VARIATIONS IN THE BASOPHILIA OF NERVE CELLS ASSOCIATED WITH INCREASED CELL ACTIVITY AND FUNCTIONAL STRESS

BY

LÁRUS EINARSON and ERIK KROGH

From the Department of Anatomy, Aarhus University, the Institute of Brain Pathology of the State Mental Hospital, Aarhus, Denmark, and the Stazione Zoologica, Naples

All the large and medium types of nerve cell as well as some small, e.g., the small pyramidal cells of the cerebral cortex, possess a high degree of cytoplasmic basophilia, while in other small nerve cells, e.g., the small granule cells of the cerebellum and cerebrum, the basophilia is more or less confined to the nucleus (Einarson, 1945).

The high degree of cytoplasmic basophilia is due to the Nissl substance, the structural pattern of which differs in the various cell types. The Nissl substance contains at least three different components: nucleoprotein, acid and basic proteins. The first of these, the ribonucleic acid compound, is formed primarily round the nucleolus inside the nucleus, migrates towards the periphery of the nucleus, and then diffuses gradually through the nuclear membrane, close to which the cytoplasmic Nissl substance is formed (Einarson, 1932, 1933, 1934, 1935; Landström, Caspersson, and Wohlfart, 1941; Hydén, 1943). With basic dyes the nucleus and Nissl substance in normal resting nerve cells of any one particular type show a fairly constant structural picture (Einarson, 1935). Within each cell type, however, this picture exhibits marked changes, with variations in the functional or pathological activity of the cell. The most conspicuous of these changes are alterations in the quantity, affinity for basic dyes, and distribution of the Nissl substance, as well as alterations in the size and staining capacity of the nucleus, nucleolus, and nuclear membrane (Einarson, 1933, 1937; Hydén, 1943). There are also spontaneous variations in the affinity for dyes of the nerve cells in the central nervous systems of normal animals and man. But such changes are greatly enhanced by experimental procedures or by disease, which may increase neuronal activity (patho-functional stress), or may decrease or abolish it (patho-functional suppression).

In studying the different depths of staining, as may be seen in preparations stained with gallocyanin-

chromalum, Einarson (1933, 1937, 1945) distinguished certain cell pictures.

Cell Conditions Visible after Gallocyanin-chromalum Staining

Hyperchromatic or Moderately Chromophilic Cells.—The cells are deeply stained. The dark, well-defined Nissl bodies make a comparatively sharp contrast against the interstitial, non-basophilic part of the cytoplasm, and several Nissl bodies are usually close to the nucleus. The nucleolus is intensely stained and often somewhat enlarged, and a relatively large number of stained particles is present in the nucleus.

Moderately Chromophobic Cells.—These show a decrease in the number and staining reaction of the Nissl bodies, and an increase in the nuclear chromatin. The nuclear membrane is intensely stained and the nucleolus enlarged. Perinuclear accumulations and nuclear caps of Nissl substance can be seen (peripheral chromophobia), but sometimes the Nissl substance is accumulated in the periphery of the cytoplasm (central chromophobia).

Extremely Chromophobic Cells.—The cells are pale and there is little or no staining substance. Both the nucleus and cytoplasm are shadow-like, and frequently the nucleus is eccentric in the cell body, which may be swollen, the picture perhaps resembling that of chromatolysis. The contours of the nucleus often appear irregular or shrivelled, and the dark, enlarged nucleolus may finally lose its capacity to stain inside the pale nucleus.

Extremely Chromophilic Cells.—These cells are intensely stained, with closely packed, dark Nissl bodies, while the interstitial non-basophilic substance of the cytoplasm is markedly reduced; sometimes the cells appear as dark, compactly stained bodies. The cell contours are well defined, and the dendrites

are often stained for a considerable distance. The nucleus is intensely stained, but the nucleolus is usually visible, despite the dark colour of the nucleus, whereas the nuclear membrane is hardly discernible (see also Einarson and Ringsted, 1938; Einarson and Lorentzen, 1946; Krogh, 1950; Lorentzen, 1950; Hartelius, 1952).

Chromoneutral Cells.—These cells represent the "normal" cell picture, showing the usual average staining capacity.

As to the functional meaning of these cell pictures, i.e., their significance as histological correlates of cellular activity, our investigations since 1932 on the staining reactions of nerve cells under various experimental conditions as well as our observations from human material have led to the following definitions:—

The chromoneutral condition represents cells at rest or in the usual state of normal activity (the indifferent phase of activity); moderate chromophilia (hyperchromasia) characterizes cells when their activity begins to increase (chromophilia of initial activity); moderate chromophobia gradually becoming more marked characterizes cells in increasing activity of longer duration (chromophobia of prolonged activity), while extreme chromophobia represents cells in a state of severe functional stress, fatigue, and exhaustion (chromophobia of fatigue and exhaustion). Extreme chromophilia characterizes cells either in a state of primary, active inhibition of prolonged duration (chromophilia of inhibition), or whose activity has been abolished for some time (chromophilia of suppressed activity) (Einarson, 1945, 1949, 1952; Krogh, 1950; Lorentzen, 1950). In pathological conditions extreme chromophobia may proceed to total dissolution of the cell, and extreme chromophilia will gradually proceed to cell sclerosis, irreparable cell atrophy, and cell shadows, or even to disappearance of the cell.

