Gut, 1961, **2**, 233

Non-esterified fatty acids and lipoprotein lipase activity in patients with cirrhosis of the liver

C. CHLOUVERAKIS AND PETER HARRIS

From the Department of Medicine, The Queen Elizabeth Hospital, Birmingham

SYNOPSIS After the intravenous injection of heparin the plasma concentration of non-esterified fatty acids rises in normal subjects and in patients with nephrosis or diabetes but not in patients with portal cirrhosis.

In 1943, Hahn observed that when blood to which heparin had been added as an anticoagulant was infused into lipaemic dogs the lipaemia cleared very rapidly. When heparin was added *in vitro* the lipaemia remained unaltered. It is now accepted that this clearing of the lipaemia after the injection of heparin is due to the appearance in the blood stream of a lipase (lipoprotein lipase) which catalyses the hydrolysis of the triglycerides contained in chylomicrons and low-density lipoproteins into glycerol and non-esterified fatty acids. Thus, after the intravenous injection of heparin, there is a rise in the concentration of non-esterified fatty acids in the plasma (Grossman, Palm, Becker, and Moeller, 1954; Gordon and Cherkes, 1956). The non-esterified fatty acids are 'free' fatty acids in the sense that they are not in covalent linkage although they are very tightly bound to albumin. Albumin, therefore, stimulates the lipolytic process by removing the fatty acids which are produced. It seems likely that heparin acts by virtue of its highly electro-negative sulphate groups, mobilizing an apo-enzyme from the tissues where it is normally present and effecting its rapid attachment to the substrate. The active enzyme is a union of heparin and apo-enzyme.

In the absence of a previous injection of heparin, the lipoprotein lipase activity of normal plasma is small or absent as measured *in vitro* by the reduction of turbidity (Engelberg, 1956a and 1958) and by the increase in the concentration of non-esterified fatty acids (Gates and Gordon, 1958) or glycerol (Cleland and Iacono, 1957). Baker, Levine, Turner, and Dubin (1958) reported that after the intravenous injection of a small (3 mg.) amount of heparin the clearing activity *in vitro* in the plasma of patients with Laennec's cirrhosis was greater than in normals. The dose of heparin used by these authors was chosen on the basis of a previous observation (Block, Mann, and Barker, 1951) that a dose of 5 mg. of heparin brings about a complete clearing of the lipaemic plasma whereas 3 mg. 'just fails to restore completely the translucency of plasma' during alimentary lipaemia. Patients with Laennec's cirrhosis and a low serum albumin level showed a maximum clearing. This was in contrast to the plasma of a nephrotic patient with hypoalbuminaemia which showed no clearing activity, an observation in keeping with the findings of Herzstein, Wang, and Aldersberg (1954). The animal experiments by Rosenman and his colleagues (Rosenman, Friedman, and Byers, 1956; Rosenman and Friedman, 1957; Rosenman and Byers, 1959) have suggested that the reduced clearing activity of the nephrotic plasma is partly attributable to the low levels of albumin which fail to remove the excess of fatty acids produced during lipolysis. The different effect of a low serum albumin level in these two different diseases is paradoxical, and becomes more so if one bears in mind that patients with cirrhosis of the liver have high levels of plasma non-esterified fatty acids (Stormont and Mackie, 1959; Wajchenberg, Hoxter, Mello, and Ulhoa Cintra, 1960), and consequently an even more reduced 'potential' amount of albumin to serve as an acceptor of the fatty acids released during lipolysis.

In the present work the lipolytic effect *in vivo* of intravenous heparin has been followed by measuring the plasma concentration of non-esterified fatty acids. Normal subjects and patients with cirrhosis of the liver were studied. The effect of heparin *in vivo* was also followed in three patients with nephrosis and in two patients with diabetes mellitus. In addition, the endogenous lipolytic activity in the plasma of cirrhotic patients has been measured *in vitro* and compared with that of normal plasma.

MATERIAL AND METHODS

Ten patients with portal cirrhosis and two patients with biliary cirrhosis were studied. The diagnosis had been confirmed histologically in each case. The clinical status and liver function tests were compatible with the histological diagnosis in all of them. Further studies were made on 11 normal subjects, on two patients with diabetes mellitus, and on three patients with a low plasma albumin level associated with nephrosis.

