molecular weights. The content of protein X in functionally immature muscles was much lower than in muscles of the adult rabbits. In other words, its concentration increases during postnatal ontogeny and during formation of the contractile reaction of the muscle fiber. The yield of MM from adult rabbit muscles was 0.5-0.6 mg/g tissue, and from newborn muscles about twice as high. However, protein X accounted for 35-45% and 3-6% of MM preparation of adult and newborn animals respectively. It must be pointed out that on the whole, as regards their fractional composition, MM preparations from muscles of newborn and adult animals differ significantly from each other.

The functional properties of protein X and its participation in the act of muscular contraction are still unknown. According to our own data, the protein possess neither ATPase, nor creatine kinase, nor cholinesterase activity. Preliminary experiments showed that in the presence of purified preparations of protein X the molecular weight of F-actin is reduced by more than half (the molecular weight was measured by intensity of light scattering). This is a problem which requires further study.

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WATER-SOLUBLE PROTEINS OF THE SUBESOPHAGEAL GANGLION COMPLEX OF *Helix pomatia* IN THE EARLY STAGES OF LEARNING

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UDC 612.822+612.8.015+577.352

KEY WORDS: water-soluble proteins; *Helix pomatia;* conditioned reflex

It was shown previously by ultra microbiochemical methods that the formation of a defensive conditioned reflex in *Helix pomatia* is accompanied by an increase in the concentration of acid water-soluble protein in the command neurons in the arc of this reflex. Unconnected stimulation and reinforcing procedures gave a substantially smaller effect [4, 11].

Since the phenomenon of formation of a new protein "phenotype" was fixed in the final stage of the reflex, when a 100% level of learning had been achieved, and it was absent in the homogenate of the whole ganglion, it was postulated that in the early stages of learning the increase in content of acid proteins reflects a general reaction of the nervous system of the snail and is observed in all cells with equal or nearly equal intensity, but later this level of protein metabolism is fixed only in a very limited number of nerve cells.

This hypothesis is supported by the results of an investigation [5] which showed a marked increase in the content of acid proteins in total homogenate of the ganglion in rela-

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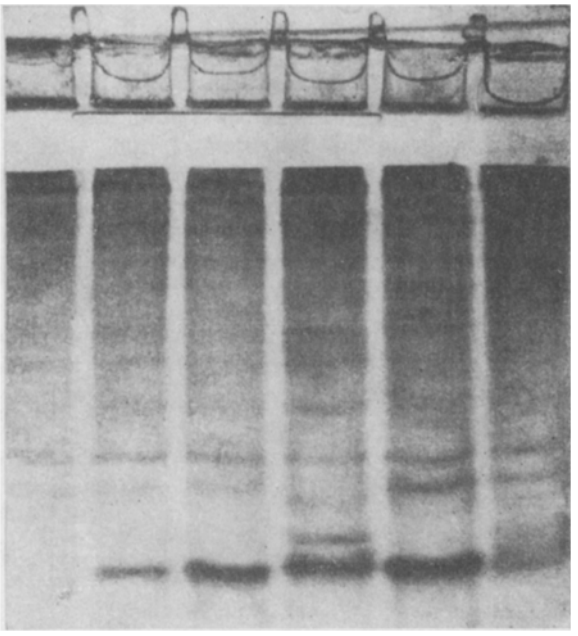

a b c d e f

Fig. 1. Photograph of polyacrylamide gel plate. Water-soluble proteins of subesophageal ganglion complex of *Helix pomatia*. a, b, f) control; c) learning; d) pseudolearning (30 combinations); e) learning (60 combinations).

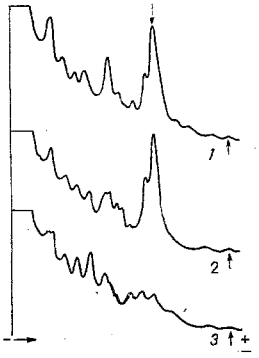

Fig. 2. Densitograms of water-soluble proteins of subesophageal ganglion complex of *Helix pomatia*. a) Learning, b) pseudolearning, c) control.

tively early stages of learning. The present investigation also was devoted to the testing of this hypothesis.

EXPERIMENTAL METHOD

A conditioned defensive spiracle closing reflex was formed in *Helix pomatia*. Light tapping on the shell was used as the conditioned stimulus, and reinforcement was a jet of air blown into the animal's spiracle. With some snails (pseudolearning) these procedures were unconnected. Usually a 100% level of learning was obtained after 160 to 240 combinations (15 a day). The reflex was described fully elsewhere [1, 2, 6-8].

After the snails had received 30, 60, 90, and 120 combined and uncombined stimuli, the subesophageal ganglion complex was removed and proteins extracted from it [4] and subjected to disk electrophoresis (PGE) [10] in polyacrylamide gel (T = 9.5%; C = 3.2%) in vertical plates measuring 100 × 110 mm, thickness of gel 2 mm, 4°C. The current was stabilized in the course of electrophoresis. The gels were stianed with Amido Black 10B (Reanal, Hungary) in 7.5% acetic acid. A Mitham (England) Scan-40 densitometer was used for scanning. In some experiments proteins in the polyacrylamide gels were subjected to isoelectric focusing.

