INTENSITY OF SYNTHESIS OF PHOSPHOLIPIDS AND CHOLESTEROL IN THE BRAIN AND SPINAL CORD OF RABBITS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Experimental allergic encephalomyelitis (EAE) was induced in rabbits by inoculation of homologous spinal cord myelin. In the terminal state of the disease acetate-2-C^{14} was injected in a dose of 50 μCi/100 g body weight and the animals were killed 2 h later. The intensity of synthesis of phospholipids and cholesterol was greatly reduced in EAE not only in the lumbar region of the spinal cord, where the injury to myelin was greatest, but also in the brain stem, where demyelinization was absent.

KEY WORDS: *allergic encephalomyelitis; brain and spinal cord; phospholipids; cholesterol.*

Experimental allergic encephalomyelitis (EAE) is used as a model of demyelinizing diseases of the CNS. Some clear views have now been put forward to explain the character of the pathomorphological process in demyelinization [3, 4], but changes in lipid metabolism which lie at the basis of the myelin lesion remain unexplained.

Considering the distinctive nature of molecular organization of the myeline sheath, the basis of which consists of phospholipid–cholesterol complexes [17], in the investigation described below changes in phospholipid and cholesterol metabolism in the spinal cord and brain stem of rabbits with a severe form of EAE were studied with the aid of acetate-2-C^{14} as labeled precursor.

TABLE 1. SA of Phospholipids and Cholesterol (in counts/min/mg dry weight) in Lumbar Spinal Cord and Brain Stem of Normal Rabbits and Rabbits with EAE (M ± m)

Fraction	Lumbar spinal cord		Brain stem	
	control	EAE	control	EAE
Phospholipids	30±6,8	41±5,5	41±4,9	55±10,4
Cholesterol	14±4,8	9,8±3,1	6,2±1,0	5,8±1,3
Homogenate*	114±32	285±32	220±32	504±36
ASF†	31±4,0	51±11,8	42±4,4	69±12,3

*SA in counts/min/mg dry residue
†SA in counts/min/ASF from 1 mg wet weight of tissue

EXPERIMENTAL METHOD

A severe form of EAE was induced in rabbits by the method described earlier [1]. Acetate-2-C^{14} was injected subcutaneously into the animals in a dose of 50 μCi/100 g body weight 2 h before sacrifice. Lipids were extracted and estimated quantitatively by the method described previously [1, 7, 8]. The cholesterol fraction was isolated from the total lipid extract (3-4 ml) by thin-layer chromatography on silica gel in a solvent system of ether–benzene–ethanol–acetic acid (40:50:2:0.2) [9]. The total phospholipid fraction was isolated by repeated chromatography of the mixture of phospholipids and galactolipids remaining at the starting line in a solvent

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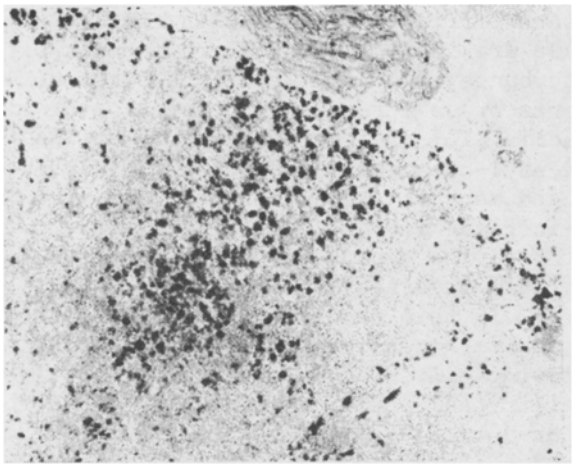

Fig. 1

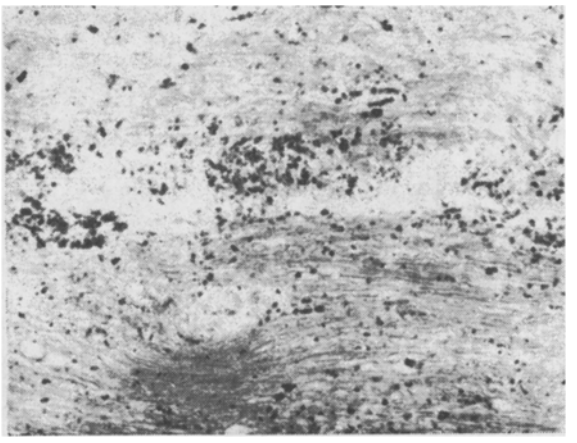

Fig. 2

Fig. 1. Focal demyelinization in lateral columns of lumbar spinal cord of rabbit with EAE (transverse section, 90 ×).

Fig. 2. Fine- and coarse-grained disintegration of myeline in lumbar spinal cord of rabbit with EAE (longitudinal section, 140 ×).

system of chloroform–methanol–NH_4OH (80:20:0.4) [6]. In this system phospholipids give two spots with the lowest mobility. To determine the total reserves of low-molecular-weight water-soluble radioactive metabolites (potential precursors for lipid synthesis) the specific radioactivity (SA) of the acid-soluble fraction (ASF) of the homogenate was determined [2]. The "Protaka" gas-flow counter was used to determine SA of the lipids and ASF.