The purpose of this paper is to present additional evidence in support of the above definitions, particularly concerning the occurrence of chromophobia in cases of functional stress as well as in animal experiments.

The Staining Method

In the tissues of mammals, and at any rate in some lower animal species too, the essential part of the gallo-cyanin-chromalum staining consists in a selective binding of the dye-lake cations to the phosphoric acid groups of the polynucleotides, i.e., a salt of the lake-cations and the nucleic acids of the cell structures is formed. The staining progresses rapidly and completely withstands alcoholic dehydration and clearing in xylol. There is no further binding of the stain to the basophilic cell

structures, even if the tissue remains in the staining solution longer than is necessary.

At the lower levels of pH, between pH 0.83 and 1.64, the staining is almost entirely caused by a binding of the dye to the nucleic acids of the cell structures; there is, however, a non-specific staining (co-staining) of the tissue, independent of the presence of nucleic acids. From approximately pH 1.75 to 1.80 the co-staining increases, and it reaches a maximum between pH 3.3 and 3.5, falling abruptly from pH 3.75 to 4.07, due partly to an adsorption of the dye, i.e., lake-sulphate, in excess of the specific cation exchange, and partly to a binding of the lake-cations to the proteins of the cell structures, e.g., the Nissl substance. The latter will not occur on the acid side of the isoelectric point of the proteins. From pH 0.83 to 1.64 staining of structures other than the nucleic acids is very slight, as in this range no binding to the proteins will take place and the unspecific adsorption is reduced to a minimum. Although negligible at these low pH values, and practically insignificant in quantitative measurements, the unspecific adsorption nevertheless withstands the usual differentiating media. In quantitative estimates of the nucleic acids of the cell structures the error due to the persistent adsorption is amply compensated for by the progressive properties of the staining, which makes it particularly suitable for photometric estimation of the degree of cellular basophilia, and, as shown by this staining, the basophilia is essentially equal to the contents of nucleic acids. In fact, the specific part of the staining is in itself a quantitative-stoichiometrical reaction for nucleic acids.

It is important to use only a suitable brand of gallo-cyanin,* and the purest obtainable chromalum. If these conditions, including a suitably low pH value, round 1.0, are fulfilled, the deep blue staining of the nucleic acids will show the highest degree of specificity, contrast, and stability, and the staining intensity is homogeneous throughout the tissue section. In these conditions, and taking into account the progressive nature of the staining as well as the error caused by the very slight unspecific adsorption, we think it justifiable to state that gallo-cyanin-chromalum is a specific stain for nucleic acids as these appear in the tissues of mammals and some lower animal species, fully acceptable for quantitative histological work. (For the theory and practice of gallo-cyanin-chromalum staining we refer especially to the paper by Einarson (1951) as well as to Pearse's (1953) book on histochemistry.)

The Occurrence of Chromophobia

The essential earlier investigations of the changes in structure and affinity for specific stains of the nerve cells following variations in the functional activity of the cells, in disease as well as in animal experiments, have been made by Einarson (1933, 1937, 1945), Einarson and Lorentzen (1946), Krogh (1945, 1950), Lorentzen (1950), Hartelius

* Satisfactory gallo-cyanin can be obtained from Hollborn and Söhne, Grübler and Co., National Aniline Division, 40 Rector Street, New York. Chromalum may be obtained from Hopkin and Williams Ltd., St. Cross Street, London, E.C.1.

FIG. 1.—Acute delirium. Giant pyramidal cells of Betz. Extreme chromophobia. Gallocyanin-chromalum. $\times 600$.

FIG. 2.—Acute delirium. Area 8 of Brodmann. Small pyramidal cells. Marked chromophobia. Gallocyanin-chromalum. $\times 800$.

(1952), and others. The theoretical and technical aspects of the problem in general will not be considered in this paper.

Illustrative Case Reports

Acute Delirium (State Mental Hospital, Viborg, No. 10438; brain examination No. 241).—A woman aged 38 had a brother who had three times been admitted to a mental hospital but otherwise had no known familial disposition. The patient herself had always been mentally normal and sociable. Appendicectomy and tonsillectomy were performed in June and July, 1943. On August 15, 1947, the patient fell from her bicycle on to her head; there was no apparent sign of cerebral concussion, but she sustained a fracture of the left radius, and was admitted to the surgical wards at Herning hospital. Five days later she suddenly became psychotic, and was admitted to the mental hospital. She was very anxious with explosive outbursts of excitement, shouting and screaming, gesticulating violently, and strongly hallucinated. She had to be restrained by a belt. After morphine-scopolamine and hypnophen she gradually calmed down, and next morning she was completely clear mentally and able to remember what had happened the evening before. Four days later, however, she became psychotic again, and on August 27, 1947, she died in exhaustion and hyperpyrexia after the utmost psychomotor exaltation, violence, and complete confusion. Laboratory examinations of the blood, spinal fluid, and urine showed nothing abnormal.

