Arresting Effect of Heparin upon Experimental Nephrosis in Rats.*† (21176)

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The injection of potent anti-rat kidney serum into rats induces a syndrome which closely resembles the nephrotic state as it occurs in human subjects(1,2). The nephrotic rat thus provides an excellent experimental medium for the study of the mechanism(s) of the abnormal plasma lipid accumulation in this syndrome(3-6). The unique effect of heparin upon the plasma lipids of human patients with nephrosis(7,8) and other hyperlipemic states led to this study of the effect of heparin upon the lipemia of experimental nephrosis in rats.

I. *Effect of Heparin upon Previously Induced Hyperlipemia and Hypercholesteremia of Nephrotic Rats. Methods.* The nephrotic state was induced in a group of 15 male rats (Long-Evans), aged 10 weeks, by intravenous injection of 0.5 ml of rabbit anti-rat kidney serum(1) on each of 2 successive days. The rats were bled from the tail for analysis of plasma total lipids(9) and total cholesterol (10) on the seventh day after the last injection of immune serum. Similar determinations were made in a group of 5 normal control rats. Sodium heparin then was administered subcutaneously, 1 mg twice daily, to 10 of the nephrotic rats and to the 5 normal controls, the other 5 nephrotic animals being kept as untreated controls. Forty-eight hours later all animals again were bled and the plasma total lipids and total cholesterol determined. The turbidity of each plasma sample was determined in galvanometer units proportional to the transmission of light in an Evelyn micro-colorimeter. Distilled water read 100% transmission and total darkness read zero per cent transmission. A 650 mμ light filter was used.

Results. The results are presented in Table I. Seven days after the final injection of immune serum the nephrotic state was well-developed in the 15 rats injected with anti-kidney serum. The subcutaneous administration of heparin to the 10 nephrotic rats led to significant decrease of turbidity of their plasma, accompanied by a marked reduction of the degree of hyperlipemia and a moderate decrease of total plasma cholesterol. In contrast, no change in plasma lipids occurred in the 5 untreated, control, nephrotic rats or in the 5 heparin-treated, normal rats. The administration of heparin for 48 hours to the nephrotic rats, however, did not appear grossly to affect their nephrotic state in other respects.

This demonstrated effect of heparin upon previously developed hyperlipemia and hypercholesteremia of nephrotic rats led to a second experiment, concerned with the effect upon experimental nephrotic hyperlipemia of heparin administered during the induction phase of the nephrotic state.

II. *Effect of Concomitantly Administered Heparin upon the Development of Hypercholesteremia and Hyperlipemia of Nephrotic Rats. Methods.* (a) Fourteen male rats, aged 10 weeks, were injected intravenously with 0.5 ml of rabbit anti-rat kidney serum on each of 2 successive days. Eight rats served as controls, the remaining 6 being daily subcutaneously injected with 2.5 mg of sodium heparin in divided doses. This was administered initially at the same time as the first injection of immune serum and then continued for 7 days. Each rat was bled from the tail on the seventh day for determination of plasma total lipids and total cholesterol. The turbidity of the plasma samples was observed grossly.

(b) In addition, 10 rats were subjected to abdominal laparotomy and cannulation of a lumbar vein with a polyethylene cannula. After closure each rat was placed in a restraining cage and the distal end of the cannula was connected to an individual syringe containing

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TABLE I. Effect of Heparin upon Plasma Lipids of Nephrotic Rats.

	Type of rat					
	Untreated nephrotic		Heparin-treated nephrotic		Heparin-treated normal	
	Before	After†	Before	After	Before	After
No. of rats	5	—	10	—	5	—
Avg wt (g)	140	—	175	—	185	—
Plasma turbidity (units)	21 (3–41)*	18 (5–39)	20 (1–50)	9 (0–24)	0	0
Total plasma lipids (mg/100 cc)	1743 (820–2700)	1657 (1140–2370)	1835 (800–3360)	940 (114–1340)	315 (220–420)	325 (230–425)
Total plasma cholesterol (mg/100 cc)	482 (344–618)	438 (412–468)	469 (242–580)	387 (266–463)	58 (47–74)	47 (38–65)

* Range of values. † 48 hr.

7 ml of isotonic saline. In 5 instances, 5 mg of sodium heparin was added to the infusion fluid. Each syringe was connected with a special continuous intravenous injection apparatus calibrated to deliver 6 ml of infusion fluid in a 24 hour interval. Each of the 10 rats was then injected intravenously with 1.0 ml of potent anti-kidney serum. After 24 hours, each rat was bled for analysis of plasma total lipids and total cholesterol.

(c) In order to confirm the data obtained from this study, a similar experiment was performed in a second series of 25 rats injected with 0.5 ml of anti-kidney serum on each of 2 successive days. Twelve nephrotic rats served as untreated controls, the remaining 13 rats receiving 1 mg of heparin subcutaneously, twice daily, beginning at the same time as the first injection of anti-rat kidney serum. Seven days after the second injection of immune serum the rats were bled from the tail. Heparin injections were stopped and each rat was bled again 4 days later. In each instance plasma total lipids, total cholesterol and degree of turbidity were determined.