The concentration of non-esterified fatty acids in plasma was estimated by Gordon, Cherkes, and Gates' method (1957). The composition of non-esterified fatty acids extracted from human plasma by this method has been studied by means of a Pye Argon chromatograph (Chlouverakis and Harris, 1960) and found to be the same in cirrhotic patients as it is in normals.

EFFECT OF HEPARIN *IN VIVO* For this purpose, heparin was injected into a peripheral vein and its effect on the concentration of non-esterified fatty acids measured in arterial blood. Arterial blood was chosen since peripheral venous levels of non-esterified fatty acids are largely dependent on local metabolism (Gordon *et al.*, 1957). Two groups of patients were studied, one with, and the other without, cirrhosis. In each group, studies were made of the effect of heparin both under fasting conditions and three hours after a fatty meal.

Subjects without cirrhosis The effect of heparin was studied under fasting conditions in three normal subjects, in two patients with diabetes, and in one patient with a low serum albumin level (2·3 g./100 ml.) associated with nephrosis. The subjects had fasted overnight for 12 hours. An indwelling arterial needle was inserted and two samples of blood drawn into heparinized syringes at an interval of five minutes. After the second sample was taken, 100 mg. of sodium heparin was injected into a peripheral vein and further samples of arterial blood taken after 15, 30, 60, and 90 minutes. The dose of 100 mg. of heparin was chosen in order to produce a maximum effect.

In a control group of five normal subjects, the same procedure was undertaken without the injection of heparin. Its purpose was to exclude the possibility that any rise of the non-esterified fatty acid level after heparin was due to continued starvation during the period of sampling.

In three normal subjects and two patients with a low serum albumin level (2·0 and 2·0 g./100 ml.) due to nephrosis a similar study was made after the administration of a fatty meal. The subjects fasted overnight. In the morning they were given 100 g. of milk cream. Three hours later an arterial needle was inserted and two samples of blood withdrawn into heparinized syringes. Then 100 mg. of sodium heparin was injected intravenously and further samples of arterial blood taken after 10, 20, 40, and 60 minutes.

Patients with cirrhosis The effect of heparin was studied under fasting conditions in five patients with portal cirrhosis and in two patients with biliary cirrhosis. The procedure was the same as that used in the subjects without cirrhosis.

In four patients with portal cirrhosis and one patient with biliary cirrhosis, the effect of heparin was studied after a fatty meal by the same method.

ENDOGENOUS LIPOLYTIC ACTIVITY *IN VITRO* The endogenous lipolytic activity was studied in the plasma of four normal subjects and five patients with portal cirrhosis. Venous blood (20 ml.) was drawn into a heparinized syringe after 12 hours' starvation overnight. The plasma from this blood was divided into six portions, each of 1 ml. The first portion was used for the estimation of non-esterified fatty acids. The other five portions were each placed in a test-tube. Nothing was added to the first tube. To the remaining four test-tubes was added respectively 0·1, 0·2, 0·3, and 0·4 ml. of an 0·2% freshly prepared emulsion of cocoanut oil. All tubes were then covered and placed in a water-bath at 37°C. for two hours. Estimations of the concentration of non-esterified fatty acids were then made on the contents of each tube.

RESULTS

The concentration of non-esterified fatty acids was 1,470 $\pm$ 312 μEq./l. (mean $\pm$ S.D.) in the arterial blood of five fasting patients with portal cirrhosis which compares with 712 $\pm$ 134 μEq./l. found in this laboratory in the arterial blood of normal subjects.

After the injection of 100 mg. of heparin the concentration of non-esterified fatty acids in the plasma rose appreciably (Fig. 1) in all the fasting subjects without cirrhosis, including the two patients with diabetes and one with nephrosis. The rise in the diabetic patients appeared to be greater than in the normals. Fig. 1 also shows the concentration of non-esterified fatty acids during the same period of time in the control group of fasting normal subjects who did not receive heparin.

Four of the five fasting patients with portal cirrhosis showed no significant change in the plasma non-esterified fatty acid concentration after the injection of heparin. One patient showed a fall. Two fasting patients with biliary cirrhosis showed a small rise (Fig. 2).

The response to heparin three hours after a fatty meal in the subjects without cirrhosis is shown in Fig. 3. In all the level of non-esterified fatty acids rose, the average of the maximal rise being 1,804 μEq./l. This response was most marked in the two nephrotic patients.

