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HISTOCHEMISTRY OF PATHOLOGICAL GLIA

The glial tissue is involved in the majority of pathological processes occurring in the central nervous system. The glial involvement may consist in damage of all types of glial cells, or only some of them together with other more sensitive cellular constituents of the central nervous system. The process may take the form of a local or generalized glial reaction to the lesions or destruction of other structural elements of the brain and spinal cord. Although all types of glia may take part in such a reaction, the process involves as a rule only astrocytes or/and Hortega's microglia.

Despite the great variety of pathomorphological syndromes underlying various disease of the central nervous system, the pathological reactions of glia can be manifested in two fundamental types of tissue impairment. One - are regressive changes taking the form of cell degeneration or atrophy, the second - progressive changes, in the form of proliferation and/or hypertrophy of glial cells.

These two groups of changes may occur separately, or they may coexist together. The nature of glial abnormality in this type of changes depends on many factors, in the first place in the character and nature of the pathogenic factor, its intensity, the duration of the pathological process, the type of changes in other nerve tissue elements, and to the greatest extent upon the sensitivity and reactivity of the glial cells involved. The same factor, e.g. hypoxia, may cause an irreversible lesion of oligodendrocytes, and a marked productive reaction of astrocytes. When the same pathogenic factor acts with greater intensity irreversible changes in all glial types and nerve cells and their processes may occur.

Besides the above mentioned secondary glial changes, there are also pathological processes in which glial abnormality is a primary pathological feature. Such a primary gliopathy is observed for instance in the Jakob-Creutzfeldt disease, in hepatogenic encephalopathy and in Wilson's hepatolenticular degeneration.

The pathological process, involving glial tissue leads to morphological changes in it, which are accompanied by impairment of histochemical properties. These histochemical changes take the form of an accumulation within the glial cells of some chemical substances, which in normal conditions are not present in this site or occur in much smaller amounts. Their occurrence or excessive accumulation may be an expression of metabolic disturbances in the involved type of glial cells as well as in the central nervous system or whole organism.

The other type of histochemical abnormality is represented by changes in enzymic activity. These consist in variations of the intensity of histochemical reactions, as compared with the same in normal conditions, disappearence of some reactions or on the contrary in the appearence of others, which in normal conditions are not found in glial cells, or their intensity is so low that they cannot be demonstrated histochemically. Such enzymic histochemical abnormalities indicate disturbances in intracellular metabolism, or serve as an indicator of the state of some subcellular organelles, such as mitochondria, lysosomes or Golgi apparatus.

The first type of disturbances, which can be described as substrate histochemical abnormality may be manifested by accumulation of various types of lipid or glycolipid substances in the cytoplasm of astrocytes and/or microglial cells in various lipid storage diseases and leucodystrophies. This phenomenon is an essential element of systemic or generalized disease.

The histochemical properties of the substances accumulated within the cytoplasm of glial cells can be revealed by various histochemical staining reactions. However, it should be kept in mind thet individual histochemical reactions, although diagnostically valid, very rarely can determine the exact chemical nature of the accumulated substances /Adams, 1965/.

The same group of substrate histochemical disturbances is characterized by the presence of iron granules in the cytoplasm of the rod-like Hortega's microglia and astrocytes in Alzheimer's disease /Hallgren,Sourander, 1960/ and of copper deposits in the hypertrophied astrocytes in Wilson's hepato-lenticular degeneration /Uzman, 1956; Okinaka et al., 1954/. To the same group of histochemical abnormalities belongs to some extent, the accumulation of glycogen deposits in astrocytes, as the result of various pathological processes. Accumulation of glycogen in astroglia has been reported in the vicinity of brain tumors /Oksche,1961/, stab wounds /Friede, 1954; Shimizu, Hamuro, 1958; Guth, Watson, 1968/ and particularly as an effect of radiation /Klatzo et al., 1961; Miquel, Haymaker, 1965/ and hypoxia /Mossakowski et al., 1968/. These changes indicating disturbances in the metabolism, transport and utilization of glucose in the central nervous system, are fully reversible. Glycogen deposits disappear completely in time intervals, varying from a few days to several weeks.