Necropsy showed nothing of particular interest in the internal organs, and there was no arteriosclerosis. Examination of the brain revealed a marked hyperaemia and oedema of the meninges as well as of the brain tissue itself. No sign of inflammation was seen anywhere, and, although congested, the blood vessels were normal everywhere; in particular, there was no sign of arteriosclerosis of the brain vessels. There were no focal changes anywhere, except a few small haemorrhages by diapedesis in the medial nuclei of the thalamus. However, in every

region of the cerebral cortex examined most of the nerve cells showed the typical picture of extreme chromophobia.

The findings in the present case serve to illustrate the chromophobic cell change as it appears in the cerebral cortex. Although the chromophobia is most pronounced in the large and medium pyramidal cells (Fig. 1), it is in the main quite conspicuous in the small pyramidal cells as well (Fig. 2). In the cells showing the most marked condition of extreme chromophobia the cytoplasm is very swollen, and there is almost no nucleoprotein left. The nucleolus is very large and darkly stained, and the nuclear contours become more or less shrivelled (Fig. 1). In our opinion and experience over many years, the interpretation must be that the power of the cell to regenerate nucleoproteins is being exhausted, as the formation of cytoplasmic nucleic acid could not possibly keep pace with the increased consumption due to the abnormal rise in functional demands on the neuron.

The chromophobic cell change is widespread in this brain. As well as in the cerebral cortex, a more or less pronounced chromophobia, in many places reaching an extreme degree, is to be seen practically everywhere; e.g., in the thalamus, hypothalamus, brain-stem, and in the Purkinje cells and dentate nucleus of the cerebellum. Numerous nerve cells show a moderate amount of chromolipoid in the cytoplasm.

Acute Delirium (State Mental Hospital, Nykøbing Sjælland, No. 11107; brain examination No. 723).—A man aged 68 was admitted to hospital on July 24, 1951. No familial disposition was known. Six years before he had been treated for prostatic hypertrophy. He had never had any head injury. The patient had always been mentally normal and sociable although of a rather

FIG. 3.—Acute delirium. Nucleus supraopticus. Extreme chromophobia. Balloon-like appearance of the cells, and depletion of cytoplasmic nucleic acid. Gallocyanin-chromalum. $\times 500$.

reserved temperament. During the last two years he had become increasingly forgetful, apathetic, and taciturn. The last two months before his mental illness he had been somewhat restless, and the last eight days before the admission he had been agitated, in high spirits, talking very much, and somewhat confused, showing a quite unusual, strange behaviour with marked emotional instability. On admission he was very agitated and reluctant, anxious, and slightly confused, with outbursts of excitement and violence, and had to be controlled in a belt; he was unable to give any relevant information. He looked older than his age; there was considerable peripheral arteriosclerosis, with marked arcus senilis and a central paresis of the left face. Next day there were marked anxiety and restlessness, and confusion with hallucinations developed. During the following days he was very excited, constantly talking, shouting, screaming, and gesticulating violently, and on July 30, 1951, he died in exhaustion and hyperpyrexia.

The spinal fluid showed normal values for albumin, globulin, and cells, and Pandy's test was negative; the Wassermann test was negative in the blood and spinal fluid; the sedimentation test was 8 mm. in one hour; serum bicarbonate and chloride levels were normal; total serum cholesterol was 138 mg. per 100 ml.

Necropsy showed hyperaemia of the brain, stasis and oedema of the lungs, atherosclerosis of the aorta and coronary arteries, several small adenomata in the cortex of the adrenals, hyperplasia of the spleen, and hyperaemia and parenchymatous degeneration of the kidneys with intracanalicular calculi of the renal medulla.

Neuropathological examination revealed considerable oedema of the brain and meninges, and only

moderate arteriosclerosis of the blood vessels at the base of the brain. No focal changes or haemorrhages were seen anywhere. The brain weighed 1,480 g.

Microscopically there is marked dilatation and congestion of veins and capillaries, and the smaller arteries and arterioles show only moderate sclerotic changes. Some atheromatous plaques are seen in only a few larger arteries of the corpus striatum. Round the ventricles the oedematous brain tissue contains numerous amyloid bodies, and in the cortex of the frontal lobes as well as in the hippocampus a few senile plaques may be seen. Many of the nerve cells, particularly in the cerebral cortex, contain a considerable amount of chromolipoid in the cytoplasm.

The most outstanding and characteristic finding, however, is the pronounced and widespread extreme degree of chromophobia of the nerve cells. This occurs practically everywhere in the brain, together with many more moderately chromophobic cells. Although in the cerebral cortex the chromophobic cell picture is not quite so pronounced as in the previous case it is nevertheless very conspicuous both in the larger and smaller pyramidal cells. The same is true of the thalamus, hypothalamus, and brainstem. In all these parts of the brain the great majority of the nerve cells show moderate to extreme chromophobia. Chromophobia is very pronounced and characteristic in the hypothalamus.

