Heparin-induced Lipemia Clearing Factor in Rats
Role of the Pancreas in its Production

H. C. Meng, M.D., Ph.D., and June L. Goldfarb, M.D., Nashville

It has long been known that hyperlipemia occurs in many patients with diabetes mellitus.[1-4] The increase in serum lipids is predominantly in triglycerides although cholesterol and phospholipids are also elevated.[5] This condition is aggravated by the presence of acidosis,[6-9] but can be restored to normal by insulin treatment.[7] Recently, an elevation in concentrations of serum lipoproteins has also been found to occur in diabetics,[10-13] but their responses to insulin have been variable.

Warren and LeCompte state that "there can be no question that diabetics are prone to develop atherosclerosis at an earlier age and to a greater extent than other persons."[14] Autopsy findings of various investigators show a much higher incidence of coronary artery disease in diabetics than that in nondiabetics,[15-20] although Bell[20] has found that development of severe vascular lesions is independent of the severity of the diabetes. Thus, a relationship between the disturbance of lipid metabolism and high incidence of atherosclerosis may well exist, although the understanding of the former is far from complete and the etiology of the latter remains obscure.

It is well documented that intravenous injection of heparin is capable of clearing alimentary lipemia and intravenously injected lipoproteins, fatty chyle and synthetic fat emulsions in vivo[21-27] and that plasma obtained after heparin injection hydrolyzes the synthetic triglyceride emulsion or triglyceride component of lipemic plasma in vitro.[28,29] Recently, we have found that the clearing activity of postheparin plasma of human diabetics is decreased.[30] It was postulated that the failure of postheparin plasma of diabetic subjects to clear or hydrolyze triglyceride may, in part, be responsible for the disturbance of lipid metabolism which contributes to the development of atherosclerosis.

From the Department of Physiology, Vanderbilt University, School of Medicine, Nashville, Tennessee. Dr. Goldfarb's present address: Institute for Psychosomatic and Psychiatric Research and Training, Michael Reese Hospital, Chicago, Illinois.

A preliminary report of this work was presented at the Annual Meetings of the American Society for the Study of Arteriosclerosis in Chicago, November 1956.

The present study was undertaken to investigate the postheparin plasma clearing activity of alloxan-diabetic rats and the role of the pancreas in the production of the heparin-induced lipemia clearing factor.

MATERIALS AND METHODS

Young adult male rats of Sprague-Dawley strain weighing 200-250 gm. were used. The animals were under light ether anesthesia during heparin injection, blood withdrawal, pancreatectomy, and alloxanization. Alloxan, 50 mg. per kilogram, was given intracardially or intravenously through a tail vein. An effort at total removal was made in the rats subjected to pancreatectomy. All rats were fed ground Purina chow. In some instances 5 per cent lyophilized beef pancreas or 0.5 per cent Lipormone* was added to the diet as indicated below. Rats with alloxan diabetes were given insulin subcutaneously in long-term experiments. Four to 8 units of insulin were given daily depending on their blood glucose level, and the time of injection was kept regular each day. Both Protamine Zinc insulin and Iletin (Insulin, Lilly) were used and showed no significant differences in their influence upon the clearing activity of postheparin plasma.

Unless otherwise specified, the dosage of heparin was 0.05 mg. per kilogram administered intracardially. All blood samples were withdrawn three to five minutes after heparin and blood was transferred into centrifuge tubes containing sodium citrate kept in ice water. plasma was determined by the method of Meng and gation for twelve minutes at 2,500 r.p.m. in a cold room. The plasmas were used immediately or stored in cold (4° C.). No plasma was stored for more than two or three days.

The *in vitro* clearing activity of pre- or postheparin plasma was determined by the method of Meng and associates[31] except that 0.5 ml. plasma was used in the present work. The decrease in optical density was recorded at intervals during a two-hour incubation period

*Lipormone, purified lipocaic of pancreas, was kindly supplied by Laboratoire Choay, Paris, France.

at 37° C. In some experiments the unesterified fatty acids were also determined in addition to the decrease in optical density. The method of Borgstrom[82] was used for unesterified fatty acid determination.

Blood glucose was measured forty-eight hours after alloxanization and whenever blood was withdrawn for plasma clearing activity. Nelson's colorimetric method modified by Somogyi[83] was used for determining blood glucose. The animals were not fasted prior to blood withdrawal for blood glucose determination.