The occurrence of PAS-positive granules, within the cytoplasm of as - trocytes cultured in vitro /Lumsden, 1958/ may be classified to the group with histochemical disturbances in the substrate, although they differ pathogenically from the previously mentioned changes. Lumsden /1958/ considers them as mucopolysaccharide or glycoprotein substances. Ogawa et al. /1960, 1961/ believe thet they are phospholipid protein complexes localized within the lysosomes. In our opinion /Mossakowski et al.,1970/ the PAS-granules in astrocytes cultured in vitro are identical with those, which Klatzo et al. /1960/ described within the cell bodies and processes of astrocytes in areas of severely edematous brain tissue. In both cases the granules are of protein nature, and in case of edema originate from the blood serum, while in cultures - from the medium.

A vast section of the neuropathological literature concerns changes in the histochemistry of reactive glia, first of all reactive astrocytes, which are the most common in human pathology and in experimental conditions.

Increase in cell diameter, in cytoplasm volume, enlargement of nuclei and their displacement towards the cell periphery are the most typical morphological characteristics of reactive astrocytes. In chronic pathological processes they take the form of typical plump cells /Fig. 1/. Identical changes occur in long-term glial cultures /Fig. 2/. The most typical feature characterizing reactive astrocytes is a remarkable increase of the activity of oxidative enzymes /Fig. 3/, which is particularly striking, because in normal astrocytes their activity is very low. This increase of oxidative enzyme activity is a universal feature, occurring in all cases of astrocytic reaction, regardless of the nature of the pathological process which are the cause of tissue reaction. In human pathology the increase of oxidative enzymes activity in astrocytes is observed in various pathological processes. This has been described in the surroundings of brain tumors /Schiffer, Vesco, 1962; Rubinstein, Sutton, 1963/ and atheromatous cerebral blood vessels /Śmiałek, Wiśniewska, 1966; Nereantiu,Tautu, 1969/, in demyelinated plaques /Ibrahim, Adams, 1963; Friede, 1961/, and in some lipid-storage diseases /Wallace et al., 1963/. Similarly in experimental conditions, the same type of changes can be seen in the majority of experimentally induced pathological processes, such as for instance, edema /Rubinstein et al., 1962/, anoxic-ischemic encephalopathy /Becker, 1961; Spector, 1963/ and many others /Friede, 1966/.

The early appearance of changes in enzymic histochemical reactions, and variations in the reaction of different enzymes deserves the special attention. Rubinstein et al. /1962/ in their studies on experimental edema note that the earliest increase of histochemical enzyme reaction appearing as early as 12 hours following injury concerns mostly glutamate dehydrogenase while the following 12 hours yield an enhancement of the activity of NAD-diaphorase and dehydrogenases linked with coenzyme I. Friede /1966/, considers that the earliest enzymic changes, occurring already 6 hours after injury, concern at first the enzymes of the glycolytic pathway and hexosemonophosphate shunt and later those of the citric acid cycle. Domańska /1970/ in this Laboratory observed an increase of glucose-6-phosphate dehydrogenase activity as soon as 3 hours after hypoxia in rats.

Besides the increase of oxidative enzymes activity, the reactive astrocytes exhibit also a markedly intensified activity of other enzymes. Such increase enzymic activity involves that of acid phosphatase /Fig. 4/ /Koenig, Barron, 1962; Schiffer et al., 1967/ beta-glucuronidase /Schiffer, Cognazzo, 1968/, butyryl cholinesterase /Roessmann, Friede, 1966/, ATP-ase /Fig. 5/ /Ibrahim, Adams, 1963/ and others. Penar /1970/ showed marked increase of aldolase activity in the reactive astrocytes within degenerating spinal cord tracts.

Since the accumulation of glycogen in astrocytes is one of the most common non-specific glial reactions, the activity of the glycogen-metabolizing enzymes in the reactive astrocytes deserves special attention. Shimizu and Hamuro /1958/ describe a rise in phosphorylase activity in astrocytes surrounding brain wounds. In our studies concerning cerebral hypoxia we have demonstrated histochemically detectable UDPG-transferase and phosphorylase a activities in astrocytes of the gray and white matter already one hour after experimental injury /Mossakowski et al.,1968/. This activity gradually increases during the whole time of glycogen presence, its reduction occurs during the next 3-5 days. The reversibility of enzymic histochemical changes depends, however, on several factors, independently of the nature of the enzyme under study. Phosphorylase activity, disappearing in 7 days from astrocytes in cases of experimental hypoxia, persists during several months in astrocytes within the degenerating spinal cord tracts /Figs 6 and 7/, following cord hemisection /Penar, 1970/. Osterberg and Wattenberg /1962/ showed a persistent increase of oxidative enzyme activity in astrocytes over one year in animals and about 4 years in human pathology. However primarily increased enzyme activity in reactive astrocytes may also be reduced; this depends on the stage of the pathological process. The intensity of enzymic reactions in the centre of an old MS-plaque is undoubtedly reduced as compared with that of a recent plaque /Friede, 1961; Ibrahim, 1965/.

