The main causes of the decrease in velocity of FA oxidation in the early stages of myocardial ischemia are therefore reductions in the concentrations of intermediates of the Krebs' cycle and of cytochrome c in isolated Mc.

The results in Fig. 1a show that when respiration of Mc with PC and H was recorded without exhaustion of endogenous substrates there was a gradual fall in the velocity of respiration, probably due to oxidation and, consequently, to a decrease in the concentration of endogenous substrates. When respiration was recorded after preliminary oxidation of endogenous substrates, the measured velocity was constant. For that reason, the initial velocity of FA oxidation measured in the presence of endogenous substrates in Mc in the control was 25-30% greater than after their preliminary oxidation. During ischemia the quantity of oxygen consumed to oxidize endogenous substrates and, consequently, their concentration in Mc, fell (Fig. 1b): by 31% after 30 min and by 52% after 60 min ($P < 0.001$). That is why the opposite picture is observed in ischemia: The velocity of respiration was lower in experiments without oxidation of endogenous substrates than in experiments with their preliminary oxidation. Measurement of the true velocities of FA oxidation is possible only after complete oxidation of endogenous substrates. This must be taken into account not only in ischemia, but also when comparing results of investigations carried out on different animals, whose Mc differ considerably in their concentration of endogenous substrates.

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CELLULAR AND REGIONAL LOCALIZATION OF NEUROSPECIFIC ANTIGEN 10-40-4 IN THE HUMAN BRAIN

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KEY WORDS: immunoenzyme assay; immunofluorescence assay; nonspecific antigen.

Neurospecific protein (NSP) 10-40-4, isolated and characterized in the writers' laboratory, has a molecular weight of 76 ± 2 kilodaltons and an isoelectric point of 4.7. The antigen contains no carbohydrate or lipid components and is precipitated by ammonium sulfate at 40-60% saturation [1]. The specificity of protein 10-40-4 for nerve tissue has been demonstrated by immunodiffusion and immunoenzyme analysis. Specimens of NSP 10-40-4 from different species of mammals are not immunologically identical [2].

The aim of this investigation was to study the cellular and regional localization of NSP 10-40-4 by the indirect immunofluorescence method of Coons and also to determine it quantitatively in extracts of various structures of the human brain by immunoenzyme assay (ELISA).

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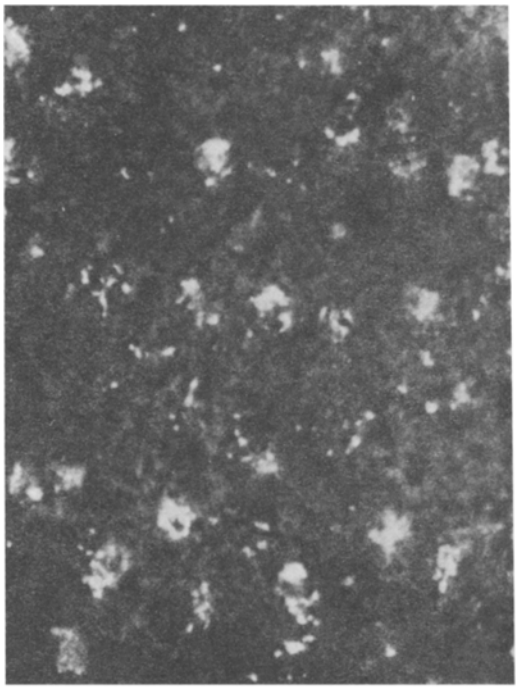

Fig. 1. Fluorescence of cytoplasm of medullary neurons against a background of weak fluorescence of neuropil. Here and in Fig. 2, magnification 250.