RESULTS

Clearing and lipolytic activities of pre- and post-heparin plasma of normal rats. It is shown in figure 1 that plasma obtained after intravenous injection of heparin (postheparin plasma) into normal rats was capable of clearing or decreasing the turbidity of synthetic triglyceride emulsion in vitro. This clearing was accompanied by an increase in the production of unesterified fatty acids. Both clearing and lipolytic activities of preheparin plasma were nil as judged by the slight decrease in optical density and the production of unesterified fatty acids. The average blood glucose of these two groups of rats (seven received no heparin and fourteen received heparin) was 113 mg. per cent, ranging from 68 to 155.

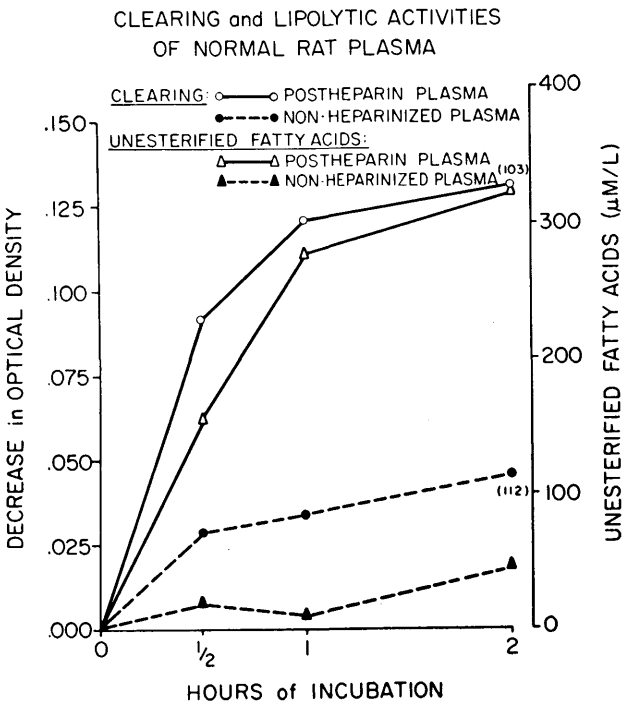

FIG. 1. Numbers in parentheses in figures 1-4 represent blood glucose in milligrams per cent.

Clearing and lipolytic activities of pre- and post-heparin plasma of alloxan-diabetic rats. The clearing activity of the preheparin plasma of alloxan-diabetic rats was similar to that of normal rats receiving no heparin. However, the clearing activity of the postheparin plasma was markedly decreased. In correlation with the poor clearing activity, there was no increase in the production of unesterified fatty acids (figure 2). Increasing the heparin dosage from 0.05 mg. to 2.0 mg. per kilogram slightly increased the clearing activity

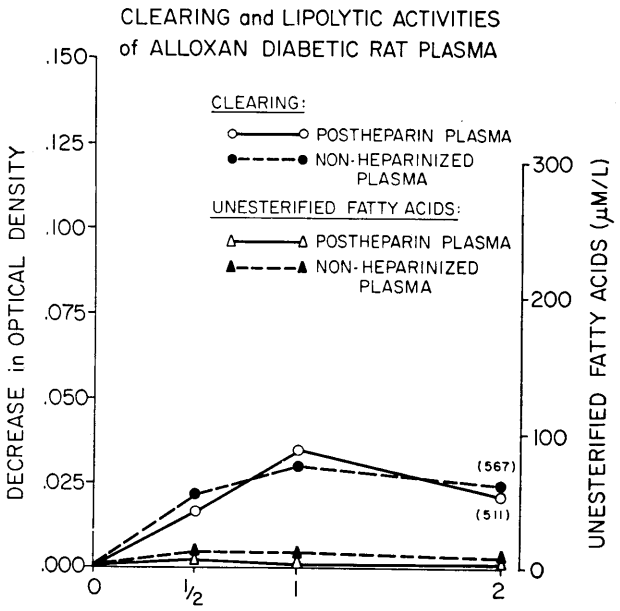

FIGURE 2

of postheparin plasma of alloxan-diabetic rats (figure 3). The over-all average of blood glucose of these two groups of rats (ten received heparin and ten no heparin) was 556 mg. per cent with the range of 252-830.

Clearing activity of postheparin plasma of depancreatized rats. The depancreatized rats were divided into two groups. (1) Nondiabetic with blood glucose of 140 mg. per cent or less, thirteen rats (blood sugar ranged from seventy-six to 140 with an average of 107); and (2) diabetic with blood glucose more than 140 mg. per cent (twelve rats). The diabetic group was subdivided into two groups: (a) six rats received 0.05 mg. heparin per kilogram, and (b) six received 2.0 mg. heparin per kilogram. The blood glucose level ranged from 152 to 405 mg. per cent with an average of 276. Blood glucose and postheparin plasma clearing were determined between one to four weeks after pancreatectomy. The results are shown in figure 4. It can

