HISTOCHEMISTRY

Triphosphopyridine Nucleotide (TPN) Diaphorase and TPN-dependent Dehydrogenase Activity of Reactive Macrophages in Tissue Necrosis

A HISTOCHEMICAL study, with the tetrazolium salt technique, of experimental cerebral œdema produced in cats by the local application of cold has shown a considerable increase of oxidation-reduction activity in reactive macroglia and microglia[1]. The fat-granule cells in the necrotic cortical lesion have furthermore been noted to display a response which differs constantly from that observed in the reactive astrocytes or the protein-carrying microglia in the œdematous white matter. In the present communication these findings are supplemented by histochemical observations on the reactive macrophage system elsewhere in the body as a result of a similarly produced necrotizing lesion.

30 adult Wistar rats, weighing 150–200 gm., were used. Circumscribed necrotic lesions were produced in the cerebral cortex by the application of dry ice to the intact skull for 90 sec., and in the skeletal muscle and spleen by its application to the exposed tissues for 30 sec. Cerebral lesions were also made in five 3-day-old rats. Animals were killed after 5 or 6 days. Rapidly frozen fresh tissues were cut on a Slee-Pearse cryostat, the brains at 16μ, and the other tissues at 8μ. Sections were incubated with the appropriate substrate and the tetrazolium salt in conditions as described elsewhere[1]. The enzymes and coenzymes investigated included diphosphopyridine nucleotide (DPN) diaphorase, triphosphopyridine nucleotide (TPN) diaphorase, and the dehydrogenases of glucose-6-phosphate, succinate, glutamate, DPN-dependent isocitrate and TPN-dependent isocitrate. In addition to nitro-BT (2,2′-di-*p*-nitro-phenyl-5,5′-diphenyl-3,3′-((3,3′-dimethoxy-4,4′-biphenylene)) ditetrazolium chloride), MTT (3-((4,5-dimethyl-thiazolyl-2))-2,5-diphenyl tetrazolium bromide) chelated with cobaltous chloride was used in a number of procedures as the tetrazolium salt, according to the technique of Pearse[2]. Conventional stains on the same tissues included colloidal sudan IV, gallocyanin, periodic acid–Schiff, methyl green-pyronin and Weil-Davenport's silver carbonate for microglia, some blocks being cut serially to compare the histochemical findings more closely with the sudan and silver carbonate stains.

The necrotic lesions in the brain, muscle and spleen, including those in the cerebral cortex of the immature rats, contained a large number of macrophages with alcohol-soluble sudanophilic material in their cytoplasm. In histochemical preparations, these cells were strongly positive for TPN diaphorase, TPN-dependent isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase, and negative for DPN diaphorase, DPN-dependent isocitrate, glutamate and succinate dehydrogenase. By contrast, the reactive astrocytes at the edge of the cerebral lesions, the overlying meninges and blood vessels were strongly positive for DPN diaphorase and glutamate dehydrogenase, though also positive for TPN diaphorase and the TPN-dependent dehydrogenases[1].

In addition 3 rats were given 0·5 ml. of *T.A.B.* vaccine intravenously in order to activate the spleen without tissue damage, and 3 were given the same dose subcutaneously into the thoracic wall in order to activate the axillary lymph nodes. In contrast to the changes associated with local necrosis, no significant alterations were noted in the reactive cells of the spleen or lymph nodes three days after the injection.

The histochemical demonstration of a predominance of TPN-dependent enzyme activity in macrophages resulting from local necrosis suggests an increased metabolic activity within these cells along one or both of two possible pathways. The first of these is fatty acid synthesis. Although the enzymes concerned in the latter have not yet been fully elucidated, the present observations are consonant with the findings of Porter *et al.*[3] and Lachance *et al.*[4] that this is effected through a TPN-dependent system, microsomal in situation, in contrast to fatty acid breakdown, in which the pathway is largely dependent on DPN and mitochondrial. The increase of TPN-dependent isocitrate dehydrogenase activity is of special interest since isocitrate has been shown to be a necessary co-factor in fatty acid synthesis[3], the first step of which would be the production, in the presence of TPNH, of a malonyl derivative through the carboxylation of acetyl coenzyme A[5–7]. Thus it seems possible that, as a result of local tissue necrosis, units of acetyl coenzyme A derived from the breakdown *in situ* of amino-acids, carbohydrates and lipids are used by the engulfing macrophages for the synthesis of neutral fat.

The present results also suggest that increased activity may involve the pentose cycle. Since adenosine triphosphate is required for fatty acid synthesis[3] and since the absence of DPN diaphorase, succinate and DPN-dependent isocitrate dehydrogenase activity suggests that the tricarboxylic cycle is not demonstrably implicated, the energy necessary for this reaction could be provided by the activity of the hexose-monophosphate shunt; this would furthermore constitute the principal source of TPNH in the extramitochondrial part of the cell, which would serve as electron donor for the reduction of unsaturated acetyl coenzyme A derivatives to their saturated counterpart, as suggested by Langdon[8]. The alternative hypothesis that the activity of the hexose-monophosphate shunt might indicate an abnormal synthesis of nucleoproteins seems unlikely in view of our failure to demonstrate any obvious increase of ribonucleic acid in the TPN diaphorase-positive macrophages by the methyl green-pyronin and gallocyanin strains.

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[1] Rubinstein, L. J., Klatzo, I., and Miquel, J., *J. Neuropath. Exp. Neurol.*, **21**, 116 (1962).
[2] Pearse, A. G. E., *Histochemistry—Theoretical and Applied*, 908 (J. and A. Churchill, London, 1960).
[3] Porter, J. W., Wakil, S. J., Tietz, A., Jacob, M. I., and Gibson, D. M., *Biochim. Biophys. Acta*, **25**, 35 (1957).
[4] Lachance, J. P., Popjak, G., and de Waard, A., *Biochem. J.*, **68**, 7, P (1958).
[5] Wakil, S. J., *J. Amer. Chem. Soc.*, **80**, 6465 (1958).
[6] Abraham, S., Matthes, K. J., and Chaikoff, I. L., *J. Biol. Chem.*, **235**, 2551 (1960).
[7] Abraham, S., Matthes, K. J., and Chaikoff, I. L., *Biochim. Biophys. Acta*, **46**, 197 (1961).
[8] Langdon, R. J., *J. Biol. Chem.*, **226**, 615 (1957).