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Role of Liver and Kidney in Development of Heparin-induced Lipemia Clearing Activity (LCA).*† (24593)

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Since the discovery that injection of heparin endows the plasma with a lipemia clearing activity (LCA)(1,2), it has been an interesting problem to find out which tissues are essential to development or inactivation of this lipolytic enzyme system. Isolated perfusion experiments showed that heparinised plasma develops LCA after it is passed through limbs, thoracic and most abdominal viscera(3-6) but not the brain(5) or the liver(6). Indeed, when plasma already endowed with LCA was perfused through the latter organ is suffered a reduction of that activity(6,9), indicating that the liver inactivates LCA. The present study was undertaken to ascertain the effects of hepatectomy or CCl_4-induced liver damage and of nephrectomy on *in vivo* development of LCA following heparin injection in rats.

Materials and methods. The same experimental plan as in a previous study(7) was employed. Heparin (Connaught's) was injected into the tail veins of control and experimental male Wistar rats weighing 220-280 g, at 0.5 mg/kg. Five minutes after heparin injection 4.5 ml of blood was withdrawn (under ether) from the abdominal aorta of every animal, mixed 1:10 (v/v) with 0.1 molar sodium citrate and centrifuged for 15′ at 3000 rpm. In each instance, 1 ml of the "postheparin" plasma thus obtained was diluted with 1 ml distilled water and mixed in a colorimeter cuvette with 0.5 ml lipemic plasma, produced as previously described(8); turbidity of the postheparin-lipemic plasma mixture was determined immediately and 10′ and 20′ after mixing in a Klett-Summerson colorimeter, against a water blank, using a red filter. Loss of turbidity ("clearing") of the mixtures at any given time was expressed as percentage of initial turbidity at mixing. The animals were injected with heparin and their plasmata obtained and tested for LCA in successive pairs consisting of one control and one experimental, following a precisely timed, staggered schedule. Since the same lipemic plasma batch was used as clearing substrate for each pair of control and experimental plasmata, the results were analyzed statistically with the Null Hypothesis(12). Thus, in every experiment, the mean difference $\pm \epsilon$ between the clearing of every control and its corresponding experimental mixture was computed for each time interval. In tabulating these differences the clearing activities exhibited by the control plasmata were arbitrarily set as references against which those of the experimental plasmata were compared. A difference with a

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TABLE I. Effects of Hemihepatectomy, CCl_4-cirrhosis, Nephrectomy and Thirst on Heparin-induced Lipemia Clearing Activity (LCA).

Group	No. animals per group	Mean body wt change during exp. (g)	Mean clearing activity (expressed as % loss of initial turbidity at mixing) ± ε 10′ after mixing	20′ after mixing	Mean difference in clearing activity between postheparin plasma of experimentals and controls ± ε 10′ after mixing	20′ after mixing
1. Hemihepatectomy, 40 hr						
Controls	6	+ 2	20.5 ± 5.5	41.8 ± 6.9		
Hepatectomy	6	−14	36.4 ± 6.4	58.0 ± 5.2	+15.9 ± 2.1 (p <.005)	+16.2 ± 2.8 (p <.005)
2. Carbon tetrachloride (0.6 ml of a 50% sol., twice weekly for 6 wk)						
Controls	5	+64	41.4 ± 1.9	67.8 ± 1.5		
Carbon tetrachloride	5	− 1	57.9 ± 1.6	76.6 ± 1.3	+16.5 ± 2.7 (p <.005)	+ 8.8 ± 1.9 (p = .01)
3. Bilateral nephrectomy, 24 hr						
Controls	8	+ 6	23.4 ± 2.4	42.7 ± 3.2		
Nephrectomy	8	− 2	37.8 ± 2.2	49.6 ± 3.5	+14.4 ± 2.2 (p <.005)	+ 6.9 ± 1.8 (p = .01-.005)
4. Thirst, 48 hr						
Controls	5	+17	25.1 ± 2.5	49.5 ± 3.6		
Thirst	5	−19	22.3 ± 2.2	47.3 ± 3.8	− 2.8 ± 1.2 (p = .1)	− 2.2 ± 1.6 (p >.1)