Altogether six control animals and five animals with the paralytic stage of EAE were investigated. The results were subjected to statistical analysis by the usual methods. A morphological control was set up on the animals which developed the disease for the presence, spread, and localization of foci of disintegration of myelin by Marchi's method [10].

EXPERIMENTAL RESULTS AND DISCUSSION

At the height of development of EAE, foci of fine-and coarse-grained destruction of myelin of different shapes were found in the anterior, posterior, and lateral columns of white matter in rabbits with a severe form of the disease on morphological examination of sections through the lumbar spinal cord impregnated with osmium (Fig. 1 and 2); the most severely affected structures were the posterior columns, in which foci of demyelinization were found most frequently. Demyelinization was absent in sections through the white matter of the brain stem.

Since the intensity of lipid metabolism in the spinal cord has hardly been investigated, it was interesting to note that in the control animals SA of phospholipids was lower in the spinal cord than in the brain stem (Table 1). Meanwhile, SA of cholesterol in the lumbar region was much higher than in the brain stem, although in both cases it was still at a fairly low level characteristic of the nerve tissue of adult animals [5].

SA of phospholipids in the terminal stage of the disease was a little increased both in the lumbar region and in the brain stem, whereas SA of cholesterol was appreciably reduced in the lumbar region and virtually unchanged in the brain stem.

An increase in SA of phospholipids and a decrease or no change in SA of cholesterol were described by Smith [13, 14], in experiments in which slices of brain stem were incubated in vitro with glucose-C^{14}; this worker concluded that the intensity of phospholipid synthesis is increased in the acute stage of EAE. However, in the absence of data for the content of radioactive precursors in the tissue, or even of any change in the value of SA of the homogenate in EAE, this conclusion can hardly be accepted as well-founded, for SA of the tissue homogenate also is changed in EAE: According to the present experiments, in both the brain stem and in the lumbar spinal cord it was almost doubled (Table 1).

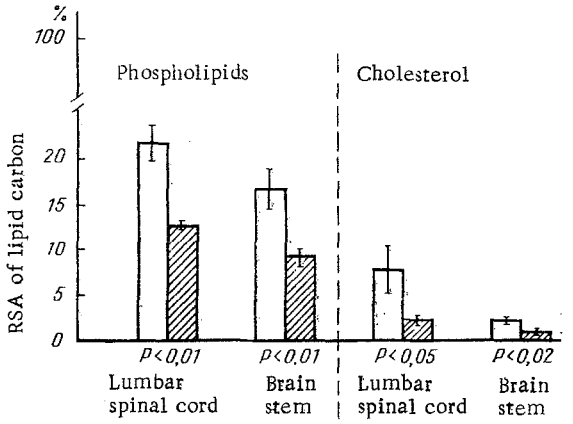

Fig. 3. Intensity of lipid synthesis in EAE. Shaded columns, EAE; unshaded columns; control. RSA of lipid carbon of homogenate taken as 100%.

Presumably the reserves of radioactive precursors for lipid synthesis in the brain tissue are increased in EAE on account of the disturbance of synthesis of the other components or as a result of damage to the mitochondria [12] and the pathways of utilization of acetyl-coenzyme A and other rapidly exchanged metabolites associated with them [15]. The increase in SA of ASF (by 64%; $P < 0.05$) confirmed the hypothesis of an increase in the total reserves of radioactive precursors in EAE. In this connection, to assess the changes in intensity of lipid synthesis the following criterion was used: the relative specific radioactivity (RSA) of lipid carbon compared with SA of carbon of the homogenate, taken as 100%.

$$\text{RSA of lipid carbon} = \frac{\text{SA of lipid carbon (counts/min/mg lipid carbon)}}{\text{SA of carbon of homogenate (counts/min/mg carbon of homogenate)}} \times 100.$$

The results of the calculation showed that in healthy animals acetate-2-C^{14} is incorporated into phospholipids and into cholesterol in the lumbar region of the spinal cord much more intensively than in the brain stem (Fig. 3). In EAE the intensity of phospholipid synthesis in the lumbar spinal cord not only was not increased but, on the contrary, it was considerably reduced (by 32%). In the brain stem the intensity of phospholipid synthesis also was reduced, although no appreciable changes were found in the phospholipid content in this part [1].

Similar results also were obtained for cholesterol: very low as it was, the intensity of its synthesis in EAE fell to an extremely low level in both the lumbar spinal cord and the brain stem. Accordingly the appearance of large quantities of cholesterol esters in the foci of demyelinization [1] can hardly be regarded as the result of increased cholesterol synthesis. It is evidently more correct to regard them as a product of "assembly" of damaged myelin lamellae phagocytosed by oligodendrocytes [11].

In experimental demyelinization induced by inoculation with homologous myelin, there is therefore marked inhibition of the synthesis of phospholipids and cholesterol in the spinal cord, correlating with a reduction in the content of those lipids. Moreover, the same metabolic response also was observed in the brain stem, where no demyelinization was present. This special type of "metabolic insult" [16] could perhaps be a specific response of the oligodendroglia, the metabolism of which is directed toward the formation and maintenance of the normal structure of myelin.

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