THE ROLE OF HEPARIN IN THE METABOLISM OF FATTY ACID ESTERS

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Heparin plays an important role in the metabolism of fat present in the blood. Hahn showed in 1943 that the addition of a small amount of heparin leads to the clearing of lipemic plasma. This phenomenon can also be produced in vitro by mixing plasma taken from a heparin-treated animal with lipemic plasma. Heparin as such does not possess this property in vitro. Therefore this action depends on some other factor. This factor has been given the name "clearing factor." The mechanism underlying the phenomenon described has not yet been elucidated. Levy and Swank [3, 4] established in experiments on dogs that within 2 minutes of administration of heparin there is a sharp rise in tributyrinase, but this rise was not statistically significant in observations on man. This communication deals with an attempt to determine the relation of heparin to serum lipase and esterase levels in man.

EXPERIMENTAL

Investigations were carried out on patients of different ages who were on a normal diet. Samples of venous blood were taken before and 15 minutes after intravenous injection of 5000 units of heparin.

The estimation of lipase and esterase was done by the titration method of Cherry and Crandall [2]. The final composition of reaction mixture in test-tube: serum 1 ml, substrate 1 ml, phosphate buffer 0.5 ml, distilled water 3 ml. This mixture was incubated at 37° and the fatty acids obtained were titrated with 0.05 N solution NaOH (100 units = 1 ml 0.05 N NaOH). 50% emulsions of olive oil, amyl acetate, and ethyl butyrate were used as substrates.

In those experiments in which esterase was inactivated by means of heating, incubation of the serum was carried out in water for 5 minutes at different temperatures.

Sodium fluoride, physostigmine, and diethyl-p-nitrophenyl phosphate were used as chemical inhibitors. These substances were added to the incubated mixture in concentrations given below.

For the determination of esterase activity, blood was taken before the administration of heparin and then 2, 20, 40, and 60 minutes after intravenous injection of 5000 units of heparin. The esterase values were determined in the first case in the presence of physostigmine, in the second, without inhibitors.

RESULTS

Table 1 shows the results of using three different substrates: olive oil, amyl acetate, and ethyl butyrate. In the case of ethyl butyrate there was a marked increase of esterase activity of the serum after heparin. No such increase was observed when the other substrates were used. Similarly, there was no increase in esterase activity after addition of heparin in vitro.

TABLE 1

Esterase Activity of Human Serum Before and After Administration of Heparin

Experimental Conditions	Subtrate			
	Olive oil	Amyl Acetate	Ethyl Butyrate	Ethyl Butyrate heparin[1] in vitro
Before administration of heparin	8	503	272	247
After administration of heparin	9	505	344	248
Average number of cases	10	10	20	-

[1]500 units of heparin

Inactivation of serum by means of heat demonstrated that the activity of both sera (normal and heparin-treated) diminished with rising temperature (Table 2). However, the activity of heparin-treated serum remains greater than that of the control serum. At 60° when the control serum shows only weak esterase activity the heparin-treated serum still shows pronounced activity.

TABLE 2

Effect of Incubation for 5 Minutes at Different Temperatures on the Esterase Activity of Serum Before and After Heparin Treatment

Experimental conditions	Esterase activity at				
	37°	45°	50°	55°	60°
Before heparin	300	297	288	253	20
After heparin	343	342	338	298	77

The results of the action of chemical inhibitors are given in Table 3. It shows that at concentration of sodium fluoride of 10^{-1} M, untreated serum completely loses its activity, while heparin-treated serum still possesses high esterase activity. This activity only ceases at molar concentration of sodium fluoride. Physostigmine inhibits untreated serum even at 10^{-6}M concentration. In this respect the esterase under investigation resembles pseudocholine esterase. The esterase activity of heparin-treated serum remains high at physostigmine concentration of 10^{-3}M, while the activity of heparin-treated serum remains high and is only diminished at 10^{-4} M concentration of the poison.

Observations on the rise in esterase activity after various intervals following administration of heparin revealed that this increase reached a maximum approximately 2 minutes after the injection, with subsequent gradual decline in the enzyme activity (Table 4). The esterase under investigation possesses some of the properties of the clearing factor mentioned above. The increase in esterase activity under the influence of heparin becomes especially pronounced in the presence of physostigmine (10^{-8} M); i.e., after the elimination of pseudocholine esterase.

TABLE 3

Effect of Chemical Inhibitors on Esterase Activity of Human Serum

Experimental conditions	Esterase activity at concentration of inhibitor					Control (without inhibitor)
	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M	1 M	
	Sodium fluoride					
Before heparin	228	163	72	18	8	268
After heparin	342	298	236	160	32	355

Experimental conditions	Esterase activity at concentration of inhibitor						Control (without inhibitor)
	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4}M	10^{-3}M	
	Physostigmine						
Before heparin	310	298	215	56	14	0	314
After heparin	346	342	286	212	194	186	348
	Diethyl-p-nitrophenyl phosphate						
Before heparin	332	308	42	10	10	10	340
After heparin	390	376	256	202	10	10	390

TABLE 4

Serum Esterase Activity at Different Intervals After Injection of Heparin With and Without Inhibitor

Experimental conditions	Esterase activity				
	Before heparin	After heparin injection			
		2 minutes	20 minutes	40 minutes	60 minutes
Without physostigmine	272	400	368	352	332
With physostigmine (10^{-3} M)	0	326	200	151	48

DISCUSSION

The present results demonstrate that heparin increases the esterase activity of human serum. This effect is observed after a relatively short interval following intravenous injection of heparin, but it cannot be produced by addition of heparin in vitro. In normal serum the esterase activity is affected by pseudocholine esterase since an inhibitory effect takes place even at low concentrations of physostigmine. After heparin administration the enzymatic spectrum of the serum undergoes changes, and the serum esterase activity is determined mainly by aliesterase. This is indicated by its enhanced stability to physostigmine. Increased stability of the esterase after intravenous injection of heparin can also be observed with respect to sodium fluoride, diethyl-p-nitrophenyl-phosphate and to inactivation by heat.

The activation of aliesterase, which exhibits appreciable affinity to ethylbutyrate, occurs not directly but is mediated by an unknown accelerating factor, since heparin does not enhance esterase activity in vitro.

It follows from the experimental results that the increase of esterase activity of human serum observed after the administration of heparin is determined mainly by aliesterase, whose effect is reinforced by an unknown factor.

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