Although the pathological significance of chromo-

FIG. 4.—Acute delirium. Nucleus supraopticus. $\times 500$. (A) Moderate chromophobia; cytoplasmic inclusions (x) stained with toluidine blue at pH 3.9. (B) Extreme chromophobia; diffuse cytoplasmic staining with toluidine blue at pH 3.9. (C) Extreme chromophobia; marked diffuse cytoplasmic staining with toluidine blue at pH 4.4. The toluidine blue stainings of the cytoplasm are superimposed on gallocyanin-chromalum.

phobia, especially its moderate stages, is difficult to estimate in the nerve cells of the hypothalamus on account of the special character of the cells, it is generally quite conspicuous. Chromophobia ranges in degree from moderate to extreme, and is widely distributed in the hypothalamic nuclei, e.g., in the supraoptic, paraventricular, medial and lateral tuberal, and posterior hypothalamic nuclei. It is most intense in the cells of the supraoptic nucleus (Figs. 3 to 5), and, on account of the structural peculiarities of these unusual nerve cells, only the extreme state of chromophobia will be emphasized.

Most of the cells of the supraoptic nucleus are extremely swollen, of an almost balloon-like appearance, with a shrunken, eccentrically displaced nucleus, and the cytoplasm is left unstained by gallocyanin-chromalum; it is really depleted of nucleic acid. In some of the cells the nucleus is still hyperchromatic, in others it has almost lost its staining capacity, and appears as a shadow (Fig. 3). When stained with toluidine blue according to Einarson (1945) there is no staining of the cytoplasm at pH values on the acid side of the isoelectric point of the Nissl substance. At pH 3.9 staining of the cytoplasm with toluidine blue becomes visible. In the more moderately chromophobic cells this appears as distinct cytoplasmic inclusions (Fig. 4A) while in the extremely chromophobic cells it appears as a faint diffuse staining (Fig. 4B) to become quite con-

spicuous at pH 4.4 (Fig. 4C). In our opinion staining with toluidine blue is probably due to the presence of some cytoplasmic protein substance. For comparison, Fig. 5 shows the extremely chromophobic cells as they appear in preparations stained by Bodian's method. In the paraventricular nucleus

FIG. 5.—Acute delirium. Nucleus supraopticus. Extreme chromophobia. Balloon-like swelling of the cells. Bodian's stain. $\times 500$.

FIG. 6.—Acute delirium. Nucleus paraventricularis. Marked chromophobia. Vacuoles and dark granular inclusions in the cytoplasm. Gallocyanin-chromalum. $\times 500$.

FIG. 7.—Nucleus supraopticus. Normal cells stained with gallocyanin-chromalum (above) and by Bodian's method (below). $\times 500$.

most of the cells present an appearance of more moderate stages of chromophobia (Fig. 6), but many of them contain big vacuoles and some dark granular inclusions in their cytoplasm.

In comprehensive surveys Greving (1928), Le Gros Clark, Beattie, Riddoch, and Dott (1938), and Scharrer and Scharrer (1940, 1954) discuss the normal cytology of the hypothalamic nuclei and the neurosecretory activity of the cells. Here it is sufficient to say that the supraoptic and paraventricular nuclei vary widely in structure, particularly in the cytoplasmic content of vacuoles, colloid droplets, and granules of various kinds, and in the mutual relation of these inclusions to the Nissl bodies and the nucleus. It is especially important to note that in these unusual nerve cells the nuclei generally take eccentric positions, the nuclear membranes are often distinctly stained, sometimes with perinuclear deposits of basophilic substance, and the coarse Nissl bodies occupy the periphery of the cell body, of which the interior shows a reduced, diffuse, or dust-like staining of the cytoplasm (Fig. 7). Thus, the normal cell pictures resemble those of moderate chromatolysis or chromophobia, although the pseudo-pathological appearance of the cells has repeatedly been pointed out by several authors. In fact, no precise line of demarcation can be drawn between the normal variations of basophilia and abnormal chromophobia, i.e., we are dealing with gradual transitions of basic staining affinity. In other words, it is difficult to tell where chromophobia of normal activity ends and that of abnormal hyperactivity begins in the cells of the hypothalamic nuclei. Thus, the significance of the more moderate stages of chromophobia remains

questionable in this particular region while sufficient data based on the galloxyanin-chromalum staining are not available. Possibly such data might reveal a significant difference between the normal and the findings in cases of acute, lethal psychoses. In fact, studies of a larger series carried out by Bom in 1954 and not yet published seem to indicate that this actually is the case. In two preliminary reports Bom (1946, 1953) pointed out the pronounced increase of the cellular vacuolization in the hypothalamus frequently met with in acute lethal psychoses as well as the marked chromophobia. So far Bom is the only other investigator who has used galloxyanin-chromalum staining in studies of this kind. Undoubtedly, however, the cell pictures of extreme chromophobia (Figs. 3 to 5) are pathologically significant as real cytological correlates of the abnormal functional stress on the neurons, and typical transitional stages of chromophobia can be seen within the same hypothalamic nucleus.

In the present paper the cell pictures of chromophobia observed in the hypothalamus have been estimated only in regard to their significance as changes following neuronal activity, intimately connected with a loss of nucleic acid from the cytoplasm, but no attempt has been made to correlate the findings with the cytological criteria of neurosecretory activity; and the hypophysis was not at our disposal.

Deaths from Convulsant Therapy with Metrazol and Insulin.—We have examined the brains from a few psychotic patients who have died as a result of convulsant drug therapy, either during or shortly after the artificially induced shocks. One patient developed spontaneous epileptic seizures following the shock therapy, and died as a result.

