ACTIVITY OF THE CLARIFICATION FACTOR IN THE PATHOGENESIS OF HYPERLIPIDEMIA IN AN EXPERIMENTAL NEPHROTIC SYNDROME

N. V. Nikiforova

UDC 661.61-008.6-092.9-06:616.153.915.01

It has previously been shown that the hyperlipidemia in experimental nephrotic syndromes is retention in character: the mobilization of fat from the fat depots is not stimulated and the content of lipids in the liver is not increased.

A deficiency in the activity of the clarification factor (CF) may play an important role in the pathogenesis of retention hyperlipidemia.

In normal conditions the triglycerides of the blood, composing the chylomicrons and liproproteins of low density, are split by an enzyme—lipoprotein lipase, which plays an important role in the removal of alimentary lipemia, causing "clarification" of the lipemic plasma. The appearance of the enzyme in the blood stream and its activity are stimulated by heparin, whether secreted endogenously or administered from outside sources. The higher free (nonesterified) fatty acids (NEFA) liberated during hydrolysis of the triglycerides by lipoprotein lipase are fixed and removed from the blood mainly by albumin, facilitating the continuation of hydrolysis. The CF is usually identified with lipoprotein lipase. However, it has been reported [7, 10], that the splitting of triglycerides, activated by heparin, is in fact associated not only with lipoprotein lipase of the blood, but also with other esterases.

Little information is available concerning the activity of CF in the nephrotic syndrome.

Rosenman and Byers [8], showed that the CF activity is not depressed in rats with an experimental nephrotic syndrome. Day and Wilkinson [1], likewise found no decrease in CF activity in two patients with a nephrotic syndrome. Gitlin and Gross [3] and Larcan and co-workers [4] found a decrease in CF activity in patients with a nephrotic syndrome.

In the present investigation the CF activity was studied at various stages of development of an experimental nephrotic syndrome in rats in order to discover the possible role of this factor in the pathogenesis of hyperlipidemia. The CF activity was judged by the degree of hydrolysis of the triglycerides of an artificial medium, by the blood of animals after receiving injections of heparin, and by the degree of the change in concentration of β-lipoproteins in the serum of these animals.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-190 g and kept on the ordinary laboratory diet. To obtain a nephrotic syndrome, a nephrotoxic rabbit serum was used, with a titer of antikidney antibodies of 1:1000 and 1:1200 by the complement fixation reaction. The serum was injected intravenously into the rats in a dose of 0.5 ml, and subcutaneously in a dose of 0.75 ml/100 g body weight on two successive days.

The animals were investigated before injection of the nephrotoxic serum, and on the 4th-5th and 8th days after its first injection. The protein content of the urine collected over a period of 18 h was estimated. To determine the severity of the nephrotic syndrome, the concentrations of the following were determined in the serum of blood taken in a fasting state from the tail: total protein (refractometrically), albumin (by electrophoresis on paper), total lipids (by Searcy's method [9]), cholesterol (by Bloor's method), and β-lipoproteins (by the method of Burstein and Samaille as modified by Link and Fassati [5]).

The activity of the CF of the blood was determined at the times indicated above by a modification of Lukasik's method [6]. As the triglyceride substrate, an emulsion of fat prepared ex tempore by the following formula was used. Dry human serum albumin in a weight of 5 g was dissolved in 50 ml of Sörensen's or Tris buffer (pH 7.4),

Department of Pathological Physiology, Central Postgraduate Medical Institute, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR E. M. Tareev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 12, pp. 29-32, December, 1966. Original article submitted November 10, 1965.

Activity of Clarification Factor of the Blood and Some Indices of Lipid and Protein Metabolism in Rats before and after Development of Nephrotic Syndrome (M ± m)

Experimental conditions		Weight (g)	Excretion of protein in urine during 18 h (in mg)	Clarification factor of blood (in μeq NEFA /ml substrate)	Serum						
					β-lipoproteins		Decrease, %	total protein	albumins	total lipids	cholesterol
					before injections of heparin	30 min after injection of heparin					
					mg %			g %		mg %	
Before injection of nephrotoxic serum		177	1,93	1,28±0,1	40,4±3,2	25,6±2,5	36,6	6,5±0,12	2,84±0,1	267±22,8	77±3,6
Nephrotic syndrome	4th day of disease (9 rats)	181	84,0	1,77±0,11 $P_1<0,01$	237,3±59,7 $P_1<0,01$	143±36,1	40,0	5,34±0,17 $P_1<0,001$	1,15±0,08 $P_1<0,001$	823±117 $P_1<0,001$	254±39,7 $P_1<0,001$
	8th day of disease (7 rats)	182	21,3	1,27±0,12 $P_2<0,01$	60,2±6,6 $P_2<0,02$	35,1±4,2	41,7	6,25±0,34 $P_2>0,05$	1,71±0,17 $P_2<0,01$	421±37,3 $P_1<0,01$	114±7,6 $P_2<0,001$