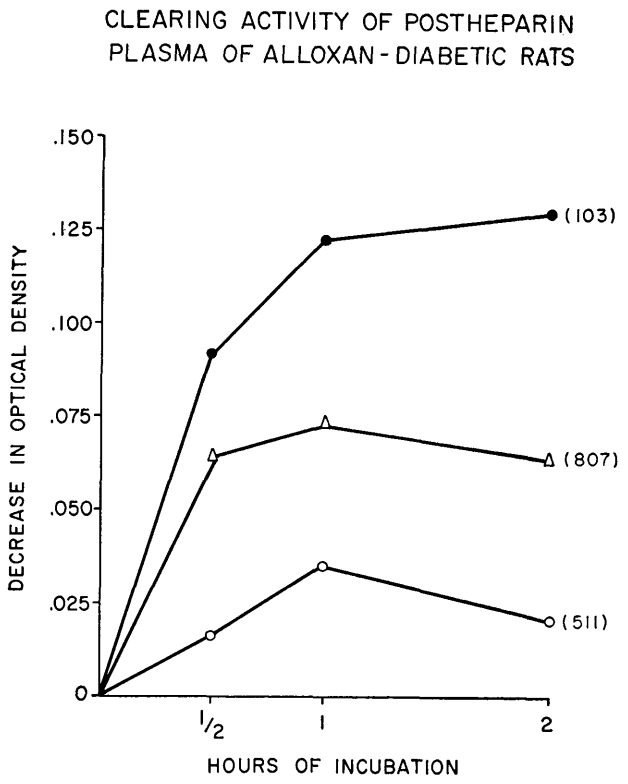

FIG. 3. [103]Normal rats 0.05 mg. heparin/kg.
[807]Alloxan diabetic rats 2 mg. heparin/kg.
[511]Alloxan diabetic rats 0.05 mg. heparin/kg.

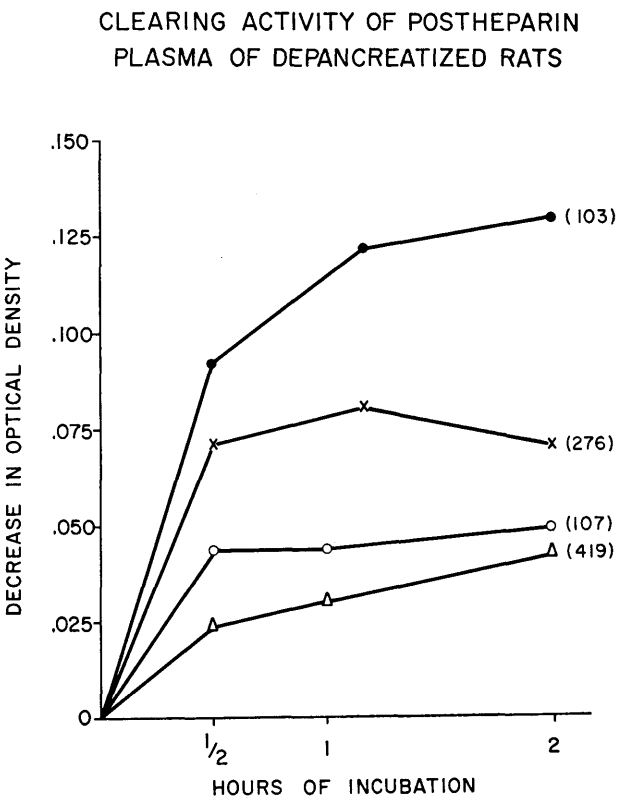

FIG. 4. [103]Normal rats 0.05 mg. heparin/kg.
[276]Depancreatized diabetic rats 2 mg. heparin/kg.
[107]Depancreatized nondiabetic rats 0.05 mg. heparin/kg.
[419]Depancreatized diabetic rats 0.05 mg. heparin/kg.

be seen that the clearing activity of postheparin plasma of depancreatized rats was also significantly reduced even in the nondiabetic group. The clearing activity of postheparin plasma of depancreatized diabetic rats receiving 0.05 mg. heparin per kilogram was further reduced. As in the alloxan-diabetic rats, an increase in the heparin dosage to 2.0 mg. per kilogram improved but did not completely correct the impairment in clearing.

Effect of insulin on the clearing activity of postheparin plasma of alloxan-diabetic rats. A total of twelve rats was included in this experiment. The blood glucose of these rats forty-eight hours after alloxanization averaged 436 mg. per cent ranging from 156 to 830. These rats were then given 4 to 8 units of insulin subcutaneously daily for two to six weeks after which blood glucose and clearing activity of postheparin plasma were determined. The blood glucose ranged from 53 to 146 mg. per cent with an average of 92 mg. The clearing activity of postheparin plasma was improved over that of alloxan-diabetic rats not being controlled with insulin, but remained significantly lower than that of normal rats (figure 5).