positive sign, therefore, implies that experimental plasmata displayed greater LCA than control plasmata, whereas a negative sign indicates the opposite. The following procedures were employed: 1) *Partial hepatectomy*: 2 major lobes of the liver (constituting approximately 50% of the total organ weight) were removed 40 hours before injection of heparin. 2) *Subtotal hepatectomy*: approximately 85% of the liver mass was removed 30 hours before injection of heparin. All hepatectomized rats and their controls were given 5 ml of a 5% dextrose solution s.c. immediately after operation and were offered the same solution to drink *ad lib.* during the post-operative period. 3) *Carbon tetrachloride cirrhosis*: 0.6 ml of a 50% solution of CCl_4 in maize oil was given by stomach tube twice weekly for 6 weeks before heparin administration. All animals become jaundiced at the end of the treatment period and histological studies demonstrated the presence of unequivocal portal cirrhosis in all livers. 4) *Nephrectomy*: both kidneys were removed 24 hours before heparin injection. 5) *Thirst*: to assess the possible role of post-operative hemoconcentration in the results of some of the above experiments, a group of animals was subjected to deprivation of water for 48 hours prior to heparin injection. 6) *Mock-operations*: to ensure comparability between control and experimental animals (where operational trauma was involved) the controls were mock-operated.

Results. Postheparin plasma of the partially hepatectomized, CCl_4-cirrhotic and nephrectomized animals displayed considerably and significantly more LCA than that of the control rats, while thirst had no significant influence on the evolution of this activity (Table I). Since only 2 sub-totally hepatectomized animals survived this drastic operation, no standard error could be computed for their mean value and their data were therefore not included in the table; nevertheless, postheparin plasma of both survivors exhibited considerably greater LCA than that of corresponding controls (averagely +18 at 10′ and +11 at 20′ after mixing).

Discussion. Our findings that removal or damage of the liver and removal of the kidney greatly increase development of LCA in response to heparin constitute indirect evidence that the integrity of the above 2 organs is associated with inhibition or inactivation of the heparin-induced lipolytic activity through some unknown mechanism. In view of the fact that the control animals were mock-operated and that water deprivation did not affect LCA significantly, it is unlikely that

surgical trauma or post-operative hemoconcentration were in any non-specific way responsible for the observed marked increments of this activity in the operated rats. Our conclusions concerning the role of the liver are thus in agreement with those of Jeffries(6) and the Spitzers(9) which were based on diminution of the LCA of heparinised or post-heparin plasma after its perfusion through the liver. Our results are also in harmony with the recent clinical findings of increased *in vivo* LCA production in patients suffering from hepatic cirrhosis and chronic alcoholism(10) and the report that liver and kidney extracts inhibited LCA *in vitro*(11).

Summary. 1) Plasma from heparin-injected hepatectomized, CCl_4-cirrhotic and nephrectomized rats exhibited significantly greater lipemia clearing activity than that of heparin-injected normal control animals, when incubated with lipemic plasma *in vitro*. 2) These results represent indirect evidence for the presence of a clearing factor inactivating system in liver and kidney.

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Reticulo-Endothelial Blockade and Capillary Resistance Response to Cortisone and STH.* (24594)

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There have been several investigations of the effect of reticulo-endothelial blockade on ACTH and cortisone induced eosinopenia. Of these, only one concluded that RE blockade diminished eosinopenic response(1). Others denied any difference in hormone induced eosinopenia(2,3,4). One of the latter, however, did note that RE block abolished eosinopenic response to various stresses(2). Kramar and associates showed a marked negative correlation between eosinopenic and capillary resistance responses to exogenous cortisone(5,6); eosinophil count decreasing as capillary resistance rises. These investigators also showed that STH causes a decrease in capillary resistance and this was verified by Wilhelmj *et al.* (7). We used capillary resistance for this investigation because of simplicity of method and because it makes possible the study of effect of RE block on this phase of STH activity.

Materials and methods. Control levels of capillary resistance were established on 14 trained dogs, 7 males and 7 females. Of these,

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