It should be pointed out that the ability of astroglia to react both morphologically and histochemically depends to a great extent on its maturity /Osterberg, Wattenberg, 1963/.

Reactive changes occur also in other types of glia. The hypertrophy of oligodendrocytes never has a form as pronounced as that of astrocytes. However there are such pathological conditions, in which the increase of enzymic activity has to be attributed to the reaction of oligodendroglia, e.g. Ibrahim and Adams /1963/ consider high enzymic activity at the edge of MS-plaques as due mostly to the reaction of oligodendrocytes. The reactive forms of microglia exhibit also a remarkable activity of oxidative enzymes /Rubinstein et al., 1962; Kreutzberg, Peters, 1962; Rubinstein Smith, 1962, and others/, mostly of NADP-diaphorase and dehydrogenases linked with coenzyme II. An increase of acid phosphatase activity in reactive forms of microglia is almost a specific feature of Hortega's cells /Anderson, Song, 1962; Wallace et al. 1963; Friede,Magee,1962; Friede,

DeJong, 1964/. A significant increase of the activity of enzymes represen-
ting the hexosemonophosphate shunt in the reactive microglia is probably
related to the intracellular metabolism of fatty acids.

It should be mentioned here, that glial tissue cultured in vitro beha-
ves as regards its enzyme histochemical properties like reactive glia in
situ. This is true for both oxidative and hydrolytic enzymes activity in
astrocytes, oligodendrocytes /Fig. 8/ and microglia /Mossakowski et al.,
1966/ Renkawek, Mossakowski, 1966; Renkawek, 1967; Kraśnicka et al., 1969/.
The histochemistry of regressive glial changes is by far less known than
that of progressive changes. The data concerning this subject are very scan-
ty and fragmentary. Zeman /1963/, Becker /1963/ and Colmant and Peters
/1965/ described reduction of enzymic activity in nervous tissue as the
consequence of severe hypoxia. Such an enzymic activity decrease involves
all the structural elements of the central nervous system, including
glial cells. The unequal damage of various types of neuroglia is a very
common and typical feature. Hypoxia causing reduction of enzymic activity
in oligodendrocytes, at the same time leads to its remarkable increase in
astrocytes and microglia. In the same conditions the decrease of butyryl
cholinesterase activity in oligodendrocytes is accompanied by its evident
enhancement in astrocytes /Roessmann, Friede, 1966/.

Our studies concerning the activity of oxidative and hydrolytic enzy-
mes in various types of glia under hypoxic and hyperoxic conditions in
tissue cultures /Kraśnicka et al., 1967; Renkawek, 1967; Kraśnicka, Ren-
kawek, 1969/ demonstrated the dynamics of regressive changes following
glial tissue injury. The greatest reduction of enzymic activity in both
astrocytes and oligodendrocytes occurs in complete anoxia and full hyper-
oxia. Young cultures /2-3 weeks/ are most sensitive towards the noxious
factors. The most pronounced drop of activity is exhibited by enzymes of
the Krebs cycle, next come those of the hexosemonophosphate shunt. The
least sensitive to injury is the activity of glycolytic enzymes. Oligo-
dendrocytes are more sensitive than astrocytes both to anoxia and hyper-
oxia. The enzyme activity disappears at first in cellular processes, then
in the perikarya /Figs 9 and 10/. Trace of enzyme activity around the nu-
clei are always present even in the most severely injured cells. The hyd-
rolytic enzymes activity exhibits a considerable increase in conditions
of moderate hypoxia, but there is marked and rapid drop of enzymic activi-
ties in extreme conditions. Non-specific esterase activity is more resi-
stant than that of acid phosphatase. Microglia exhibits the greatest re-
sistance against both anoxia and hypoxia. In all conditions all morpho-
logical forms of Hortega´s microglia show a high enzymic activity.