Effects of insulin plus lyophilized beef pancreas feeding on the clearing activity of postheparin plasma of alloxan-diabetic rats. Eighteen diabetic rats were maintained on 4 to 8 units of insulin daily beginning forty-eight hours after alloxanization. The amounts of insulin given depended on the level of blood glucose. The animals were immediately fed ground Purina chow containing 5 per cent lyophilized beef pancreas. Eleven rats were on this regimen for four to six weeks and seven for eleven to sixteen days. The blood glucose determined forty-eight hours after alloxanization averaged 537 mg. per cent (ranging 163-906) and 458 mg. per cent (ranging 373-540) for the former (long-term) and the latter (short-term) groups respectively. At the end of the appropriate period, blood glucose was again measured and clearing activity of postheparin plasma determined. It was found that the blood glucose of the long-term group averaged 164 mg. per cent (ranging 47-397) and that of the short-term group averaged 44 mg. per cent (ranging 28-68). The clearing activity

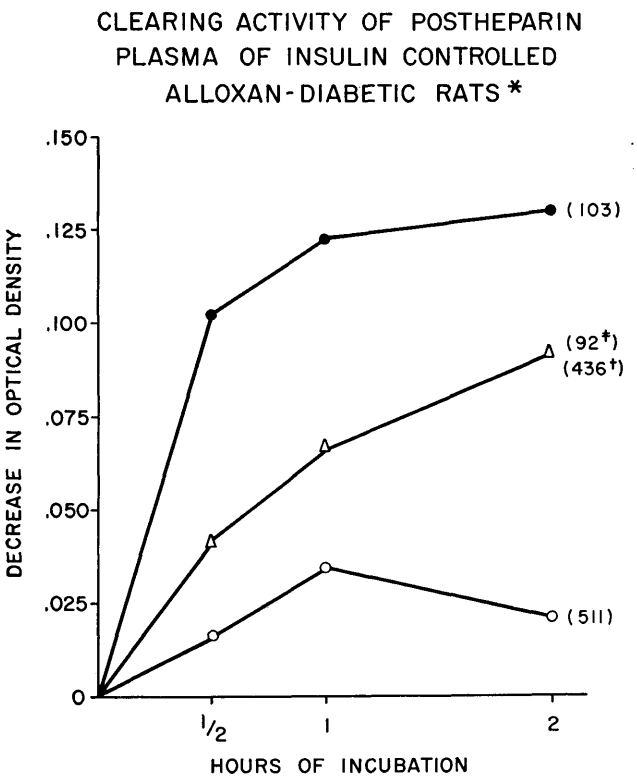

FIG. 5. *All rats were given 0.05 mg. heparin/kg.
†Blood glucose forty-eight hours after alloxanization.
‡Blood glucose at the time of blood withdrawal for clearing activity after two to eight weeks of insulin treatment.
[103]Normal rats.
[92, 436]Insulin controlled alloxan-diabetic rats.
[511]Alloxan-diabetic rats.

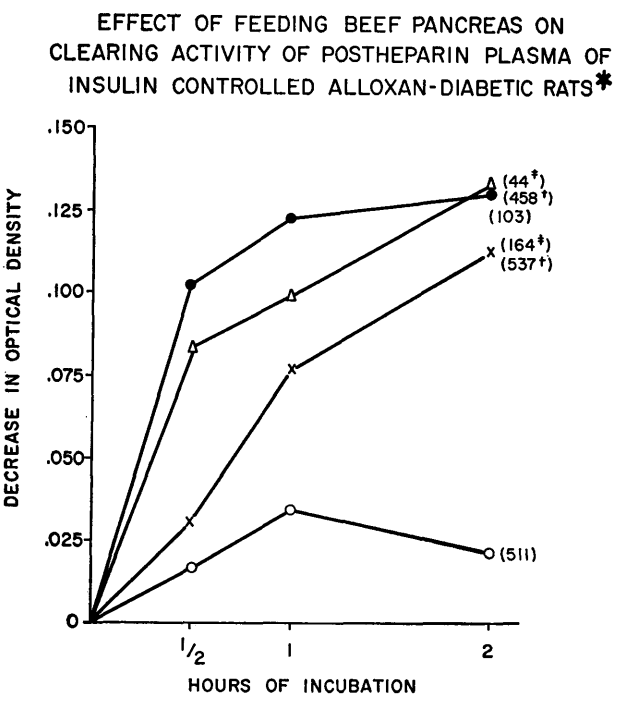

FIG. 6. Insulin controlled diabetic rats were fed beef pancreas for four to six weeks. Insulin controlled alloxan-diabetic rats were fed beef pancreas eleven to sixteen days.
*All rats were given 0.05 mg. heparin/kg.
†Blood glucose forty-eight hours after alloxanization.
‡Blood glucose at the time of blood withdrawal for clearing activity determination.
[44]Insulin controlled diabetic rats.
[103]Normal rats.
[164, 537]Insulin controlled alloxan-diabetic rats.
[511]Alloxan-diabetic rats.

