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M. J. MOSSAKOWSKI, B. PENAR

SOME ASPECTS OF THE HISTOCHEMISTRY OF THE REACTIVE GLIA *)

Department of Neuropathology, Experimental and Clinical Medical Research Centre, Polish Academy of Sciences, Warsaw, *Poland.*
Head of Department: Prof. dr M. J. Mossakowski

Glial reaction is one of the most common features in the pathology of the central nervous system. This accompanies a great number of the pathological processes of the nervous tissue and as a rule may involve all types of glia. However, the richest literature concerns astroglial reactions. This type of glial reaction forms also the subject of the present report.

Increase in cell diameter and cytoplasm volume, enlargement of nuclei and their displacement towards the cell periphery, hypertrophy of cellular processes are the most typical morphological characteristics of reactive astrocytes. In chronic pathological processes they take form of typical plumb cells (Fig. 1).

The vast section of neuropathological literature concerns changes in the histochemical properties of the reactive astrocytes under various pathological and experimental conditions. However, it has to be pointed out that histochemical understanding of reactive astrocytes is not univocal with the morphological one. Histochemical reactive changes often appear earlier than the above mentioned morphological transformations (Rubinstein et al. 1962, Mossakowski 1963, Domańska 1970, Petrescu 1972) and in many instances they are reversible in nature and not accompanied by any modifications in morphological structure of the cells (Mossakowski et al. 1968, Mossakowski, Zelman 1971).

The most typical feature characterizing reactive astrocytes is a remarkable increase of the activity of oxidizing-reducing enzymes (Figs. 2 and 3), which is particularly striking, since in normal astrocytes their activity is very low. In human pathology, an increase of oxidizing-redu-

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cing enzymes was observed in various pathological processes. This was described in the surroundings of brain tumours (Schiffer, Vesco 1962, Rubinstein, Sutton 1963, Mossakowski 1962) and atheromatous cerebral blood vessels (Śmiałek, Wiśniewska 1966, Nareantiu, Tantu 1969), in demyelinated plaques (Ibrahim, Adams 1963, Friede 1961) and in some lipid storage diseases (Wallace et al. 1963). Similarly the same types of changes were seen in the majority of experimentally induced pathological processes, such as edema (Rubinstein et al. 1962) anoxic-ischemic encephalopathy (Becker 1961, Spector 1963) and many other (Friede 1966).

The time of appearance of changes in enzyme histochemical reactios and variations in the activity of different enzymes deserves a special attention. Rubinstein et al. (1962) in their studies on experimental edema noted that the earliest increase of enzyme-histochemical reaction, appearing as early as 12 hrs following injury concerned mostly glutamate dehydrogenase, while the following 12 hrs yielded an enhancement of the activity of NAD-diaphorase and dehydrogenases linked with coenzyme I. Friede (1966) considered that the earliest enzymic changes occuring already 6 hrs after injury concerned at first the enzymes of glycolytic pathway and hexosemonophosphate shunt and later those of the citric acid cycle. Domańska (1970) in our laboratory observed an increase of glucose-6-phosphate dehydrogenase activity as early as 3 hrs after hypoxia in rats.

Besides the increase of oxidizing-reducing enzyme activity, the reactive astrocytes exhibit also a markedly intensified activity of other enzymes such as acid monophosphatase (Fig. 4) (Koenig, Barron 1962, Schiffer et al. 1967), beta-glucuronidase (Schiffer, Cognazzo 1968), butyryl-cholinesterase (Roessmann, Friede 1966), ATP-ase (Ibrahim, Adams 1963) and others.

This increase of the activity of various enzymes is considered univocally to be an universal feature, occuring in all cases of astrocytic reaction regardless the nature of the pathological processes which are the cause of tissue reaction. On the other hand, however, there is a number of observations by different authors and those by our own, which point at the histochemical variances of the reactive astrocytes, despite of morphologically identical substrate involved.

In that respect the problem of glycogen accumulation in the reactive astrocytes deserves a special attention, as this feature constitutes the most common non-specific glial reaction resulting from a great number of pathological processes. The glycogen deposits were reported in astrocytes in the vicinity of brain tumours (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).

