nuclear RNA polymerase activity. However, during the first hour (Fig. 2a), before any interferon had been synthesized, and 7.5 h after induction (Fig. 2b), when active interferon synthesis was in progress, the course of the curves and the level of RNA synthesis were the same for nuclei isolated from L_{929} cells producing interferon in the presence of adult and neonatal mouse serum. In other words, the nuclear RNA polymerase activity of cells synthesizing interferon in the presence of adult and neonatal mouse sera was identical.

These results are evidence, in our opinion, that the low level of interferon formation in the presence of neonatal blood serum is not due to disturbances of transcription of RNA for interferon. Results obtained previously indicate the role of post-translation mechanisms in the inhibition of interferon formation by serum factors. It has been shown, for instance, that inhibition of cathepsin D in the presence of adult mouse serum and its activation and release in the presence of neonatal serum determined the level and composition of the molecular populations of interferons [2]. It may be that the mechanism of serum regulation of interferon production consists of the proteolytic degradation of interferon in the presence of neonatal serum.

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DETERMINATION OF MYELIN MARKER ENZYMES IN THE BLOOD SERUM OF PATIENTS WITH PERIPHERAL NERVOUS DISEASES

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Insufficient attention has been paid to the study of the enzyme composition of myelin. The presence of at least two enzymes — leucine aminopeptidase (LAP) and 2',3'-cyclic nucleotide-3-phosphohydrolase (CNP), nowadays considered to be myelin markers, has been reliably demonstrated [1]. LAP activity has been found in various tissues of the body. As regards CNP, its activity in the myelin sheath is known to be some 10 times higher than in other tissues [5]. It has been shown that this enzyme, which carries out hydrolysis of 2',3'-cyclic nucleotides to the 2'-derivatives, exhibits maximal activity during ontogeny in the period of myelination of the nervous system.

In demyelinating diseases and, in particular, in multiple sclerosis, CNP activity falls in the substance of the sclerotic plaques, possibly due to passage of the enzyme into the blood. No CNP can be found in healthy human blood [2].

The object of this investigation was to study activity of myelin marker enzymes in the blood serum of patients with diseases of the peripheral nervous system and in animals with experimental injury to nerve trunks.

EXPERIMENTAL METHOD

LAP activity was determined by the classical method using ready-made kits supplied by Fermognost (East Germany).

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TABLE 1. Determination of CNP Activity in Blood Serum of Healthy Subjects and Patients with Various Neuromuscular Diseases

Group test	Number tested	Number of patients in whom CNP activity was discovered	CNP activity, mmoles/min/liter
Patients:			
with neural amyotrophy	35	34	4,44—44,4
with polyneuropathies	23	7	4,44—22,2
with muscular dystrophies	18	1	8,88
Healthy subjects	20	—	—

To determine CNP activity in the tissues, several laborious methods have been used [4, 7]. The present writer suggests a method of determining CNP activity in blood serum spectrophotometrically, using adenosine-2',3'-cyclic phosphoric acid (sodium salt) as the substrate, being a modification of the method in [3].

Into a centrifuge tube containing 0.2 ml blood serum 0.1 ml of a 50 mM solution of citrate buffer, pH 6.2, and 0.1 ml of a 0.5 mM solution of adenosine-2',3'-cyclic phosphoric acid (sodium salt) were added. The mixture was incubated for 20 min at 37°C. Deproteinization was carried out with cold 0.6 N perchloric acid, after which the sample was centrifuged for 10 min at 1000g, and the supernatant was treated with 3 ml citrate buffer. Blood serum in the control test was treated in the same way as in the experimental test but the substrate was added at the end of incubation. Optical density was measured at 286 nm. The quantity of enzyme capable of hydrolyzing 1 millimole of substrate in 1 liter blood serum in 1 min was taken as the unit of activity.

EXPERIMENTAL RESULTS

CNP activity and the composition of the patients by diagnosis are given in Table 1.

It will be clear that no enzyme activity was found in the blood of healthy subjects or of patients with muscular dystrophies with no signs of lesion of the peripheral nervous system, constituting the control group. In patients with neural amyotrophy, in whom the myelin sheath of the peripheral nerves is primarily affected, with necrosis and deformation of the structures, CNP activity was detected in the blood serum. The wide fluctuations in the level of this activity were evidently due to differences in the severity of the lesions of the myelin. In polyneuritis of varied etiology, when the symptom-complex may be connected with different types of morphological changes not only in the nervous, but also in the vascular system, CNP activity was recorded in the blood serum of only 30.4% of subjects.

A morphological study of experimental material carried out parallel with the blood biochemical tests revealed definite correlation between the results. A ligature was tied around the sciatic nerve of a rabbit and 48 h later CNP activity was recorded, ranging between 4.44 and 13.32 mmoles/min/liter, with a maximum on the 12th-14th day. Morphologically, signs of destruction of the myelin sheath of the nerve were found during this period below the site of the ligature.

Investigation of LAP activity in healthy human blood confirmed data in the literature: The values obtained were between 2 and 6 units, which is normal. No significant deviations of LAP activity in the blood serum of patients or laboratory animals were found. This may be connected with the high background activity of this enzyme (of hepatic origin) and the relatively low LAP content in the tissues of the nervous system.

The possibility that CNP, a specific marker for myelin, may pass into the blood serum from foci of destruction of this structure, has thus been demonstrated for the first time. Determination of the activity of this enzyme in the blood serum can be used as a test for the diagnosis of demyelinating diseases of the peripheral nervous system.

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