Results. The injection of immune serum into the untreated control rats of both series induced ascites, lipemic-turbidity of the plasma and occasionally subcutaneous edema within 48 hours following the last injection. At the time of the initial bleeding (7 days after injection of immune serum), marked ascites and a variable degree of subcutaneous edema could be observed. These findings contrasted sharply with the apparent complete absence of ascites and subcutaneous edema in the heparin-treated rats.

The results of the analyses of plasma lipids in the first series of rats are presented in Table II. Simultaneous administration of heparin subcutaneously during the induction and development of the nephrotic state in 8 rats pre-

TABLE II.
Effect upon Plasma Lipids of Heparin Given during Induction of Nephrosis in Rats.

	Subcutaneous administration of heparin		I.V. administration of heparin	
	Untreated controls	Heparin-treated†	Untreated controls	Heparin-treated‡
No. of rats	8	6	5	5
Avg wt (g)	120	125	130	128
Plasma				
Turbidity (units)	Present	Absent	Present	Absent
Total lipids (mg/100 cc)	2225 (1540–3400)*	538 (320–814)	496 (305–760)	213 (167–240)
Total cholesterol (mg/100 cc)	428 (264–618)	261 (73–592)	144 (93–210)	69 (62–74)

* Range of values. † 7 days. ‡ 24 hr.

TABLE III. Effect of Heparin Given during Induction of Nephrosis in Rats.

	Heparin-treated rats		Non-heparin-treated rats	
	Day 7*	Day 11	Day 7	Day 11
No. of rats	13	13	12	12
Avg wt (g)	203	—	201	—
Plasma				
Turbidity (units)	0	14 (6–23)†	9.5 (5–17)	21 (8–46)
Total lipids (mg/100 cc)	570 (250–1225)	1975 (910–3040)	1548 (780–2570)	5367 (1300–8870)
Total cholesterol (mg/100 cc)	229 (88–485)	362 (280–505)	373 (230–585)	385 (290–480)

* Heparin stopped on day 7. † Range of values.

vented turbidity of their plasma, in contrast to marked turbidity present in the plasma of the 6 control rats. The heparin-treated group also exhibited a significantly lesser rise of plasma total lipids and total cholesterol. Table II also shows that continuous intravenous administration of heparin appeared completely to suppress the rise of plasma total lipids and cholesterol observed in their paired control rats, 24 hours after injection of immune serum; the values obtained in the heparin-treated group are normal levels for this strain of rats.

The results of the second experiment confirmed those of the first one. It again was observed that simultaneous subcutaneous administration of heparin during the induction phase of the nephrotic state significantly retarded the development of the hypercholesteremia and hyperlipemia of the treated group when compared to the untreated group of rats (Table III). Furthermore, as observed above, the plasma of the heparin-treated rats in each instance was of normal clarity in contrast to variable turbidity of the samples from the untreated rats. However, when heparin administration was stopped in the treated rats for 4 days, their plasma exhibited markedly increased turbidity and a significant rise of total lipids and total cholesterol, although the degree of turbidity and hyperlipemia was much less than that observed at this time in the untreated nephrotic rats.

III. *Effect of heparin upon the clinical picture of experimental nephrosis in rats.* The apparent arresting effect of heparin upon the lipemia and other manifestation of nephrosis observed in the preceding experiments suggested that heparin might be affecting the fundamental pathogenesis of the abnormalities observed in the nephrotic state. This hypothesis was studied in the following experiment.

Material and methods. Ten male rats, aged 8 weeks, were each injected intravenously with 0.5 ml of anti-kidney serum on each of 2 successive days. Five of the rats also received subcutaneous injections of 1 mg of sodium heparin twice daily, the others remaining untreated. Four days later each rat was sacrificed and analysis made of the plasma total lipids and total cholesterol.

Results. From Table IV it can be noted that the heparin-treated rats injected with immune serum did not exhibit gain of weight, ascites, subcutaneous edema, or turbidity of their plasma. A moderate rise of cholesterol and of total lipids was found in these rats. In contrast, the control rats which similarly were injected with immune serum but which did *not* receive heparin exhibited marked ascites, a variable degree of subcutaneous edema and a consequent average gain of weight of 39 g, and marked lipemic-turbidity of their plasma. A considerably greater rise of plasma lipids and of cholesterol also was observed in these animals.

Discussion. The present data indicate that heparin apparently is capable of profoundly influencing the nephrotic state in rats. It was found initially that heparin induced a significant reduction of plasma lipids and lipemic-turbidity in rats previously made nephrotic (Table I). This response was anticipated in

TABLE IV. Effect of Heparin on Lipids and Other Signs of Nephrosis in Rats.