In our studies carried out on various models of experimental hypoxia (Mossakowski et al. 1968 Mossakowski, Zelman 1971, Pronaszko et al. 1971, Long et al. 1972) we have also stated the occurrence of glycogen deposits within astrocytes (Fig. 5). These changes indicating disturbances in metabolism, transport and utilization of glucose in the central nervous system (Mossakowski et al. 1968, Klatzo et al. 1970) were fully reversible. Astrocytic glycogen deposits appeared and disappeared in characteristic time sequences, depending upon the type of experimentally induced hypoxia. Usually they appeared at 6^{th} — 10^{th} hour after experiment and disappeared completely at time intervals varying from few to several days. However, in striking contrast to that there were areas, mostly those surrounding necrotic foci, where astrocytic glycogen deposits persisted for several weeks. It has to be noticed, that none of the glycogen bearing astrocytes, except those within necrotic foci and their vicinity, had manifested any features of cellular hypertrophy and after the disappearance of polysacharide deposits, they were morphologically identical with those in the undamaged tissue. On the other hand, in astrocytes undergoing gemistocytic transformation granular glycogen deposits also disappeared in the course of several days; however, their cytoplasm remained PAS-dimedon positive during many weeks following hypoxia (Fig. 6). This morphological alteration of glycogen might, in turn, indicate variation in its biochemical or physical state and/or its binding with cellular proteins.

In young animals with immature central nervous system the astrocytic accumulation of glycogen deposits occured both in the gray and white matter, which was in a striking contrast with previous observations of Miquel et al. (1965) dealing with irradiation and those of ours concerning the effect of hypoxia on the mature nervous system.

Miquel et al. (1965) considered that glycogen storage was a specific property of the protoplasmatic astroglia. The limitation of astrocytic accumulation of glycogen in the white matter to the areas undergoing an active myelination, might indicate that glycogen accumulation depended more on the functional state of astrocytes that upon their histological type.

In the some series of experiments we noticed parallel changes in the activity of glycogen-metabolizing enzymes (Mossakowski et al. 1968, Mossakowski, Zelman 1971). These consisted in the appearance of histochemically detectable UDPG-transferase and phosphorylase a activities already within 1 hr following hypoxia (Figs. 7, 8) and disappeared completly within 3 days for UDPG-transferase and within 7 days for phosphorylase a. However, the reversibility of the enzyme changes depended on several factors. The phosphorylase activity, found to disappear from

astrocytes within 7 days in the case of experimental hypoxia, persisted several months in hypertrophied astrocytes within degenerating spinal cord tracts (see below).

In our recent studies on the histochemical properties of the reactive astrocytes in hemisectomized spinal cord in cats we turned our attention to the differences between the astrocytic reaction in areas of the glial scar surrounding necrotic foci, and that within degenerating spinal tracts. The differences reflected already on the morphological picture of cells. Within perinecrotic areas the reactive astrocytes took the form of typical gemistocytes already in the course of several weeks after cord injury whereas in degenerating spinal pathways, the glial proliferation was accompanied by considerable cellular hypertrophy, but without formation of typical plumb cells. Gemistocytes within glial scars revealed strong intracytoplasmatic, granular glycogen aggregations which persisted in here considerably longer than in the surroundings of the experimental stab wounds, as reported by Friede (1954) and Shimizu and Hamuro (1958). Glycogen deposits within gemistocytes cytoplasm in this group of experiments differed in their granular nature from those in gemistocytes in hypoxic experiments. At the same time hypertrophied astrocytes in degenerating spinal tracts showed no glycogen deposits.

The variations in histochemical glycogen reactions were accompanied by differences in the activity of glycogen metabolizing enzymes, first of all in that of the phosphorylases. These differences between gemistocytes from the glial scars arround necrotic foci and hypertrophied astrocytes from the degenerating spinal tracts consisted in the intensity and cellular localization of phosphorylase activity (Figs. 9, 10), which was found to be much more intensive and compactly distributed in the latter localization. The persistence of increased phosphorylase activity in this case has been already mentioned. Similar differences concerned the activity of other enzymes, such as aldolase (Fig. 11) and succinate, lactate (Fig. 12) and glucose-6 P-dehydrogenases.

