THE INFLUENCE OF THE FEBRILE REACTION AND DINITROPHENOL HYPERTHERMIA ON PROCESSES OF PROTEIN REGENERATION IN RABBIT LIVER AND SPLEEN

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Elucidation of the influence of the febrile reaction on the rate of metabolic processes in different organs is very important for solving the problem of the biological significance of fever [1].

Recent work has shown [2-5, 6, 8, 10, 11] that fever produced by pyrogens of low toxicity raises the level of energy metabolism (conjugated phosphorylation) in the liver.

However there exist no data in the literature concerning the rate of protein regeneration in various organs during fever. Nevertheless this is very important for characterizing its significance.

The goal of the present study was to investigate the rate of incorporation of radioactive sulfur (S^{35}) labelled methionine into proteins of rabbit liver and spleen during a febrile reaction.

METHODS

The febrile reaction was produced in all experiments by intravenous injection of killed microbial vaccine of B. mesentericus [9] in a dose of two million microbes per kg of body weight. In series I (basic) (16 rabbits) at $1^1/_2$-2 hours after pyrogen injection S^{35}-methionine was injected intravenously as a dilution in physiological saline of 100 microcuries/kg. Two hours after this the animals were killed by decapitation. Samples were taken from their organs for study. In separate experiments saline perfusion via the aorta for five min had been carried out previously to wash out the blood. No difference was noted in the results of these experiments and those without perfusion.

In the second series of experiments a solution of S^{35} labelled methionine, in the same quantity, was given to eight rabbits 24 h after a single injection of pyrogen. Two hours later the rabbits were killed.

In series III ten rabbits were given intravenous solution of 2,4-dinitrophenol in a dose of three mg/kg. At the peak of hyperthermia the animals were injected with labelled methionine in the same quantity as in the first series and then were killed after the same interval.

In all series of experiments the isotope was given in a parallel manner in equal amounts to intact control rabbits.

Isolation of the proteins from the tissue samples was performed with 10% trichloracetic acid with subsequent washing three times with five per cent and twice with three percent trichloracetic acid and centrifugation. Ethyl alcohol (96%) was added to the precipitate. On the following day the samples were washed twice with alcohol, then with a mixture of alcohol and ether, ratios 1 :1 and 1:2 and with ether. The precipitates were dried in an incubator at 50-60° to constant weight and were ground in a mortar. Ten mg of the powder were placed on foil discs and several drops of ether were added. After drying under an infrared lamp a quantity of proteins was obtained on the discs which was spread evenly over the bottom of foil plates. The radioactivity of the protein was counted on a "B"

Effect of Fever Vaccine and Hyperthermia Produced by Dinitrophenol on the Incorporation of Sulfur-Labelled Methionine into Proteins of Rabbit Liver and Spleen

Condition of experiment	Group of animals	Liver		Spleen	
		number of animals	number of counts/min per 10 mg of protein	number of animals	number of counts/min per 10 mg of protein
Injection of S^{35} at the height of vaccine fever (series I)	Experimental	16	478 ± 54.5	11	920 ± 31.2
	Control	16	437 ± 41	11	608 ± 25.6
Injection of S^{35} 24 h after vaccine (series II)	Experimental	8	504 ± 73.5	8	678 ± 133
	Control	8	409 ± 68	8	678 ± 196
Injection of S^{35} at the height of hyperthermia produced by dinitrophenol (series III)	Experimental	10	562 ± 81	10	727 ± 111.3
	Control	10	642 ± 80	10	862 ± 105.7

apparatus equipped with a pavement counter of type T-25-BFL (thickness of mica window 1.1 mg/mm^2). The results were expressed in counts/min per 10 mg of protein.

RESULTS

The data obtained are presented in the Table.

As the table shows, when the isotope is given at the height of the febrile reaction there is a regular (statistically valid) increase in the radioactivity of proteins in the spleens of animals with fever in comparison to samples taken from control animals.

In liver proteins from febrile animals, on the average, somewhat more labelled sulfur is incorporated than in proteins from control animals. However this increase is not statistically valid.

When the methionine is given at 24 h after the vaccine (i.e. 16-18 h after the termination of fever) a more intensive incorporation of radioactive sulfur into proteins was observed in separate experiments (five out of eight) in the liver of the animals which had fever. The differences from the control group, however, were not statistically valid.

The incorporation of labelled sulfur into spleen proteins of animals with fever and healthy animals in this series of experiments appeared to be identical.

At the peak of the fever produced by injection of dinitrophenol, a tendency to less intensive incorporation of S^{35}-methionine was noted in the biosynthesis of spleen and liver proteins, although there was no statistically verified difference from the controls.

Naturally, our data is not yet complete. All the same we wish to make some preliminary conclusions. First, attention is called to the sharp rise in rate of proteins regeneration in the spleen during the first hours after injection of vaccine, with an extremely small and statistically invalid increase in this process in the liver. If it is taken in account that the spleen is richer in reticulo-endothelial elements than the liver, then, evidently, the increase in protein metabolism in the spleen reflects mainly the activity of the reticulo-endothelial system. It remains unclear, however, whether this activity is related to the direct or indirect effect of the febrile reaction itself on protein metabolism and the high temperature or whether it is related to the increased phagocytic activity of splenic macrophages as a result of introducing microorganisms into the blood. It is possible that both these factors are of importance, in any case the protein metabolism in the spleen after vaccine fever is significantly increased and rapidly returns to normal at its conclusion.

The more marked tendency to increased proteins metabolism in the liver, on the other hand, appears not at the time of fever but 15-18 h later. The mechanism of this "following" activation is also nuclear and probably is complex. In any event, it is impossible to relate to the indirect effect of high temperature on the course of metabolic processes.

The tendency to decrease in methionine sulfur incorporation into proteins of liver and spleen during dinitrophenol hyperthermia may be related to the decrease in energy resources in cells produced by the uncoupling of respiration and phosphorylation that is effected by dinitrophenol [7]. It is known that dinitrophenol generally lowers the incorporation of labelled amino acids into tissue proteins [12].

Our experiments once more indicate the principal differences in processes which take place in the organism during fever and dinitrophenol hyperthermia that are related to the toxic nature of thermogenesis. This difference was first noted by S. A. Neifakh and E. P. Zdrodovskaya [6, 8].

The significance of the direct metabolic affect of the temperature factor itself in these two conditions requires further investigation.

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