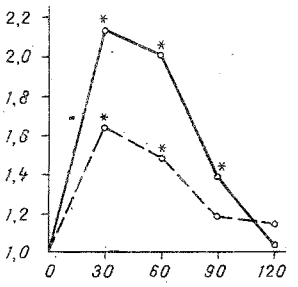

Fig. 3. Relative percentage of acid neurospecific protein of *H. pomatia* at different stages of learning. Continuous line, learning; broken line, active control (pseudolearning). Abscissa, number of combined stimuli; ordinate, protein content (in per cent of control).

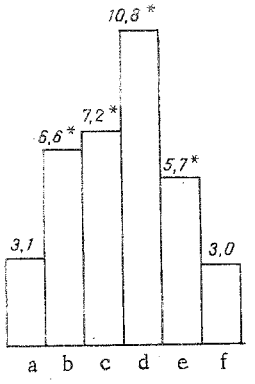

Fig. 4. Percentage content of acid neurospecific protein of *H. pomatia* at different times after arousal. a) 0 h, b) 2 h, c) 3 h, d) 6 h, e) 24 h, f) 2 weeks. *P < 0.05 compared with control (a).

EXPERIMENTAL RESULTS

During PGE of water-soluble proteins of the subesophageal ganglion complex of snails receiving 30-60 combined stimuli a sharp increase was observed in the content of acid protein with mobility of 0.58 relative to the front and 0.66 relative to the extreme anodal protein (Figs. 1 and 2). Whereas in the control the content of this protein was 3.1 ± 0.4% (calculated relative to total proteins entering the gel), in the conditioned snails its level rose to 8-12%. After 90 combinations the relative content of this protein fell, and after 120 combinations it was virtually back at the normal level (Fig. 3). During pseudolearning a similar pattern was observed, with lower average values and wider scatter of the relative content of this protein (Fig. 3). The stages tested are the first step in learning by the snails and are characterized by a very high level of the orienting reflex, and by a low and unstable number of positive reactions to the conditioned stimulus.

To verify correlation between the increase in the content of this protein and formation and extinction of orienting reflexes a series of experiments was carried out involving arousal of the snails. Sleeping snails were placed in an aquarium and left there for 2, 3, 6, and 24 h. Proteins of the subesophageal complex were studied by the method described above. The results of analysis of the spectra showed a sharp increase in the content of a protein with mobility of 0.58, measured 2, 3, and 6 h after arousal. By the end of 24 h the level of this protein was falling (Fig. 4). This effect was very strong, and the relative protein content after 6 h of activity was 4-6 times higher than in sleeping snails and in snails awake for a long time (2 weeks). Isoelectric focusing in polyacrylamide gel revealed similar quantitative changes and also demonstrated the homogeneity of the fraction studied. The protein tested was not present in the kidneys, liver, or gonads of the snails, and is evidently tissue-specific.

Since the orienting reflex is characterized by nonspecificity of the reflexogenic zone and by a low threshold of sensitivity [3, 9], the great majority of neurons of the snail is involved in the reaction and their excitability is enhanced; it is probably this which leads to such a sharp increase in the content of this protein in the whole of the animal's CNS, both in the early stages of learning and on arousal. During further learning the raised protein level persists only in a limited number of neurons directly concerned with the reflex formed [4, 11]. It can thus be postulated that this effect of an increase in content of acid protein is connected with the orienting reflex and virtually disappears when that reflex is extinguished.

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CHANGES IN DISTRIBUTION OF ISOCITRATE DEHYDROGENASE ACTIVITY WITH TIME IN RAT LIVER

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KEY WORDS: liver; isocitrate dehydrogenases; enzyme activity

Enzyme activity (EA) in hepatocytes fluctuates in the 24-h period and differs in different parts of the hepatic lobule [1, 4-7]. However, correlation between the topographic distribution of enzymes in the hepatic lobule and circadium rhythms of their activity has virtually never been studied. The presence of such correlation was established previously as changes in the distribution of activity of β-hydroxybutyrate dehydrogenase activity with time in rat hepatocytes [1]. The aim of this investigation was to determine the character of changes in the distribution of activity of NAD- and NADP-dependent isocitrate dehydrogenase (IC-NAD and IC-NADP respectively) in the hepatic lobule or normal rats with time.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred albino rats weighing 180-200 g. Before sacrifice the animals were kept for 2 weeks in artificial light with alternation of 12 h light (from 9 a.m. to 9 p.m., intensity of illumination 300 lx) and 12 h darkness. The rats were decapitated at 9 a.m., 1, 5, and 9 p.m., and 1 and 5 a.m. At each experimental point 4 or 5 animals were used. IC-NAD IC-NADP activity in the hepatocytes was determined by the method in [3] in frozen sections 12 μ thick. The technique of quantitative determination and calculation of the parameters for subsequent analysis was described previously [1]. To record changes in the EA level among the cell positions graphically, differences in EA between neighboring cells were expressed as percentages, and changes exceeding the coefficient of variation of EA in the zone of the lobule at each stage of the experiment were taken to be

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