of postheparin plasma was corrected from the marked low level of diabetic rats to that of normal animals. Feeding a diet containing 5 per cent lyophilized beef pancreas for eleven to sixteen days was almost as effective as for four to six weeks (figure 6).

Effect of insulin plus feeding of Lipormone on the clearing activity of postheparin plasma of alloxan-diabetic rats. The blood glucose of nine rats determined forty-eight hours after injection of alloxan averaged 528 mg. per cent (ranging 242-810). The animals were then given 4 to 8 units of insulin daily and fed Purina chow containing 0.5 per cent Lipormone. After they were maintained on this regimen for three to five weeks, blood glucose and clearing activity of postheparin plasma were determined. It was found that the clearing activity of postheparin plasma was also markedly increased over that of the diabetic rats as in those receiving pancreas diet. The blood glucose ranged from 75-363 mg. per cent with an average of 208 at the time of blood withdrawal for clearing determinations.

Summary of clearing activity of postheparin plasma of various groups of rats. Figure 7 summarizes the clearing activity of postheparin plasma of various experimental groups of rats. The results represent the mean decrease in optical density (clearing activity) of the buffer system containing pre- or postheparin plasma and synthetic triglyceride emulsion after two-hour incubation at 37° C. A comparison between the clearing of postheparin plasma of normal rats and that of other groups is shown in table 1.

Lack of correlation between blood glucose level and clearing activity of postheparin plasma of alloxan-diabetic rats. In figure 8 a vertical line is drawn at the blood glucose level of 140 mg. per cent and a horizontal line at the decrease in optical density of 0.10. It can be seen that the clearing activity of postheparin plasma did not correlate with the blood glucose levels in rats of various groups. It is of interest to note that all normal

TABLE 1

Clearing activity of postheparin plasma of various groups of rats: a comparison between normal and other groups

Experimental group	Decrease in optical density (in two hours) Mean ± S.E.	Experimental group	Mean ± S.E.	Difference in mean	P
		Alloxan-diabetic (10)	0.021 ± 0.008*	0.109	0.001
		Alloxan-diabetic (10)	0.064 ± 0.018†	0.066	0.001
		Depancreatized nondiabetic (13)	0.051 ± 0.009*	0.079	0.001
Normal (14)	0.130 ± 0.008	Depancreatized diabetic (6)	0.043 ± 0.016*	0.087	0.001
		Depancreatized diabetic (6)	0.070 ± 0.022†	0.060	0.01
		Alloxan-diabetic + insulin (12)	0.095 ± 0.012*	0.035	0.02
		Alloxan-diabetic + insulin and pancreas diet‡ (11)	0.134 ± 0.017*	0.004	0.8
		Alloxan-diabetic + insulin and pancreas diet§ (7)	0.113 ± 0.005*	0.017	0.05
		Alloxan-diabetic + insulin and Lipormone diet (9)	0.112 ± 0.007*	0.018	0.1

*0.05 mg. heparin per kg. †2.0 mg. heparin per kg. ‡On diet containing pancreas for four to six weeks.
§On diet containing pancreas for eleven to sixteen days.
Numbers in parentheses in columns 1 and 3 (Experimental Group) represent the number of animals.