Despite of all well known reservations, concerning interpretation of section histochemical findings, it seems justified to consider, that differences in histochemically detectable activity of various enzymes and in the intracellular contents of some chemical substances, like e.g. glycogen, between normal and reactive astrocytes are indicating changes in the metabolism of these cellular components in the central nervous system occuring under the influence of various pathological processes. Therefore, the above presented variations of histochemical properties of the reactive astroglia itself, might, in turn, indicate the differences in these metabolic disturbances, depending upon the type of reacting glia, its functional state, localization and on the kind, nature and duration of the noxious

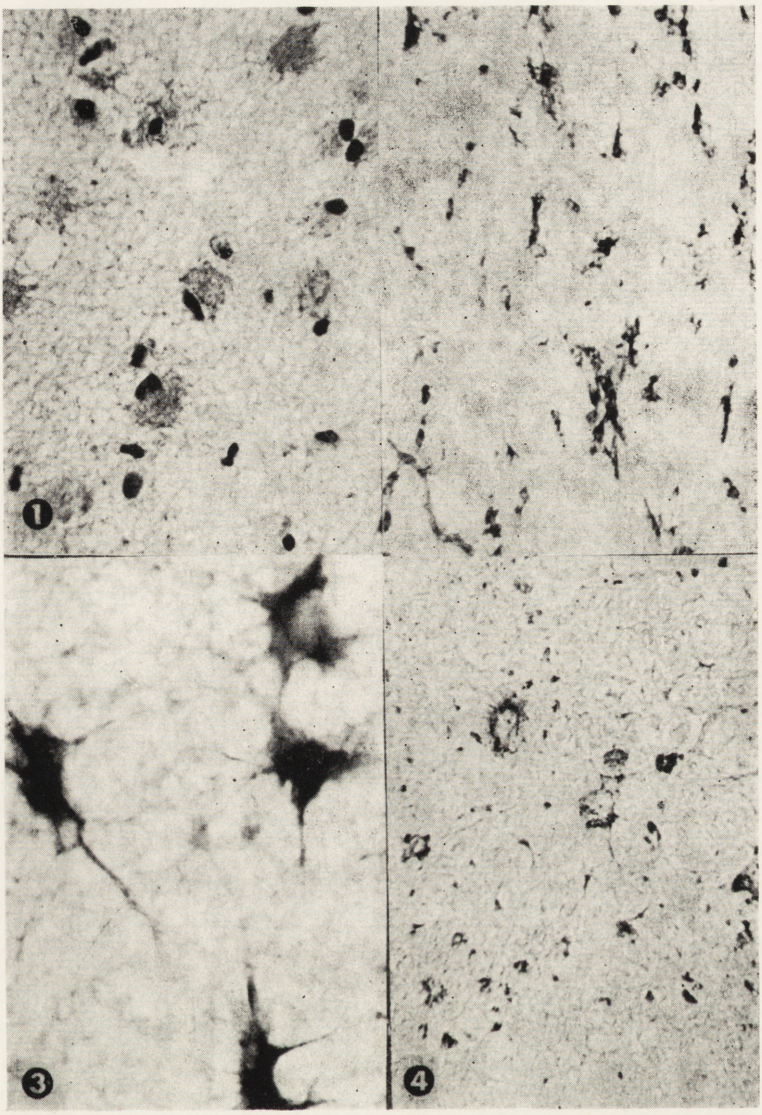

Fig. 1. Plump-astrocytes in the white matter of cerebral hemisphere in a case of diffuse sclerosis. H-E. × 400.

Fig. 2. Glucose-6-phosphate dehydrogenase activity in astrocytes of the cerebral white matter in a case of experimental hypoxic-ischemic encephalopathy (rat), 24 hrs following hypoxia. × 160.

Fig. 3. Succinate dehydrogenase activity in the hypertrophied astrocytes, surrounding a glial brain tumour in man. × 800.

Fig. 4. Acid phosphatase activity in hypertrophied astrocytes within a degenerating tract of the hemisectomized spinal cord in cat. × 400.