	Heparin-treated rats	Non-heparin-treated rats
No. of rats	5	5
Avg wt (g)		
Day 0	139	142
" 7	143	181
Ascites	Absent	Present
Subcut. edema	"	"
Plasma		
Turbidity (units)	"	"
Total lipids (mg/100 cc)	376 (335–460)*	1333 (520–2570)
Total cholesterol (mg/100 cc)	141 (121–191)	338 (226–487)

* Range of values.

view of the findings by others that heparin similarly lowered the plasma lipids of nephrotic humans(7,8). It seems likely that the changes induced by heparin in the physicochemical state of the plasma lipids(11,12) facilitates the removal of excess lipids from the plasma by the liver, with consequent reduction of the degree of hyperlipemia and hypercholesteremia.

Heparin appeared to exert a greater effect upon plasma lipids when administered during the induction and development phase of the nephrotic state in rats. Thus, clear plasma and markedly lower levels of plasma lipids were observed in the animals treated with heparin following injection of anti-kidney serum, in contrast to untreated rats also injected with anti-kidney serum (Table II). This inhibitory effect was striking when heparin was administered by continuous intravenous infusion following injection of immune serum. On the other hand, the suppressive effect of heparin upon retention of excess lipids in the plasma of the nephrotic rat apparently was of a temporary nature, as cessation of heparin therapy was followed by progressive rise of hyperlipemia, hypercholesteremia, and the appearance of lipemic-turbidity of the plasma (Table III).

Although heparin treatment consistently reduced lipid levels in the rat with previously induced nephrosis, it did not appear grossly to affect other aspects of the nephrotic state.

However, administration of heparin during the induction phase of the nephrotic state appeared to exert a profound arresting effect upon other aspects of the nephrotic syndrome. Administration of heparin during the development phase completely or almost completely prevented such other "clinical" manifestations of the nephrotic state as subcutaneous edema, ascites, and weight gain (Table IV). In Fig. 1 can be seen the typical appearance of the edematous nephrotic rat 7 days after injection of immune serum, initially similar in weight and appearance to the heparin-treated rat in which edema and ascites were not observed at this time. However, when heparin was stopped, there was a delayed appearance of moderate ascites and occasionally subcutaneous edema, in association with a rise of plasma lipids. In subsequent studies we have observed that much larger doses of heparin are required to suppress the "clinical" signs and the hyperlipemia induced by injection of exceedingly high potency anti-kidney serum, smaller doses of heparin sufficing when less potent immune serum is used.

It seems likely that the degree of nephrotic lipemia is proportional to the severity of the underlying abnormality, and therefore it appears unlikely, that the apparent suppressive effect of heparin upon the development of the nephrotic state is merely secondary to its effect upon the plasma lipids. At present it seems plausible that heparin has some effect upon the causal mechanism whereby nephrosis is induced in rats by injection of rabbit anti-rat kidney serum. Ehrich and his associates (2) extensively studied the pathological

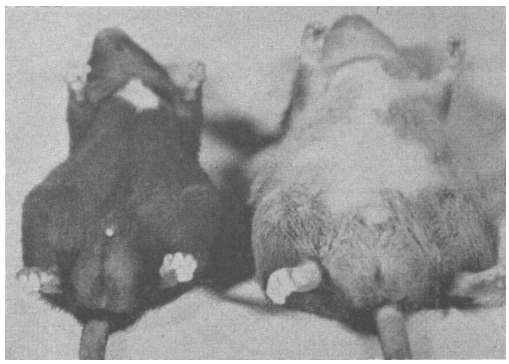

FIG. 1. Two rats injected with anti-kidney serum 7 days previously: Left, treated with heparin daily for 7 days. Right, untreated control.

changes in the kidneys of nephrotic rats. They emphasized the close resemblance to the morphological changes observed in the kidneys of humans with lipid nephrosis and suggested that the immunologic reaction induced by injection of anti-rat kidney serum is of the nature of a Shwartzman reaction. Thus, heparin might act by preventing such a Shwartzman reaction since it has been shown that heparin is fully capable of preventing both the local and the generalized Shwartzman reaction(14,15). Heparin also might influence the reduction of serum complement induced by injection of anti-kidney serum, shown to occur in rats which develop nephrosis following injection of immune serum(13). Finally, heparin might act in a different manner by suppressing thrombosis of the capillaries and alterations of the basement membranes of the glomeruli which follow injection of anti-kidney serum(2).

Our results agree in part with the report of Kleinerman(16) that heparin was found to prevent or markedly suppress "clinical" signs and pathological renal lesions induced in rabbits following injection of (duck) anti-rabbit kidney serum. Ehrich and associates(2) have pointed out that although anti-kidney disease in rabbits more closely resembles glomerular nephritis in humans with an immunologic reaction likened to the Arthus phenomenon, the response to injected anti-kidney serum in rats and in rabbits appears to be a different immunologic reaction to the same factor(2).

Summary. Heparin administration decreases the hyperlipemia and hypercholesteremia of rats with established nephrosis, induced by injection of rabbit anti-rat kidney serum. Heparinization during the *induction* phase of the nephrotic state appears to suppress or prevent ascites and edema, and to reduce markedly the degree of lipemia, hypercholesteremia and lipemic-turbidity of the plasma. Possible mechanisms of this arresting effect of heparin are discussed briefly and it is suggested that heparin suppresses the causal mechanism whereby nephrosis is induced in rats by injection of anti-kidney serum.

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