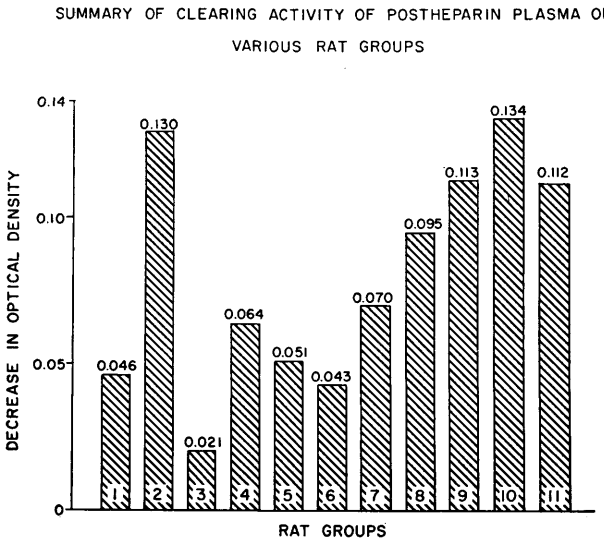

FIG. 7. Figures above the bars are mean decrease in optical density after two-hour incubation at 37° C. of various groups of rats. 1. Nonheparinized plasma of normal rats 2. Postheparin plasma of normal rats 3. Postheparin plasma of alloxan diabetic rats receiving 0.05 mg. heparin/kilogram 4. Postheparin plasma of alloxan-diabetic rats receiving 2.0 mg. heparin/kilogram 5. Depancreatized nondiabetic rats receiving 0.05 mg. heparin/kilogram 6. Depancreatized diabetic rats receiving 0.05 mg. heparin/kilogram 7. Depancreatized diabetic rats receiving 2.0 mg. heparin/kilogram 8. Insulin controlled alloxan-diabetic rats 9. Insulin controlled alloxan-diabetic rats fed beef pancreas for four to six weeks 10. Insulin controlled alloxan-diabetic rats fed beef pancreas for eleven to sixteen days 11. Insulin controlled alloxan-diabetic rats fed Lipormone. All rats in groups 8, 9, 10 and 11 were given 0.05 mg. heparin/ kilogram.

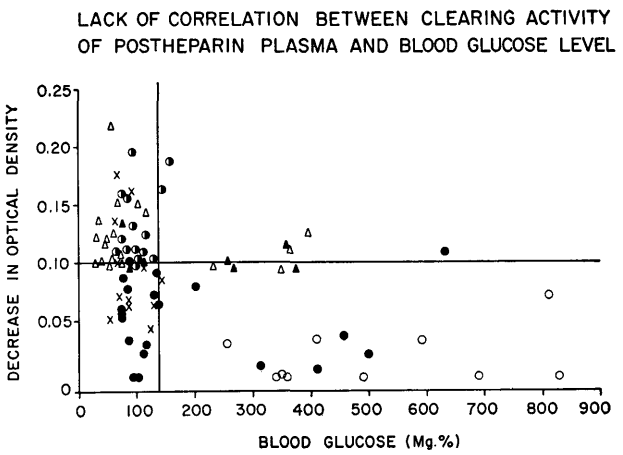

FIG. 8. Half-closed circle—normal rats.
Open circle—alloxan-diabetic rats.
Closed circle—depancreatized rats.
×—insulin controlled alloxan-diabetic rats.
Open triangle—insulin controlled alloxan-diabetic rats plus beef pancreas feeding.
Closed triangle—insulin controlled alloxan-diabetic rats plus Lipormone feeding.

rats are in or near the upper left quadrant, and all alloxan-diabetic rats are in the lower right quadrant. However, the clearing activity of postheparin plasma of the depancreatized rats was all below the horizontal line except in two instances, yet their blood glucose levels ranged from lower normal to as high as 630 mg. per cent. The blood glucose of the insulin controlled alloxan-diabetic rats ranged from 55 to 145 mg. per cent; but the clearing activity of postheparin

plasma was below the horizontal line in eight of the twelve animals. It was further noted that the clearing activity of postheparin plasma of insulin controlled alloxan-diabetic rats fed a diet containing lyophilized beef pancreas or Lipormone was all above or near the horizontal line, yet the blood glucose of these rats ranged from 28-400 mg. per cent.

DISCUSSION

The finding that failure of postheparin plasma of alloxan diabetic rats to clear or lipolyze the synthetic triglyceride emulsion in vitro is in accord with our observations obtained in patients with diabetes mellitus.[30] Since the postheparin plasma of depancreatized rats was also incapable of exerting the clearing activity, a relation between pancreas and the clearing phenomenon seems apparent. It is not known, however, whether alloxanization or pancreatectomy causes a decrease in the production of the heparin-induced lipemia clearing factor per se or whether these procedures provoke a reaction which hinders the production of clearing factor or inhibits its activity. Increasing dosage of heparin does improve the clearing activity of postheparin plasma of both alloxanized and depancreatized rats. These findings would suggest that the production of the heparin-induced clearing factor can be enhanced either by stimulating the remaining normal cells of the pancreas or by affecting cells of other organs. We have shown that addition of heparin to the perfusate of the isolated normal rat heart is capable of producing clearing factor.[31] It is not known if heparin could induce the production of clearing factor in a perfused diabetic rat heart.

The fact that insulin treatment improved the clearing activity of postheparin plasma of alloxan-diabetic rats would indicate that the defect in producing the clearing factor can be partially corrected. This improvement might have been due to the increased utilization of glucose alone or in combination with other metabolic processes. However, insulin was unable to correct completely the impairment in clearing by postheparin plasma although it lowered the blood glucose level to normal. The findings are not surprising since the clearing activity of postheparin plasma did not correlate with the blood glucose level.

