THE FORMATION OF TISSUE HEMOLYSINS IN LIVER EXTRACTS OF WHITE RATS FED WITH LINSEED OIL

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The first observations on the hemolytic activity of extracts from various animal tissues were made by I. I. Mechnikov in the course of classical investigations of phagocytosis [5, 7]. Studying the chemical nature of hemolysins, Levaditi [12] established that fatty acids and their salts manifest hemolytic activity. Later, in the investigations of a number of authors [2, 13], adequate proof was obtained that hemolysin can exist as a free, unsaturated fatty acid, or as a complex of the acid with protein.

It was shown [6] that, under the influence of ionizing radiation, toxic agents arise in the animal organism, particularly in the liver, which cause hemolysis of erythrocytes in vitro (tissue hemolysins, hemolytic factor). In the opinion of a number of authors [3, 4, 8, 9], tissue hemolysins, appearing as unsaturated fatty acids and their products of oxidation – peroxides, epoxides, etc., play the role of cytotoxins in the irradiated organism.

Earlier, we established [1] that tissue hemolysins in the liver extracts of irradiated rats arise in vitro as a result of oxidation of biolipids in the process of their incubation. The hypothesis was advanced that the formation of hemolytically active substances in the tissue extracts of irradiated animals is connected with a disturbance in their fat metabolism.

It appeared of interest to study the possibility of tissue hemolysins forming upon injection of unsaturated fatty acids into the organism.

EXPERIMENTAL METHOD

The experiments were performed on 315 white rats of both sexes, weighing 100-300 grams. The animals were maintained on a full food ration, which was strictly followed during the course of the experiment. The rats were divided into 3 groups: the first contained intact animals, the second – rats injected subcutaneously with apricot oil (1.5 ml), and in the third – rats which received from 0.5 to 4 grams of linseed oil per animal per day in their food, over a long period of time (15-96 days). From the third group, 35 rats were sacrificed for determination of the hemolytic activity of thier liver, and 105 rats, in order to study their radio resistance, were subjected to roentgen irradiation in doses of 500 and 800 r. The animals were irradiated on the RUM-3 apparatus, under the following conditions: 180 kv, 15 ma, filters – Cu 0.5 mm and Al 1 mm, focal distance of 35 cm, output of the apparatus of 42.5 r/min.

The tissue hemolysins were determined according to the method described by Yu. B. Kudryashov [3] and A. S. Mochalina [6]. The animals were decapitated, and the liver pulverized under refrigeration in a five-fold volume of 0.9% NaCl solution. The homogenate (5 ml) was centrifuged for 15 min at 3000 rpm. The centrifugate was then diluted in 0.9% NaCl. We usually prepared 10-12 dilutions, the last samples representing dilutions of from 1:512 to 1:2048. Subsequently, we added 0.5 ml of an erythrocyte suspension (4%) to the liver extract (1 ml). The individual tests (extract with erythrocytes) were incubated at 37° for one hour, and were then kept at 5° for the next 48 h. The degree of hemolysis was determined by visual comparison of the sample with standards, and expressed in percents.

EXPERIMENTAL RESULTS

Table 1 shows that, in the group of intact rats, only isolated animals showed hemolytically active substances in the extracts of their livers. Injection of oil subcutaneously led to almost 80% of the rats showing hemolysins in their liver extracts. Finally, hemolytic activity of the extracts rose sharply when the animals were sacrificed following prolonged feeding with linseed oil. In this case, the hemolytic factor was found in the same quantity as in the rats that underwent exposure to roentgen rats in fatal dosage.

TABLE 1. The Hemolytic Activity of Aqueous-Saline Liver Extracts (Diluted from 1:32 to 1:128) from Rats, Following Injection of Apricot Oil or Prolonged Feeding with Linseed Oil

Total number of animals	Time of investigation		Percent hemolysis		
	after injection of oil	after oil feeding	0-5	15-30	50-75
12	After 1-2 h	–	2	5	5
11	After 49-168 h	–	1	4	6
14	–	After 15-45 days	–	7	7
12	–	After 50-96 days	–	3	9
17	Control	–	14	3	–

The increase in hemolytic activity of the liver extracts can probably be explained by the increase in their concentration of fat. The injection of apricot oil subcutaneously, or feeding of the animals with linseed oil, leads to deposition of unsaturated fatty acids in the liver, and in the final account, to an increase in the concentration of fat within the extracts [11]. To confirm this, we set up experiments in which we added 0.1-0.2 ml of apricot or linseed oil to liver extracts from intact rats. The addition of fat to the extracts always led to formation of a significant quantity of hemolytically active substances within them. Apparently, in these experiments the increased fat concentration in the liver was one of the main reasons for the formation of hemolysins in vitro.

Attention is drawn to the fact that maximum hemolysis was observed in the tests with extract dilutions of 1:32, 1:64, and 1:128. In the tests with lower dilutions (1:2, 1:4, etc.), as well as with higher dilutions of the extracts (1:512, 1:1024, etc.), hemolysis was not seen. It must be postulated that, at the optimal extract dilutions (1:64, etc.), the relationship between the amount of natural antioxidants and the tissue lipids is disturbed, and creates favorable conditions for the development of the chain reaction of biolipid oxidation. The outcome of the hemolysins depended on how the concentration of antioxidants in the biolipids was altered upon dilution during the period of incubation and the subsequent maintenance at 5°. In the first test mixtures of the natural antioxidants it was completely sufficient to protect the biolipids from oxidation, and thus inhibit the formation of hemolytically active substances. With further dilutions of the extracts, there was not only a decrease in the antioxidants, but also in the amount of biolipids – the base product for hemolysin formation. This probably also explains the absence of hemolysins in the tests with extract dilutions of 1:512 and greater.