Legend: P_1 – by comparison with indices in animals before development of nephrotic syndrome; P_2 – by comparison with indices on the 4th day of the disease.

2 ml of refined sunflower oil was added, and the volume was made up to 100 ml with buffer solution. The mixture was emulsified in an emulsifier for 30 min; the pH of the prepared emulsion was adjusted to 7.41 with a 1 N solution of NaOH, after which heparin was added as anticoagulant in a dose of 1 unit/ml.

Blood for investigation was taken from the tail of the experimental rat before and 30 min after intraperitoneal injection of heparin* in a dose of 50 units/100 g body weight. Samples of blood, 0.1 ml in volume, were mixed in a test tube with 1 ml of emulsion and incubated in a water bath for 60 min at 37° with constant agitation. At the end of incubation, the content of NEFA was determined in each sample by Dole's method [2]. The index of the CF activity of the blood was the difference between the NEFA content in the samples to which blood taken before and 30 min after injection of heparin into the animal had been added. The CF activity was expressed in μeq NEFA/ml substrate.

Parallel with the CF activity, the concentration of β-lipoproteins was determined in the serum of the experimental animals before and after injection of heparin by the method described above.

EXPERIMENTAL RESULTS AND DISCUSSION

As the table shows, the nephrotic syndrome in the rats on the 4th day of the disease was well marked: a considerable proteinuria, hypoproteinemia, and hypoalbuminemia and a distinct hyperlipidemia were present.

During incubation of the postheparin blood of the sick rats with the emulsion of triglyceride, the amount of NEFA formed in it was much greater than during incubation with the postheparin blood of the same animals before development of the disease. The result of estimation of the concentration of β-lipoproteins, the natural substrate for lipoprotein lipase, in the serum showed that injection of heparin into the rats caused the same decrease in the concentration of the serum β-lipoproteins both before and after development of the nephrotic syndrome.

Judging by the degree of the decrease in concentration of the serum β-lipoproteins in response to injections of heparin, the activity of the lipoprotein lipase was not depressed in the nephrotic syndrome.

On the 8th day of the disease, as it was subsiding (decrease in proteinuria, increase in total serum protein concentration and decrease in hyperlipidemia), the CF activity determined from the splitting of the triglycerides of the emulsion, was restored to normal. The degree of lowering of the level of the serum β-lipoproteins after injection of heparin in this period was the same as before the disease.

These results show that the CF activity not only was not lowered in rats with an experimental nephrotic syndrome, but was actually increased; the degree of splitting of the β-lipoproteins of the serum after injection of heparin was no less than before the development of the disease. The possibility is not ruled out, however, that in the nephrotic syndrome the formation of endogenous heparin may be depressed. Further investigations are being carried out to shed light on this problem.

LITERATURE CITED

1. A. J. Day and G. K. Wilkinson, Circulation, 18 (1958), p. 76.
2. V. P. Dole, J. Clin. Invest., 35 (1956), p. 150.
3. D. Gitlin and P. A. Gross (1957). Cited by A. Larcan, et al.
4. A. Larcan, et al., Pathol. et Biol., 10 (1962), p. 417.
5. J. Link and P. Fassati, Z. Ges. Inn. Med., 19 (1964), p. 400.
6. S. Lukasik, Arch. Immunol. Terr. Dosw., 9 (1961), p. 847.
7. J. Pav and J. Wenkeova, Clin. Chim. Acta, 5 (1960), p. 90.
8. R. H. Rosenman and S. O. Byers, Proc. Soc. Exp. Biol., New York, 103 (1960), p. 31.
9. R. L. Searcy, J. L. Korotzer, and L. M. Berquist, Clin. Chim. Acta, 8 (1963), p. 376.
10. B. Shore and V. Shore, Am. J. Physiol., 201 (1961), p. 915.

* Later, for the sake of brevity, the blood and plasma of the animals which had received an injection of heparin will be called "postheparin."