It may well be possible that the reduced clearing activity of postheparin plasma in alloxanized or depancreatized rats stems from lack of a certain essential factor ordinarily supplied by the pancreas. Success in correcting this defect of postheparin plasma clearing by means of feeding the animals a diet containing lyophilized beef pancreas constitutes good evidence for such a possibility. The nature of this pancreatic factor is not known. The defect in clearing in alloxan-diabetic rats could also be corrected by feeding of Lipormone, a purified preparation of lipocaic of Dragstedt.[35] At present it is not possible to state whether the two substances are identical. However, since it has been observed in this laboratory that destruction of acinous tissues of the pancreas did not reduce the clearing activity of postheparin plasma,[36] the islets seem to be the source of this factor.

The findings reported must be considered preliminary in nature. Further studies are necessary to investigate the role of pancreas, particularly the islets of Langerhans in the production of heparin-induced lipemia clearing factor in vivo and in lipid metabolism.

SUMMARY

The clearing activity of postheparin plasma of alloxan-diabetic and depancreatized rats was markedly reduced. Increase in heparin dosage administered to, or insulin treatment of, these rats improved but failed to correct this defect. Insulin treatment plus feeding of a diet containing lyophilized beef pancreas or Lipormone, a purified lipocaic, was capable of restoring the postheparin plasma clearing of alloxanized rats to that of normal animals. It is suggested that a pancreatic factor may be necessary in the in vivo production of the heparin-induced clearing factor.

SUMMARIO IN INTERLINGUA

Le Rolo Del Pancreas In Le Production, Inducite Per Heparina, De Factor De Clarage De Lipemia

Le activitate de clarage in plasma post-heparinic ab rattos alloxano-diabetic e dispancreatisate esseva marcatemente reducite. Augmento del dosage de heparina o administration de insulina a iste rattos meliorava sed non normalisava le activitate in question. Le administration de insulina in association con un dieta continente lyophilisate pancreas bovin o liphormon (un purificate lipocaico) esseva capace a restaurar le activitate de clarage in plasma post-heparinic ab alloxanisate rattos al nivello trovate in animales normal. Es suggerite que un factor pancreatic es possibilemente necessari in le production in vivo del factor de clarage inducite per heparina.

ACKNOWLEDGMENT

The work reported here was supported by research grants from the American Heart Association; Eli Lilly and Company, Indianapolis; and Mid-Tennessee Heart Association, Nashville, Tennessee.

The authors wish to express their appreciation to Dr. Margaret P. Martin and Mr. Ben Couch, Department of Preventive Medicine, Vanderbilt University School of Medicine, for statistical analysis of the data presented in table 1.

REFERENCES

[1] Joslin, E. P., Bloor, W. R., and Gray, H.: The blood lipids in diabetes. J.A.M.A. 69:375-78, 1917.

[2] Rabinowitch, I. M.: The cholesterol content of blood plasma in diabetes mellitus: A statistical study based on two thousand observations in three hundred and eighty-five cases. Arch. Int. Med. 43:363-71, 1929.

[3] Blix, G.: Studies on diabetic lipemia. I. Acta Med. Scand. 64:142-74, 1926.

[4] Gray, H.: Lipoids in 1,000 diabetic bloods, with special regard to prognosis. Am. J. Med. Sc. 168:35-46, 1924.

[5] Pomeranze, J., and Kunkel, H. G.: Serum lipids in diabetes mellitus. Proc. Amer. Diabetes Assoc. 10:217-31, 1950.

[6] Man, E. B., and Albrink, M. J.: Serum lipids in different phases of carbohydrate metabolism. Yale J. Biol. & Med. 29: 316-34, 1956.

[7] Hirch, E. F., Phibbs, B. P., and Carbonaro, L.: Parallel relation of hyperglycemia and hyperlipemia (esterified fatty acids) in diabetes. A.M.A. Arch. Int. Med. 91:106-17, 1953.

[8] Harris, L. V. D., Albrink, M. J., Van Euk, W. F., Man, E. B., and Peters, J. P.: Serum lipids in diabetic acidosis. Metabolism 2:120-32, 1953.

[9] Herbert, F. K.: Observations on the blood fats in diabetic lipemia. Biochem. J. 29:1887-93, 1935.

[10] Barach, J. H., and Lowy, A. D.: Lipoprotein molecules, cholesterol and atherosclerosis in diabetes mellitus. Diabetes 1:441-46, 1952.