The changes of the nerve cells in cases of death from metrazol shock have been briefly reported and illustrated by Einarson and Lorentzen (1946) and by Krogh and Einarson (1954), and the cell changes in cases of death from insulin shock have been studied by Lorentzen (1954; still unpublished). It is sufficient to note here that the cell picture of chromophobia, ranging from moderate to extreme degrees, is a most conspicuous and widely distributed feature in the cerebral cortex and the brain-stem. In the motor areas of the cortex it has reached its most advanced stages, affecting the great majority of the cells in all the cortical layers. The giant pyramidal cells of Betz are extremely swollen, with a more or less shrivelled and eccentrically displaced nucleus, and their cytoplasm is ghost-like, with practically no remnants of nucleic acid left. In some of the cells the large nucleolus is still darkly stained; in others

it has become quite pale. The cell picture is fundamentally the same as shown in Figs. 1 and 2.

Traumatic Encephalopathy and Symptomatic Epilepsy (State Mental Hospital, Aarhus, No. 18790; brain examination No. 272).—A woman aged 25 had no known familial history of mental disorder, but an aunt has been a patient in the Viborg Mental Hospital for 10 years. When 5 years old, while playing, the patient was hit hard on the right temple with a wooden shoe; however, there was no loss of consciousness and no nausea or vomiting. At the age of 7 she was run down by a motor-cycle, an accident that involved cerebral concussion. From her sixth year she has had periods of unconsciousness and epileptic attacks. From 1930 to 1937 she was a patient at Moltke's Infirmary and there had numerous Jacksonian epileptic attacks with myoclonus in the left arm, platysma, and left side of the face. A light paresis of the left orbicularis oculi muscle and left arm, reduced sensibility on the left side of the body (face, neck, shoulder, arm), and light parageusia developed.

From 1937 to 1941 she stayed at Filadelfia, the Danish hospital for epileptics, and from there she was twice (July, 1940, and January, 1941) transferred to the Department of Neurosurgery, National Hospital, Copenhagen, for examination for a possible cortical encephalitis, an atrophic brain affection (traumatic?), or a vascular malformation, causing partial epilepsy. On January 17, 1941, she had numerous epileptic fits with loss of consciousness, opisthotonos, and involuntary urination, and gradually there developed status epilepticus, from which she was finally relieved by narcosis. During the following two days she had nine epileptic fits, between each of which she was completely awake and talked rationally; each fit lasted three to 10 minutes. On January 20, 1941, she was operated on, the central part of the right cerebral hemisphere being exposed. The cortex looked congested with arachnoidal adhesions in the Sylvian fissure. About 10 cm. from the median line there was a small, atrophic portion of cortex in the precentral sulcus, with a deep-set, spiral vein. The vein with the surrounding tissue was coagulated, and a piece of arachnoid was removed (Prof. Busch). Subsequent microscopical examination showed a thickened arachnoid with vascular growth into it; the dura was normal (Dr. Christensen). On February 10 the patient was transferred to Filadelfia, and stayed there until December 15, 1941, when she left for her home.

In the following years she was repeatedly admitted to various hospital departments, including the Aarhus State Mental Hospital, on account of epilepsy and several suicidal attempts, and on July 7, 1947, she was for the third time admitted to the Aarhus State Mental Hospital. In hospital her condition varied from normal behaviour to an intense verbal and motor restlessness, and she had numerous epileptic attacks. After an epileptic seizure during the night between September 15 and 16, she slept quietly, and died in her bed early in the morning of September 16, 1947, without further convulsions or any premonitory symptoms. Laboratory examinations

FIG. 8.—Traumatic epilepsy. Diagram indicating the approximate sites (the lined areas A and B) of meningeal adhesions and the most marked glial changes.

showed nothing abnormal, either in the blood, spinal fluid, or urine. The temperature was always normal.

Necropsy showed stasis and oedema of the lungs, ecchymoses of the pericardium, and acute stasis of the abdominal organs. No arteriosclerosis was seen anywhere.

FIG. 9.—Traumatic epilepsy. Middle frontal gyrus. Very pronounced hyperplasia of the astrocytes in the subcortical white matter and the deepest layers of the cortex. Bodian's stain. $\times 95$.

On examination of the brain there were meningeal adhesions to the cortex of the right hemisphere in two places: first, to a slightly depressed area round the inferior frontal sulcus, extending a little backwards into the anterior central gyrus (A, Fig. 8); secondly, to an area comprising the hind part of the superior temporal gyrus and the opercular parts of the parietal lobe, extending upwards into the post-central gyrus (B, Fig. 8). In both these areas and the adjacent parts of the cortex there is a considerable glial proliferation, with hyperplasia of the small astrocytes, and some increase in glial fibres, distributed in the most superficial cortical layer and just beneath the pia. In the subcortical white matter and the deepest layer of the cortex, particularly in area A (Fig. 8) and its adjacent parts, there is, however, a most pronounced proliferation and hyperplasia of the large astrocytes, many of which are extremely

FIG. 10.—Traumatic epilepsy. Area 4 of Brodmann. Giant pyramidal cells. Marked perinuclear deposition of basophilic substance (nucleic acid). Gallocyanin-chromalum. $\times 600$.

swollen (Fig. 9). For the rest, the meninges are oedematous, and the meningeal blood vessels are dilated and congested, but there is no actual sign of inflammation anywhere. The vessel walls are normal both in the meninges and the brain tissue itself. In several places the perivascular spaces are dilated on account of the oedema.