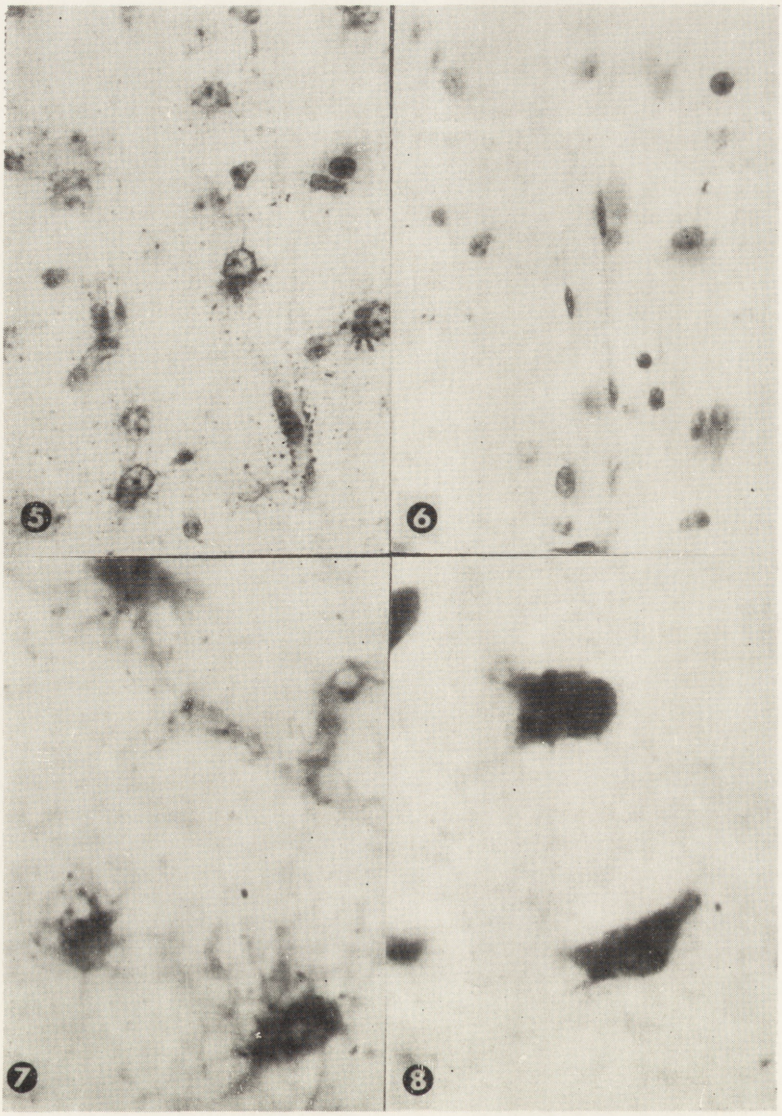

Fig. 5. Glycogen accumulation within the cytoplasm and cellular processes of the white matter astrocytes in an asphyxiated newborn monkey, 24 hrs following asphyxiation. × 780.

Fig. 6. PAS-dimedon positive cytoplasm of gemistocytic astrocytes within 7 day old necrotic focus of the brain in an asphyxiated newborn monkey. × 600.

Fig. 7. UDPG-transferase activity within the white matter astrocytes in an asphyxiated newborn monkey, 1 hr following asphyxiation. PAS staining, × 760.

Fig. 8. Glycogen phosphorylase a activity within the white matter astrocytes in an asphyxiated newborn monkey 1 hr following asphyxiation. PAS staining. × 760.