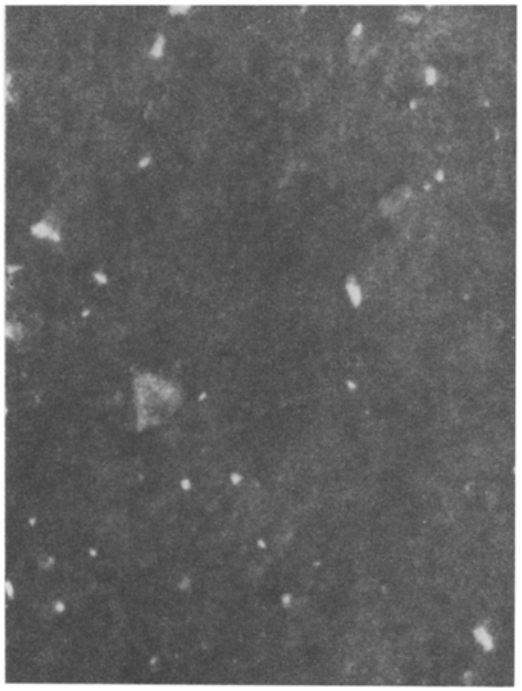

Fig. 2. Weak fluorescence of cytoplasm of cortical neurons.

EXPERIMENTAL METHOD

Brains taken from persons dying accidentally, not more than 2-4 h after death, were used. To study protein 10-40-4, 13 different structures of the human brain were used.

The localization of protein 10-40-4 was determined in parallel frozen brain sections 10 μ thick. Unfixed sections were treated with specific antibodies. Monospecific antiserum against human NSP 10-40-4 was used in a working dilution of 1:40. The original titer of the serum, determined by immunodiffusion assay by titration of the antigen with an initial concentration of 5 mg/ml was 1:256. Fluorescein-labeled serum against rabbit γ-globulins (FLS), in a working dilution of 1:8 (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) was used as the second antibodies.

Each series of experiments was accompanied by the following controls. Sections were treated: 1) with FLS, 2) with nonimmune rabbit serum followed by treatment with FLS, 3) with serum against NSP 10-40-4 and with serum against rabbit γ-globulins, followed by treatment with FLS, 4) with serum against NSP 14-3-2 followed by treatment with FLS. This last control was due to the fact that NSP 14-3-2 is an exclusively neuronal protein and is not found in glial cells.

The preparations were examined and photographed by means of the "Univar" microscope (Reichert). For quantitative determination of NSP 10-40-4 in different structures of the human brain an immunoenzyme method was used. Immunoenzyme assay was performed by the method in [4] in polystyrene plates (Linbro, England). To activate the plates, antibodies against NSP 10-40-4, isolated from monospecific sera with the aid of an immunosorbent prepared on the basis of Sepharose 4B, and activated with cyanogen bromide, and a purified preparation of protein 10-40-4 were used. The conditions for preparation of the immunosorbent were described previously [1]. Antibodies against NSP 10-40-4 were conjugated with horseradish peroxidase (type IV, from Sigma, USA) by the periodate method [3]. A 0.08% solution of 5-aminosalicyclic acid (from Serva, West Germany) was used as the substrate.

Brain tissue from the corresponding structures was minced and homogenized in 10 volumes of 0.005 M Na-phosphate buffer, pH 7.7, using a "Virtis-45" knife homogenizer (USA), at 20,000 rpm, and centrifuged at 120,000 rpm for 1 h. The resulting supernatant was lyophilized. Before immunoenzyme assay the lyophilized preparations were dissolved in physiological saline, pH 7.2, to a concentration of 0.1 mg/ml and serial double dilutions were prepared.

TABLE 1. Study of Regional Localization of Neurospecific Antigen 10-40-4 (M ± m)

Brain region	Immuno-fluorescence staining	Immunoenzyme assay, μg/mg extracts
Forebrain:		
Frontal cortex	+,±	18,7±3,50
Occipital cortex	+,±	11,3±1,16
Basal ganglia	+	40,8±4,60
Hippocampus	+	70,5±8,64
Medulla:		
Thalamus	++	120,0±19,98
Hypothalamus	+	57,0±12,60
Midbrain:		
Corpora quadrigemina	++	106,0±13,70
Cerebral peduncles	+,++	87,5±10,30
Hindbrain		
Cerebellum	+,±	38,3±5,62
Pons	++	60,0±18,80
Medulla - agglomeration of nuclei	+++	160,9±30,50

Legend. To obtain preparations of brain zones for immunofluorescence staining and for immunoenzyme assay, two and five post-mortem human brains respectively were used.