In our opinion there can be no doubt that the glial changes developed in connexion with a traumatic lesion which also provoked post-traumatic epilepsy, although its incidence in closed head injuries is comparatively low (Russell, 1951; Russell and Whitty, 1952; Bertrand and Harriman, 1953).

In this case the cell pictures are interesting, particularly in the cerebral cortex. Generally, chromophobia is the most outstanding feature not only in the cortex but in the thalamus and brain-stem as well. It is most prominent in the cortex of the frontal lobes, where the vast majority of the pyramidal cells show it in various stages. The characteristic distribution of the stainable substance (nucleic acid) in the cell body and its structural relation to the unstained part of the

FIG. 12.—Traumatic epilepsy. Area 6 of Brodmann. Middle-sized pyramidal cells with perinuclear accumulations and nuclear caps of basophilic substance. Gallocyanin-chromalum. $\times 600$.

FIG. 11.—Traumatic epilepsy. Area 44 of Brodmann. Large pyramidal cells showing marked deposition of nucleic acid round the nucleus (peripheral chromophobia). Gallocyanin-chromalum. $\times 600$.

cytoplasm are especially to be emphasized (Figs. 10 to 13). Thus, the cell picture of moderate chromophobia, with an accumulation of deeply stained substance (nucleic acid) round the nucleus, surrounded by a relatively broad peripheral zone of non-basophilic, very light or indifferently stained cytoplasm is a prominent feature. The cells are but slightly swollen, and their pyramidal form is mostly well preserved (Figs. 10 to 13). This picture resembles the so-called "peripheral chromatolysis", but for several reasons we prefer to call it "peripheral chromophobia". In some of the largest pyramidal cells, especially the giant

cells of Betz (Fig. 13), the cytoplasm is also vacuolized.

The cell picture described is especially conspicuous in the large and medium-sized pyramidal cells, most characteristically in the areas 4, 6, 8, 44, 45, and 46 of Brodmann. In every area examined, however,

FIG. 13.—Traumatic epilepsy. Giant pyramidal cells of Betz showing perinuclear distribution of basophilic substance and vacuolization of the cytoplasm. Gallocyanin-chromalum. $\times 600$.

some moderately chromophobic cells showing an accumulation of basophilic substance in the periphery of the cytoplasm with a light inner or perinuclear zone may be seen ("central chromophobia"), and still other cells show both peripheral and perinuclear accumulations of basophilic substance, with a light intermediary zone. In fact, we are dealing with transitional cell pictures of moderate chromophobia, but whichever the pattern observed the nuclear membrane is always strongly basophilic. Some markedly swollen cells, resembling extreme chromophobia, may also be seen.

Our interpretation of the appearances in Figs. 10 to 13 is that the cells are in a state of restoration from a more advanced stage of chromophobia, i.e., of the regeneration of the Nissl substance after the abnormal neuronal discharge which constitutes the epileptic fit. In fact, the sections indicate very clearly how cytoplasmic nucleic acid is deposited round the nucleus, making the nuclear membrane strongly basophilic. This observation provides convincing evidence in support of the theory that the nucleic acid component of the cytoplasm is formed primarily round the nucleolus inside the nucleus, migrates towards the periphery of the nucleus, and then diffuses through the nuclear membrane into the cytoplasm where it is incorporated as a constituent of the basophilic elements (Einarson, 1933, 1935). Thus, we are dealing with the structural picture of a certain phase of material interchange between nucleus and cytoplasm.

Animal Experiments

Our experimental material is derived from the usual experimental animals (rats, rabbits, cats, dogs), but similar structural effects of neuronal hyperactivity can be shown in molluscs, and in this paper we are especially concerned with the latter.

Stimulation of the Ganglia of *Aplysia* (Sea Hare).—The specimens of this big marine snail used in our experiments weighed about 500 g. The greater part of the nervous system of *Aplysia* consists of a ring of ganglia situated around the oesophagus. The nerve cells of these ganglia are very large, ranging from 150 to 400 microns in diameter. The nuclei vary with the size of the cells, and may reach a maximum of about 250 microns. The ganglion cells were subjected to electric stimulation, and stained with gallocyanin-chromalum.

A wire of stainless steel enclosed in a small glass tube was inserted through the mouth of the animal and fixed there in such a way that the point of the wire protruding from the glass tube was exactly at a level with the ganglia. The animal was placed in

a small aquarium in which the water was renewed every 10 seconds by a constant flow of fresh oxygenated sea water. The second electrode was a plate of stainless steel placed at the bottom of the aquarium. The stimulator used was the Harvard induction coil.

During stimulation the animal responded with a very strong tetanic contraction of all muscles, to such an extent that the animal almost resembled a ball. After a stimulation period of about one and a half to three hours the muscles relaxed, and stimulation was then discontinued and the animal killed. The ganglia were immediately removed and fixed in a saturated solution of picric acid in sea water and

FIG. 14.—Ganglion cells of *Aplysia*. Normal cells (above) showing considerable cytoplasmic basophilia. After stimulation for two hours the cells (below) show a very pronounced chromophobia. Gallocyanin-chromalum. $\times 100$.

embedded in paraffin. Ganglia from stimulated animals and from normal control animals were embedded in the same block and cut together at a thickness of 10 microns.