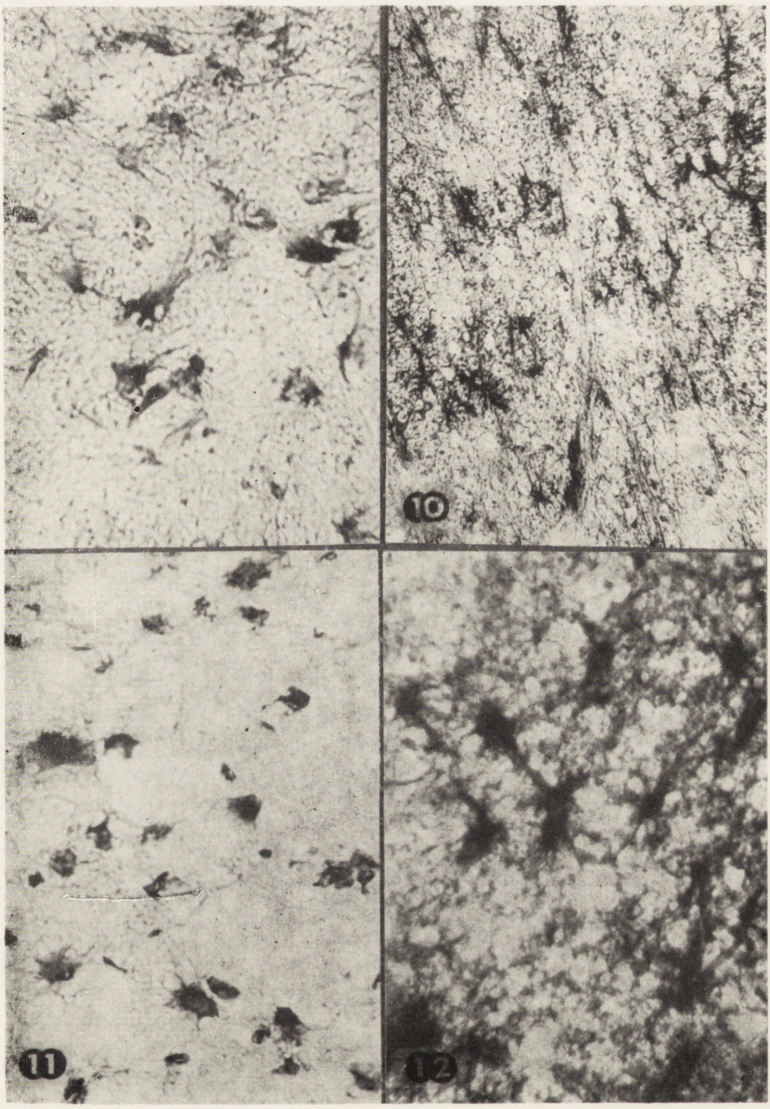

Fig. 9. Glycogen phosphorylase activity in reactive astrocytes from the vicinity of necrotic focus within hemisectomized spinal cord in cat. Iodine staining. × 600.

Fig. 10. Glycogen phosphorylase activity in hypertrophied astrocytes from the degenerating tract of the hemisectomized spinal cord in cat. Iodine staining. × 600.

Fig. 11. Aldolase activity in the reactive astrocytes surrounding necrotic focus within hemisectomized spinal cord in cat. × 400.

Fig. 12. Lactate dehydrogenase activity in the reactive astrocytes from the glial scar in the hemisectomized spinal cord in cat. × 600.

factors, provoking glial response. The data, presented at this Symposium by Szydłowska and Kałuża (1972), concerning the histochemistry of protein functional (terminal) groups in reactive glia in the areas surrounding various pathological foci in the brain are strongly supporting this view. This would point to the possibility that the morphologically homogenous pathological feature, known under the name of reactive astrocytes, corresponds to various, changeable biological states.

M. J. Mossakowski, B. Penar

WYBRANE ZAGADNIENIA HISTOCHEMII GLEJU ODCZYNOWEGO

Streszczenie

Na podstawie przeglądu piśmiennictwa i własnych obserwacji prowadzonych na różnych modelach doświadczalnych autorzy wykazują zróżnicowanie właściwości histochemicznych odczynowych astrocytów.

Autorzy wykazują, że gromadzenie się glikogenu w astrocytach, stanowiące jedną z najpospolitszych nieswoistych reakcji tkanki nerwowej na działanie różnorodnych czynników uszkadzających, wykazuje zróżnicowanie uwarunkowane z jednej strony rodzajem gleju, jego stanem czynnościowym, położeniem, a rodzajem, czasem trwania i charakterem czynnika uszkadzającego z drugiej. Takie same różnice dotyczą aktywności enzymów metabolizujących glikogen i niektórych innych enzymów takich jak aldolaza, dehydrogenaza bursztynianowa, mleczanowa i glukozo-6-fosforanowa.

Autorzy postulują, że zróżnicowanie cech histochemicznych, stanowiących wykładniki metabolicznych właściwości tkanki, wskazuje na niejednorodność pojęcia „glej odczynowy", mimo niejednokrotnej identyczności obrazów morfologicznych.

М. Е. Моссаковски, Б. Пенар

ИЗБРАННЫЕ ВОПРОСЫ ГИСТОХИМИИ РЕАКТИВНОЙ ГЛИИ

Резюме

На основании литературного обзора и собственных наблюдений, проводимых на разных экспериментальных моделях, авторы доказывают дифференциацию гистохимических свойств реактивных астроцитов.

Авторы доказывают, что скопление гликогена в астроцитах, составляющее одну из самых распространенных неспецифических реакций нервной ткани на воздействие разных повреждающих агентов, обнаруживает дифференциацию, обусловленную — с одной стороны — видом глии, ее функциональным состоянием, положением и видом, временем воздействия и характером повреждающего агента — с другой стороны. Такие же разницы касаются активности энзимов метаболизирующих гликоген и некоторых других факторов, таких как альдолаза, сукцинатдегидрогеназа, лактатдегидрогеназа и глюкозо-6-фосфатдегидрогеназа.

Авторы полагают, что дифференцирование гистохимических свойств, составляющих показатели метаболических свойств ткани, указывает на неоднородность понятия „реактивная глия" несмотря на неоднократную идентичность морфологической картины.

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Authors' address: Department of Neuropathology, Experimental and Clinical Medical Research Centre of Polish Academy of Sciences, Warszawa, ul. Dworkowa 3.