THE BLOOD-CLEARING FACTOR IN ACUTE EXPERIMENTAL TOXIC HEPATITIS

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Recent research has shown that the so-called blood-clearing factor, or lipoprotein lipase, plays a great part in fat metabolism.

Clearing of lipemic serum after injection of a small dose of heparin was first reported in 1943 [8]. This observation was also confirmed in other investigations [5, 13, 14]. Heparin added to lipemic plasma in vitro did not clear it. This reaction occurred only on mixing with plasma obtained from an animal previously given heparin [5].

The results of a study of the clearing reaction in vitro and in vivo enable us to advance the hypothesis that this reaction is not caused by heparin directly, but by the special chylolytic substance which it activates; this substance causes cleavage of the chylomicron triglycerides and thus leads to clearing of the plasma [12]. This enzyme was termed the clearing factor [6]. It was demonstrated that the clearing factor catalyzes the hydrolysis of protein-bonded triglycerides [9] and this furnished grounds for calling it lipoprotein lipase.

The fact that clearing of lipemic serum begins immediately after injection of heparin (within 3-5 min) enabled a number of authors [10, 11] to advance the hypothesis that lipoprotein lipase is formed in the vascular wall.

Hyperlipemia is apparently an adequate stimulus to the mast cells located along the blood vessels and promotes liberation of endogenous heparin from them [4, 7]. The latter activates the lipoprotein lipase, inducing cleavage of the chylomicron triglycerides and lipoproteins to form free (nonesterified) fatty acids (NEFA), which are adsorbed on the albumin and, in complex with it, easily escape from the vascular bed.

A number of investigations enable us to assume that a decrease in lipoprotein lipase activity occurs in the pathogenesis of the protracted alimentary hyperlipemia which occurs in atherosclerosis [3, 11], as well as in the etiology of certain forms of so-called essential hyperlipemia [11].

Proceeding from the role of the clearing factor in fat metabolism, we set outselves the task of investigating the change in its activity in acute experimental toxic hepatitis, which is accompanied by fatty infiltration of the liver.

EXPERIMENTAL METHOD

Our research was conducted on male white rats weighing 150-280 g, which were fed the usual laboratory diet.

We induced fatty infiltration of the liver by subcutaneous injection of carbon tetrachloride (CCl_4) in doses of 0.3 mg/100 g of body weight at 1 day intervals. We investigated clearing factor activity during the acute period of the toxic hepatitis, 4 and 48 h after the first CCl_4 injection, 24 h after the 2nd injection, and 24 h after the 3rd (i.e., on the 5th and 7th days after the onset of the poisoning); we also studied the clearing factor activity during the recovery period—5, 9, and 14 days after the final CCl_4 injection. An investigation of clearing factor activity in the same animals prior to injection of CCl_4 served as the control.

As the substrate in determining clearing factor activity we used a fatty emulsion prepared ex tempore in the

Changes in Blood-Clearing Factor Activity in Control Experiments and after Single and Repeated Injections of CCl_4, as well as During the Recovery Period after Cessation of CCl_4 Injections (averaged data: M±m)

Experimental conditions	Time after injection	No. of animals	Clearing-factor activity (in μ equivalents/ml)
Control	-	17	11.6 ±0.3
Injection of CCl_4 (0.3/100 g)	4 h after 1st injection	18	8.2 ±0.32 P<0.001
The same	48 h after 1st injection	15	9.3 ±0.54 P<0.001
Injection of CCl_4 (0.3/100 g), 2 injections on alternate days	5 days after 1st injection	16	9.4 ±0.42 P<0.001
Injection of CCl_4 (0.3/100 g), 3 injections on alternate days	7 days after 1st injection	15	7.9 ±0.4 P<0.001
Recovery	5 days after last injection	11	9.0 ±0.44 P> 0.05
The same	9 days after last injection	14	11.9 ±0.67 P<0.001
" "	14 days after last injection	12	11.16±0.92 P<0.01

following manner: 5 g of serum albumin was dissolved in approximately 50 ml of Sorenson buffer solution (pH-7.4). To this we added 2 ml of apricot oil, Sorenson buffer solution then being added to make a final volume of 100 ml. The emulsion was treated in an emulsifier for 30 min and the pH of the final emulsion was reduced to 8.0 with 1 N NaOH. To chemical test tubes containing 3 ml of the final emulsion we added 0.1 ml of blood taken from the tail of an experimental rat 30 min after the preliminary intraperitoneal heparin injection (in a dose of 50 units/100 g of body weight). We determined the NEFA content in 1 ml of this mixture before incubation and then sealed the test tubes with foil or cellophane and placed them in a Warburg water bath for 120 min at 37°, with continuous agitation. We then again determined the NEFA content in 1 ml of the incubated mixture. A dummy experiment was conducted at the same time: we incubated 3 ml of pure emulsion under the aforementioned regime and determined the NEFA content of 1 ml of the incubated emulsion.

The difference in the NEFA contents of 1 ml of the incubated mixture (after 2 h of incubation) and of 1 ml of the emulsion with no blood added (dummy test) served as the index of clearing factor activity.

The NEFA content was determined by a previously described method [2].

EXPERIMENTAL RESULTS

As may be seen from the data given in the table, a statistically reliable decrease in blood-clearing factor activity was observed 4 h after the initial CCl_4 injection. It persisted for 48 h after the first injection and was present on the days following the 2nd and 3rd CCl_4 injections.

An increase in hepatic fat content was noted at the same times [1]: this increase averaged approximately 23.3 g-% 4 h after the 1st CCl_4 injection (normal—19.87 g-%), approximately 27.8g-% 48 h after the 1st injection, 31.9 g-% on the day after the 2nd injection, and approximately 34.7 g-% on the day after the 3rd injection.

During the recovery period clearing factor activity was still reduced 5 days after the last CCl_4 injection. It reverted to normal on the 9th day after poisoning ceased and remained at that level.

Toxic hepatitis is thus characterized by a decrease in blood-clearing factor activity within 4 h after subcutaneous injection of CCl_4.

SUMMARY

Toxic hepatitis was provoked in rats by triple subcutaneous injection (every other day) of CCl_4. Four hours after the first injection there was a drop in the blood clearance factor activity. The same reduction in the blood clearing factor activity was observed 48 h after the 1st injection and the next day after the 2nd and 3rd CCl_4 injections. The activity of the factor investigated was restored on the 9th day after discontinuing of the CCl_4 poisoning.

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