The hypothesis described was verified by experiments in which the antioxidant, n-propyl ether 3,4,5-trioxybenzoic acid (propylgallate), was added to the liver extracts before incubation. This

TABLE 2. Changes in the Weight of the Rats and the State of the Peripheral Blood for the Experimental and Control Animals

Group of animals	Number of animals	Duration of feeding with oil (in days)	Original body weight (in grams)	Increase in body weight (in grams) after 60 days	Blood indices on the 60th day						
					erythrocytes	leukocytes	eosinophiles (in percents)	bands (in percents)	neutrophiles (segmented nuclei) (in percents)	lymphocytes (in percents)	monocytes (in percents)
Experimental	15	60	40—56 (48)	90—116 (101,7)	5 500 000—7 100 000 (6 300 000)	10 800—23 800 (16 900)	0—5 (2,2)	0—3 (1,6)	10—32 (18,1)	63—86 (77,9)	0—1 (0,2)
Control	15	—	45—55 (50)	36—109 (78,3)	5 700 000—8 000 000 (6 700 000)	14 000—23 800 (19 300)	0—2 (1,0)	0—4 (1,5)	15—57 (27,9)	46—84 (69,5)	0—1 (0,1)

Note. Mean values are shown in parentheses.

TABLE 3. Resistance of White Rats Fed Linseed Oil in Their Food to Roentgen Rays

Group of animals	No. of animals	Duration of feeding (in days)	Dose of irradiation (in r)	Number of animals dying from irradiation			Surviving on the 30th day	Percent survival
				on the 3rd-7th day	on the 8th-14th day	on the 15th-127th day		
Control	25	–	800	8	11	3	3	12
Experimental	25	30	800	6	9	5	5	20
Control	64	–	500	–	6	6	52	81.3
Experimental	76	15–86	500	1	6	5	64	84.7

agent stops the oxidation of fats at any stage, and does not affect already formed peroxide compounds. The addition of 0.1 mg of propylgallate to 1.5 ml of extract and a suspension of erythrocytes completely prevented the formation of hemolytically active substances. The experiments showed that the hemolysins are formed in vitro (if they arose in vivo, then the addition of propylgallate would not impede their hemolytic activity in vitro), and that their appearance in the dilutions of 1:32, 1:64, and 1:128 is connected with a disturbance in the relationship between the antioxidants and the biolipids, specifically with a decrease in the concentration of antioxidants in the biolipids.

It is interesting to note that while in the liver extracts from the rats that were fed with oil maximum hemolysis was recorded in the tests with dilutions of 1:64 and 1:128, in the irradiated animals it was observed in the tests with dilutions of 1:8 and 1:16. The increase in extract dilution for appearance of its maximum hemolytic activity, seen in the rats fed with oil, as compared with irradiated rats, can be explained in the following manner. Linseed oil contains a large quantity of natural antioxidants. The possibility is not excluded that in the liver, in this case, there occurs both a deposition of unsaturated fatty acids and an accumulation of antioxidants. With irradiation, however, it is known that one observes only an elevated concentration of fat in the liver, with a parallel increase in antioxidants. Thus, with feeding the animals oil, a greater dilution of the extract is needed to disturb the relationship between the antioxidants and the biolipids, in vitro, and also to obtain optimal conditions for oxidation of the biolipids, than in the case of the irradiated rats.

Prolonged feeding of the rats with linseed oil led to the formation of hemolytically active substances in the liver extracts of the indicated animals. In this case, the rats looked well, and gained weight faster than the control animals (Table 2). In addition, no pathological changes of any kind were noted in the peripheral blood.

As has already been mentioned, 105 rats of the third group were irradiated with roentgen rays. It was interesting to determine the radioresistance of the animals in whose liver extracts we detected the hemolysins which, in the opinion of a number of authors, possess cytotoxic properties. Table 3 shows that the radioresistance of the rats irradiated with roentgen rays in a dose of 800 and 500 r and fed with linseed oil was practically the same as for the control animals.

The data presented conforms with the results of investigations [10] in which an elevation in radioresistance was also not seen in rats fed cottonseed oil and irradiated once in doses close to the LD_{100}.

Thus, the hemolysins observed in liver extracts from rats that have received unsaturated fatty acids in their food for a long time cannot be regarded as cytotoxins. Their appearance in the extracts is probably connected with the accumulation of unsaturated fatty acids in the liver in the process of the animals' being fed linseed oil, and with the subsequent oxidation of the acids during the period of incubation. The experiments confirmed the data which we obtained earlier, indicating that an increase in fat concentration within the liver by various means can lead to the formation of hemolysins in vitro.

SUMMARY

This work was done to ascertain the possibility of inducing the tissue hemolysins formation in administration of fatty acids of unsaturated series into the organism. Experiments were staged on 315 albino rats. Tissue hemolysins were determined both in the extracts from the liver of both experimental animals, who had been given vegetable oil, and of control – intact animals. As demonstrated experimentally, prolonged feeding of rats with linseed oil or subcutaneous injection thereof led to a detection of hemolysins in the extracts from the liver of sacrificed animals. According to the data analysed, tissue hemolysins were formed in vitro and could not be regarded as cytotoxins.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