The cellular glial forms occurring in hepato-lenticular degeneration
and hepatogenic encephalopathies known in the neuropathological literature
as Alzheimer cells, type I and II, and Opalski cells, represent the second
type of glial degeneration, namely primary gliopathy.

It is well known, that in some types of hepatogenic encephalopathy, in-
tranuclear glycogen inclusions occur in Alzheimer cells, type II /Inose et
al., 1960/. Similar inclusions of a histochemically confirmed glycogen na-
ture, and PAS-positive, non-glycogen inclusions, both intracytoplasmic and
intranuclear have been observed by Shiraki and Yamamoto /1960/ in astrocy-
tes in cases of Inose hepato-lenticular degeneration. In our material /Mos-
sakowski, 1966/ exclusively intranuclear, glycogen inclusions /Fig.11/ in

naked nuclei are present in encephalopathies related to various non-specific lesions of the liver, with and without porto-caval shunt performed. They are never seen in Alzheimer cells type II in Wilson's disease. In hepatogenic encephalopathy the naked nuclei are surrounded by aggregates of granular PAS-positive material, which stains also positively with Sudan black B and Alcian blue. As this material accumulates also in the vicinity of morphologically unchanged astrocytic nuclei, we would consider these aggregates rather as products of cell degeneration then those of cell breakdown. No enzymic activity of any type directly related to naked nuclei could be demonstrated either in experiments on animals, in tissue cultures.

The most typical feature in the histochemical picture of Opalski cells is accumulation within their cytoplasm of strongly PAS-positive granules /Fig. 12/, which distinguish Opalski cells from those of Alzheimer, type I /Mossakowski, 1965/. These intracytoplasmic granules are histochemically identified as acid and neutral mucopolysaccharides bound up with cellular proteins. The presence of copper in the cytoplasm of Opalski cells in Wilson's disease is the only difference between these cells in hepato-lenticular degeneration and in hepatogenic encephalopathy.

So far we did not have any human material suitable for enzymes histochemical studies of Alzheimer cells, type I and Opalski cells. The only information in this respect is given by Friede /1965/ who demonstrates a very low SDH activity in the hypertrophied astrocytes of Alzheimer type I in a case of Wilson's disease.

Our observations concerning enzyme histochemistry of these cellular forms in tissue culture are the subject of a separate report. In experiments carried out on glial tissue cultures, we are able to obtain cellular forms which from the morphological and histochemical points of view are identical with Opalski cells in hepatogenic encephalopathy. The cytoplasm of these cells, like that of their analogues "in situ" is filled with granular or filamentous aggregates of PAS-positive substances /Fig. 13/ which can be identified histochemically as neutral and partly acid mucopolysaccharides, bound up with protein material. They do not contain any histochemically detectable lipid substances. Copper is also absent even in cases in which exogenous copper salts have been added to the incubating medium. As far as enzyme histochemistry is concerned, the Opalski cells in cultures exhibit a high G6PDH activity /Fig. 14/ and very low activity of SDH /Fig. 15/ and GDH as compared with normal astrocytes both in vivo and in vitro. Acid phosphatase activity /Fig. 16/ is markedly elevated, what is specially noticeable in view of the usually rather low acid phosphatase activity in tissue cultures /Renkawek, 1967/ and its moderate increase in reactive astrocytes /Anderson, Song, 1962/.

Besides Opalski cells, the cultures contain numerous glial cells representing intermediate stages beteen normal astrocytes and fully developed Opalski cells. Their substrate-histochemical and enzyme-histochemical pictures combine in various proportions the features of both types of cells. Single, Alzheimer cells, type I – occurring only in cultures with sera from Wilsonian patients do not contain in their cytoplasm any mucopolysaccharide material; their enzymic activity is more or less the same as in normal astroglia, or even slightly lower as far as SDH activity is concerned.

Despite of the well known limitations in comparability of experimental material, especially that from tissue cultures with human pathology, we believe that the striking morphological and histochemical similarity of the above discussed changes entitle us to suggest some hypotheses, concerning the possible pathogenesis of glial changes in these conditions. It seems to us that the pathogenic factor or factors /with no limitation to copper and ammonia/, which disturb glial carbohydrate metabolism at the

level of the Krebs cycle, may cause abnormalities in intracellular metabolism, which lead to the production of mucopolysaccharides. These in turn, are stored within the astrocytes cytoplasm and are responsible for their morphological transformation.

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