The histological findings were very uniform. In all the stimulated animals the nerve cells were almost empty of cytoplasmic nucleic acid, as compared with the normal control animals in which the nerve cells contained a considerable amount of nucleic acid in

the cytoplasm (Fig. 14). After gallocyanin-chromalum staining this is indicated by the difference in the affinity for staining of the nerve cells of the normal and the stimulated animals respectively.

In the cells of the normal group there is marked cytoplasmic basophilia, while those of the stimulated group show a highly reduced basophilia, i.e., there is pronounced chromophobia of the cells (Fig. 14). (The sections were photographed on the same film and produced under exactly the same conditions.)

Within each group of ganglia, i.e., the normal and the stimulated, the cells show certain mutual variations in their degree of basophilia. Moreover, the basophilia varies at different spots in the cytoplasm of the same cell, but the difference between the average degrees of basophilia of the normal and stimulated groups is always very clear.

FIG. 15.—Graph showing the mean photometric values of 120 and 105 measurements respectively of the cytoplasmic basophilia in 40 normal (above) and 35 stimulated ganglion cells of *Aplysia* (below). Gallocyanin-chromalum; pH 1.64.

In order to elucidate these observations, and to put them on a relative-quantitative basis, we subjected our material to microphotometric determinations of the basophilia by means of the technique used for many years in our laboratory and described by Einarson (1945). It should be mentioned that in the beginning we used the ocular photo-cell of Dr. B. Lange, Berlin, but now we are using the selenium photo-cell, type A, of Evans Electro-selenium Ltd., and a mirror galvanometer of Siemens Halske, Berlin, with a scale distance of 1.5 metres. The stabilizer used is the "Stavolt" of Struers

Laboratory, Copenhagen. The maximum light absorption of the gallocyanin-chromalum solution is at approximately 560 m μ , as determined by the Beckman spectrophotometer; this corresponds with the maximum relative sensitivity of the photo-cell used.

Fig. 15 illustrates our measurements in 40 normal and 35 stimulated cells from the same section. The number of cells is entered along the abscissa. The ordinate indicates the amount of light transmitted by the stained cytoplasm as a percentage of that transmitted by the slide, the layer of balsam, and the cover glass. Each point on the graph represents the mean value of three determinations at different spots in the same cell. Thus, the total of determinations in this particular section is 120 and 105 respectively. It is to be seen that the average light transmission by the normal cells (above) is about 42 to 43%, while that of the stimulated cells (below) is about 79 to 80%. This is a clear indication of the reduced basophilia of the stimulated cells.

Chronic Vitamin-E Deficiency.—Variations in the basophilia of nerve cells, including chromophobia, are particularly conspicuous in vitamin-E deficiency as produced in adult rats by Einarson and Ringsted (1938). Although somewhat variable, the changes of basophilia mostly pass through several consecutive structural stages. From the normal condition of chromoneutrality the nerve cells pass through stages of hyperchromasia and chromophobia followed by increasing basophilia leading to extreme chromophilia, and finally to irreparable cell atrophy, with a marked reduction or even loss of their affinity for staining. These cell changes may roughly be correlated with the clinical symptoms displayed by the rats during the progress of the deficiency disease from its outset to the end. The cell pictures are complicated by the occurrence of deposits of acid-fast, fluorescent products in the cytoplasm (Einarson, 1953, 1954). As these increase the nucleic acid disappears from the cytoplasm (cellular lipodystrophy). Thus, this metabolic derangement induces a secondary reduction in the basophilia of the nerve cells in addition to the primary changes.

Notwithstanding this complication we have subjected our material to renewed photometric determinations of the basophilia, and so far the results definitely tend to corroborate our concepts of the functional meaning of the cell pictures. These results will be reported in a separate paper.

Acute Hypoxia, Insulin Shock, and Electrically Induced Convulsions.—Studies of the variations in the basophilia of nerve cells in experimental acute

hypoxia (Krogh, 1945, 1950), in insulin shock (Lorentzen, 1950), and in electrically induced convulsions (Hartelius, 1952) were carried out by means of gallocyanin-chromalum staining.

In all three categories of experiments the nerve-cell changes showed a considerable variability, largely according to the varied experimental conditions. Generally, however, various stages of chromophobia were quite conspicuous, especially in Krogh's and Lorentzen's material (rabbits). In the material of Hartelius (cats) the nerve-cell changes noted were slight, but there was a statistically significant difference between the control animals and those subjected to electric convulsant treatment with regard to the variability of the cell changes, which were in the form of various stages of chromophobia, frequently with coincident nuclear hyperchromatism; the arrangement of the cells was mainly focal.