To construct a calibration curve purified NSP 10-40-4 was used. The results of immunoenzyme assay were recorded on a "Titertek Multiscan MC" multichannel spectrophotometer (Flow Laboratories, England) at 450 nm.

EXPERIMENTAL RESULTS

The study of the cellular and regional localization of NSP 10-40-4 (by Coons' method) gave the following results. Only weak fluorescence of the neuropil was found in all the control sections, with darker neuron bodies distinguishable against it. Monospecific antiserum against protein 14-3-2 induced only weak fluorescence (±) of the neuropil, with somewhat more intensive fluorescence of neurons (+, ++).

The use of monospecific antiserum against protein 10-40-4 revealed diffuse fluorescence of the neuropil in sections of all parts of the brain studied, with immunoreactive neurons, giving different intensities of fluorescence (+, ++, +++) distinguishable against this background.

In sections of brain structures such as the medulla, thalamus, and pons more intensive fluorescence of the perikarya of individual neurons was visible against the background of weak fluorescence of the neuropil (Fig. 1). Conversely, in the cortex, cerebellum, hypothalamus, and basal ganglia, intensive fluorescence was not found during the reaction with the test serum, and its brightness in the neurons did not exceed that in the neuropil (Fig. 2).

It can be concluded from these results that NSP 10-40-4 is detectable in significant quantities in different types of neurons in the medulla, thalamus, and pons. The cortex, cerebellum, hypothalamus, and basal ganglia do not contain any significant quantities of NSP 10-40-4.

The results of immunofluorescence staining of the various structures of the human brain are given in Table 1. Quantitative determination of the test antigen in these brain structures was undertaken by immunoenzyme assay, using extracts from zones of the human brain and antibodies against NSP 10-40-4, isolated by immunoaffinity chromatography on the immunosorbent Sepharose 4B–NSP 10-40-4. Maximal sensitivity of the method of immunoenzyme assay of NSP 10-40-4 (2 ng/ml) was obtained with the specific antibodies in a concentration of 25 μg/ml and with the conjugate in a dilution of 1:50.

It will be clear from Table 1 that the content of NSP 10-40-4 in 1 mg extract of water-soluble brain proteins differed considerably in different structures. Its concentration was

highest in the medulla (15-18% of water-soluble proteins), somewhat lower in the thalamus, corpora quadrigemina, cerebral peduncles, and hippocampus (5-10% of water-soluble proteins). The lowest concentration of NSP 10-40-4 was discovered in the cerebral cortex (frontal, occipital) and cerebellum (1-4% of water-soluble proteins).

It can thus be concluded from the results that the content of NSP 10-40-4 differs in different parts of the human brain. On the basis of the results it is impossible to identify the localization of the protein in any particular cell type. It is evidently specific for individual types of neurons. Since diffuse fluorescence of the neuropil was observed in all sections examined, the possibility of its localization in glial cells also cannot be ruled out. Moreover, immunoenzyme assay revealed that its concentration is highest in structures containing predominantly glial cells.

A final conclusion on the cellular localization of NSP 10-40-4 can be drawn after a study of its content in neuronal and glial tumors, and also a study of its appearance in the course of ontogeny.

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EFFECT OF HEPARIN ON DNA

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The widespread use of heparin in medicine has necessitated active research into its action on various biological objects [1, 2, 7].

The writers have studied the effect of heparin on DNA. To analyze DNA disturbances by mutagenic factors and the effectiveness of cell repair systems under normal and pathological conditions, centrifugation of cell lysates in alkaline density gradients is widely used. Since liberation of DNA from cells and unwinding of its strands in alkali is a long process (up to 24 h [10]), to speed it up in order to prevent degradation of the DNA in alkali, the use of heparin has been suggested [15]. However, our data show that heparin modifies the sedimentation characteristics of DNA. The causes of this phenomenon are analyzed in the investigation described below by centrifugation of DNA in alkaline and neutral sucrose gradients and by viscosimetry.

EXPERIMENTAL METHOD

DNA was isolated as described previously [3] from pig lymphocytes (obtained by the method in [13]) and calf thymocytes after preliminary isolation of chromatin [5]. Sedimentation analysis was carried out in a 5-20% sucrose density gradient in 0.1 M NaOH + 0.01 M

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