[11] Keiding, N. R., Mann, G. V., Root, H. F., Lawry, E. Y., and Marble, A.: Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications. Diabetes 1:434-40, 1952.

[12] Tuller, E. F., Mann, G. V., Schertenleib, F., Roehring, C. B., and Root, H. F.: The effects of diabetic acidosis and coma upon the serum lipoproteins and cholesterol. Diabetes 3: 279-86, 1954.

[13] Kolb, F. O., deLalla, O. F., and Goffman, J. W.: The hyperlipemias in disorders of carbohydrate metabolism: Serial lipoprotein studies in diabetic acidosis with xanthomatosis and glycogen storage disease. Metabolism 4:310-17, 1955.

[14] Warren, S., and LeCompte, P. M.: The Pathology of Diabetes Mellitus. Philadelphia, Lea and Febiger, 1952, p. 146.

[15] Root, H. F., Bland, E. F., Gordon, W. H., and White, P. D.: Coronary atherosclerosis in diabetes mellitus; postmortem study. J.A.M.A. 113:27-30, 1939.

[16] Stearn, S., Schlesinger, M. J., and Rudy, A.: Incidence and clinical significance of coronary artery disease in diabetes mellitus. Arch. Int. Med. 80:463-74, 1947.

[17] Clawson, B. J., and Bell, E. T.: Incidence of fatal coronary disease in nondiabetic and diabetic persons. Arch. Path. 48: 105-06, 1949.

[18] Millard, E. B., and Root, H. F.: Degenerative vascular lesions and diabetes mellitus. Am. J. Digest. Dis. 15:41-51, 1948.

[19] Liebow, I. M., and Hellerstein, H. K.: Cardiac complications of diabetes mellitus. Am. J. Med. 7:660-70, 1949.

[20] Bell, E. T.: A post-mortem study of 1,214 diabetic subjects with special reference to the vascular lesions. Proc. Am. Diabetes Assoc. 10:62-82, 1950.

[21] Hahn, P. F.: Abolishment of alimentary lipemia following injection of heparin. Science 98:19-20, 1943.

[22] Anderson, N. G., and Fawcett, B.: An antichylomicronemic substance produced by heparin injection. Proc. Soc. Exper. Biol. & Med. 74:768-71, 1950.

[23] Bragdon, J. H., and Havel, R. J.: In vivo effect of antiheparin agents on serum lipids and lipoproteins. Am. J. Physiol. 177:128-33, 1954.

[24] French, J. E., Morris, B., and Robinson, D. S.: Observations on the removal of chylomicra from the blood. Third Internat. Conference on Biochem. Problems of Lipids. Brussels, 1956, pp. 323-31.

[25] Grossman, M. I., and Strub, I. H.: Effect of heparin on fate of intravenously administered fat emulsion in rats. Proc. Soc. Exper. Biol. & Med. 85:356-59, 1954.

[26] Meng, H. C., and Youmans, J. B.: Effect of heparin on serum lipids following intravenous administration of fat emulsion in dogs. Proc. Soc. Exper. Biol. & Med. 97:691-93, 1958.

[27] Shoulders, H. H., Jr., Meng, H. C., and Tuggle, S.: Effects of heparin on body temperature and plasma lipids following intravenous administration of fat emulsion in man. J. Lab. and Clin. Med. In press.

[28] Anfinsen, C. B.: On the role of lipemia clearing factor in lipid transport. A Symposium on Fat Metabolism, edited by Victor A. Najjar. Baltimore, the Johns Hopkins Press, 1954, pp. 93-110.

[29] Hollett, C., and Meng, H. C.: Comparison of fat emulsion clearing activities of postheparin plasma and pancreatic lipase. Am. J. Physiol. 184:428-32, 1956.

[30] Meng, H. C.: Unpublished results.

[31] Meng, H. C., Hollett, C., and Cole, W. E.: Some factors affecting the clearing of neutral fat emulsion by postheparin plasma. Am. J. Physiol. 179:314-18, 1954.

[32] Borgstrom, B.: On the action of pancreatic lipase on triglycerides in vivo and in vitro. Acta Physiol. Scand. 25:328-47, 1952.

[33] Somogyi, M.: Notes on sugar determination. J. Biol. Chem. 195:19-23, 1952.

[34] Meng, H. C.: Production of heparin-induced lipemia clearing factor in perfused rat heart. Third World Congress of Cardiology, Abstract. September, 1958.

[35] Dragstedt, L. R., Prohoska, J. V., and Harms, H. P.: Observations on a substance in pancreas (a fat-metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. Am. J. Physiol. 117:175-81, 1936.

[36] Meng, H. C., and Hadley, J. L.: Further studies of pancreas and the production of heparin-induced lipemia clearing factor. To be published.