It is to be emphasized in particular that at the milder degrees of hypoxia which Krogh obtained by cutting off the blood flow to the lumbar part of the spinal cord (compression of the abdominal aorta) for shorter periods of time, e.g., 20 minutes, many of the animals developed spastic paralysis of the hind legs, and Krogh correlated the clinical pictures, electromyograms, and cell changes of the animals. The surviving anterior horn cells, which innervated the spastic muscles, invariably showed much lower contents of cytoplasmic nucleic acid than normal cells do, and they were very sensitive to proprioceptive impulses. By alternately stretching and bending the spastic hind legs for a long period the cells in question can be exhausted and made to show the picture of extreme chromophobia (Krogh and Einarson, 1954). These experiments indicate that the cell pictures of chromophobia are to be interpreted as structural correlates of increased and abnormal neuronal discharge.

Summary

After staining nerve cells with gallocyanin-chromalum, a quantitative and stoichiometrical staining reaction for nucleic acids, the cell pictures exhibit marked changes with variations in the functional and pathological activity of the cells. These changes consist in variations in the basophilia of the cytoplasm, nucleus, nucleolus, and nuclear membrane, accompanied by alterations in the form of the cell body and nucleus, and size of the nucleolus, as well as characteristic changes in the distribution of the basophilic elements in the cytoplasm and displacement of the nucleus inside the cell body. The fundamental cell stages of basophilia (chromo-

neutrality, hyperchromasia, chromophobia, chromophilia) are described.

Spontaneous variations in the basic staining reaction of the nerve cells constantly occur in the central nervous systems of normal animals and man, but are greatly enhanced by experimental procedures or in disease.

Chromophobia in certain human cases of patho-functional stress (acute delirium, death from convulsant treatment, traumatic epilepsy), as well as in the ganglion cells of *Aplysia* after electric stimulation, is described.

All the evidence indicates that the cell pictures of chromophobia are to be interpreted as structural correlates of increased activity and abnormal neuronal discharge. Certain characteristic cell pictures provide strong evidence in support of the theory (Einarson, 1933, 1935) that the nucleic acid of the cytoplasm is formed primarily round the nucleolus inside the nucleus and then diffuses through the nuclear membrane to become a constituent of the basophilic elements in the cytoplasm.

REFERENCES

- Bertrand, I., and Harriman, D. G. F. (1953). *Rev. Neurol., Paris*, 88, 249.
 Bom, F. (1946). *Acta psychiat., Kbh.*, 21, 57.
 — (1953). *Nord. psykiat. Medlemsbl.*, 7, 71.
 — (1954). Personal communication.
 Clark, W. E. Le Gros, Beattie, J., Riddoch, G., and Dott, N. M. (1938). *The Hypothalamus*. Oliver and Boyd, Edinburgh.
 Einarson, L. (1932). *Amer. J. Path.*, 8, 295.
 — (1933). *Amer. J. Anat.*, 53, 141.
 — (1934). *Laeknabladid*, 20, 142.
 — (1935). *J. comp. Neurol.*, 61, 101.
 — (1937). *Hospitalstidende*, 80, 1.
 — (1945). *Acta Jutlandica—Aarskr. Aarhus Univ.*, 17, no. 1, pp. 31–54 and 108.
 — (1949). *Acta orthop. scand.*, 19, 27.
 — (1951). *Acta path. microbiol. scand.*, 28, 82.
 — (1952). *Acta psychiat., Kbh.*, Suppl. 78, pp. 11–27.
 — (1953). *Journal of Neurology, Neurosurgery and Psychiatry*, 16, 98.
 — (1954). *Anat. Skrifter*, 1, 1.
 —, and Lorentzen, K. A. (1946). *Acta Jutlandica—Aarskr. Aarhus Univ.*, 18, No. 4, pp. 11–48.
 —, and Ringsted, A. (1938). *Effect of Chronic Vitamin E Deficiency on the Nervous System and the Skeletal Musculature in Adult Rats*. Copenhagen and London.
 Greving, R. (1928). In *Handbuch der mikroskopischen Anatomie des Menschen*, bd. 4, teil 1, p. 917, ed. W. von Möllendorff. Springer, Berlin.
 Hartelius, H. (1952). *Acta psychiat., Kbh.*, Suppl. 77.
 Hydén, H. (1943). *Acta physiol. scand.*, Suppl. 17.
 Krogh, E. (1945). *Acta Jutlandica—Aarskr. Aarhus Univ.*, 17, Suppl., pp. 1–40.
 — (1950). *Acta physiol. scand.*, 20, 263.
 —, and Einarson, L. (1954). *Anat. Skrifter*, 1, 65.
 Landström, H., Caspersson, T., and Wohlfart, G. (1941). *Z. mikr.-anat. Forsch.*, 49, 534.
 Lorentzen, K. A. (1950). *Acta psychiat., Kbh.*, Suppl. 64, pp. 29–30.
 — (1954). Personal communication.
 Pearse, A. G. E. (1953). *Histochemistry*, pp. 117 and 428. Churchill, London.
 Russell, W. R. (1951). *Journal of Neurology, Neurosurgery and Psychiatry*, 14, 35.
 —, and Whitty, C. W. M. (1952). *Ibid.*, 15, 93.
 Scharrer, E., and Scharrer, B. (1940). In *The Hypothalamus. Res. Publ. Ass. nerv. ment. Dis.*, 20, 170.
 — (1954). In *Handbuch der mikroskopischen Anatomie des Menschen*, bd. 6, teil 5, p. 953, ed. W. von Möllendorff. Springer, Berlin.