückzuführen, dass die peripheren Zellen dicht am arteriellen Ende der Kapillaren liegen, während die zentralen Zellen dicht an den venösen

Enden liegen. Wenn der Bedarf an Sauerstoff und E-Vitamin bei Poliomyelitis vermehrt ist, wird vielleicht die zur Verfügung stehende Menge im arteriellen Ende der Kapillaren verbraucht, weshalb für die zentralen Zellen, die um die venösen Enden herum liegen, nur wenig oder gar nichts mehr übrig bleibt.

In den chromoneutralen Zellen liegt der isoelektrische Punkt für die Nissl'schen Körperchen bei einem pH von 2,7; seine Lage ist für die Färbung von Bedeutung. Auf der sauren Seite des isoelektrischen Punktes verbindet sich das Gallocyanin-Lack-Ion nur mit den Nucleinsäuren der Zellenstruktur; innerhalb eines pH von 0,83—2,7 ist die Bindung des positiv geladenen Farblackes unabhängig von der Ionisation der Proteine. Bei einem entsprechenden pH ergibt die Intensität der Färbung mit Gallocyanin-Chromalum ein genaues Mass für den Gehalt der Zellenstruktur an Nucleinsäuren; das Gallocyanin-Lack-Ion wird aller Wahrscheinlichkeit nach an die Phosphorsäure-Gruppe der Polynucleotiden gebunden.

Die extreme Chromophilie einer Nervenzelle kann bis zur irreparablen Atrophie und Lipodystrophie andauern, bei der die mit Gallocyanin färbbare Substanz von Degenerationsprodukten der Lipoide und Lipo-Proteine erstattet ist. Letztere lassen sich mit Toluidinblau und Hämatoxylin intensiv färben, können aber mit Gallocyanin-Chromalum überhaupt nicht gefärbt werden, was eine scharfe Unterscheidung gestattet zwischen einem Zustande wirklicher extremer Chromophilie und einem Zustande von Lipoid-Protein-Dystrophie einer Nervenzelle. Höchstwahrscheinlich verursachen diese Lipoid-Protein-Substanzen eine intensive ultraviolette Absorption infolge einer Lichtzerstreuung und eines unspezifischen Lichtverlustes in den Präparaten, aber in solchen Fällen darf man die dunklen ultravioletten Zellen-Bilder oder Toluidin-Blau-Bilder nicht mit wirklich extremer Chromophilie verwechseln. Bei einem richtigen pH (1,64 oder darunter) gibt Gallocyanin-Chromalum keine unspezifische Mitfärbung.

REFERENCES

Alsterberg, G.: Mikroskopie 1948, 3, H. 5/6, p. 136.
Bicknell, F. & F. Prescott: The vitamins in medicine. William Heinemann, London 1942; p. 553.
Bodian, D.: Symposia Soc. exp. Biol. 1947, Nr. 1, p. 163.
Einarson, L.: Am. J. Pathol. 1932, *8*, 295.
— Am. J. Anat. 1933, *53*, 141.
— Læknabladid 1934, *20*, 142.
— J. comp. Neurol. 1935, *61*, 101.
— Hospitalstidende 1937, *80*, 1.
— & *A. Ringsted:* Effect of chronic vitamin E deficiency on the nervous system and the skeletal musculature in adult rats. A neurotropic factor in wheat germ oil.—Copenhagen, 1938, 163 pp.
— Nordisk Medicin, 1941, *11*, 2082.
— Acta Jutlandica 1945, XVII, 1, 150 pp.
— & *K. A. Lorentzen:* Acta Jutlandica 1946, XVIII, 4, 116 pp.
— Ugeskr. f. Læger 1947, Nr. 6, February, p. 143.
— Acta orthopaed. scand. 1949, in press.
Elliott, H. C.: Am. J. Pathol. 1945, *21*, 87.
Eppstein, S. H. & S. Morgulis: J. Nutrition, 1941, *22*, 415.
— — Ibid, 1942, *23*, 473.
Gurdjian, E. S., J. E. Webster & W. E. Stone: Surg. Gynecol. and Obstet. 1944, *78*, 618.
Häggqvist, G.: Ztschr. f. mikr.-anat. Forsch. 1938, *44*, 169.
— Hygiea, 1938, *100*, 2, p. 224.
Hochberg, I. & H. Hydén: Acta physiol. scand. 1949, *17*, Suppl. 60. p. 46-51 a. 58.
Horányi-Hechst, B.: Dtsch. Ztschr. f. Nervenheilk. 1935, *137*, 1.
Houchin, O. B.: J. Biol. Chem. Chem. 1942, *146*, 313.
— & *H. A. Mattill:* Proc. Soc. Exp. Biol. a. Med. 1942, *50*, 216.
— — J. Biol. Chem. 1942, *146*, 309.
— — J. Biol. Chem. 1942, *146*, 301.
Hove, E. L., K. Hickman & P. L. Harris: Arch. Biochem. 1945, *8*, 395.
Krogh, Erik: Studier over Vascularisationsforholdene i Rygmarvens Lumbaldel. — Festskrift til Aug. Krogh, København, 1944, p. 13.
— Acta Jutlandica, 1045, XVII, Suppl. pp. 40.
— Acta physiol. scand. 1945, *10*, 271.
Lagerstedt, S.: Acta anatomica 1947, II, Fasc. 3/4, p. 392.
— Ibid, 1947, III, Fasc. 1, p. 84.
— Nordisk Medicin, 1948, *38*, Nr. 20, p. 997.
— Acta anatomica, 1948, *5*, no. 3, p. 217.
Milhorat, A. T. & W. E. Bartels: Science 1945, *101*, 93.
Morrison, L. R.: Arch. Neurol. & Psychiat., 1946, *55*, Nr. 1, p. 1.
Schwalbe, E.: Beitr. z. pathol. Anat. u. z. allg. Pathol., 1902, *32*, 485.

Stoerk, H. C. & E. Morpeth: Proc. Soc. Exp. Biol. a. Med., 1944, *57*, 154.
Stone, W. E., C. Marshall & L. F. Nims: Am. J. Physiol., 1941, *132*, 770.
Torda, C. & H. G. Wolff: Proc. Soc. Exp. Biol. a. Med., 1945, *58*, 163.
Victor, J.: Am. J. Physiol., 1934, *108*, 229.
Warburg, B.: Arch. Neurol. & Psychiat., 1931, *25*, 1191.