Fig. 4 shows the effect of heparin during an alimentary lipaemia in patients with cirrhosis of the liver. Two patients with portal cirrhosis showed a rise in the concentration of non-esterified fatty acids similar to that found in normals. One patient showed a fall and another a very slight rise. The only patient with biliary cirrhosis who was studied in this fashion responded by a slight rise.

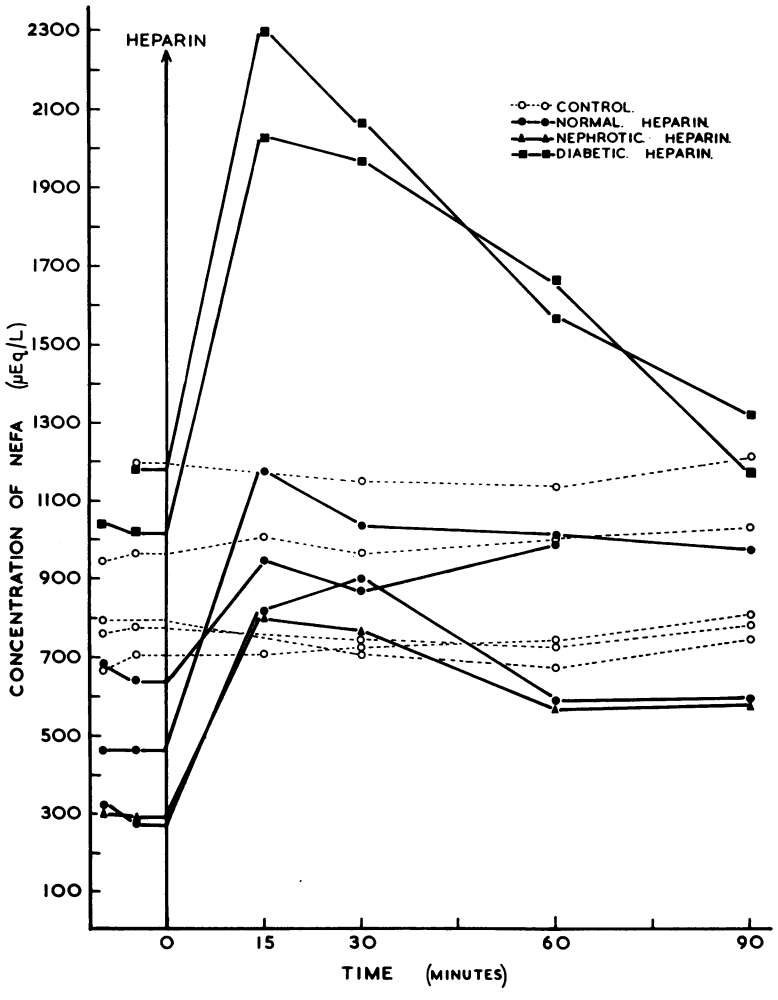

FIG. 1. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of fasting, non-cirrhotic subjects.*

Fig. 5 shows the results of the estimation *in vitro* of endogenous plasma lipolytic activity on four normal subjects and five patients with portal cirrhosis. None of the normal subjects showed any lipolytic activity. On the other hand, only one of the five cirrhotic patients did not show enzyme activity. The other four showed a substantial activity which varied with the different concentrations of the exogenously added substrate.

DISCUSSION

The central finding of the studies *in vivo* is that, in the fasting state, heparin causes a rise in the plasma concentration of non-esterified fatty acids in normal people but no rise in patients with portal cirrhosis. The cause of this lack of response on the part of the cirrhotic patients does not appear to be a lack of plasma albumin, since a nephrotic patient with a low serum albumin level responded normally. Moreover, in two of the cirrhotic patients, the serum albumin had been restored to normal levels by means of infusions of human albumin before the heparin test was undertaken.

In diabetic patients who had a high level of plasma non-esterified fatty acids the response to heparin appeared to be greater than normal, so that the lack of response in the cirrhotic patients is not due to the high initial levels of plasma non-esterified fatty acids, which might occupy most of the available sites for the attachment of the fatty acids in the albumin molecule.

A further possible reason for the difference between cirrhotics and normals could be a relatively low concentration of available lipid substrate (Baker *et al.*, 1958). When this possibility was checked by

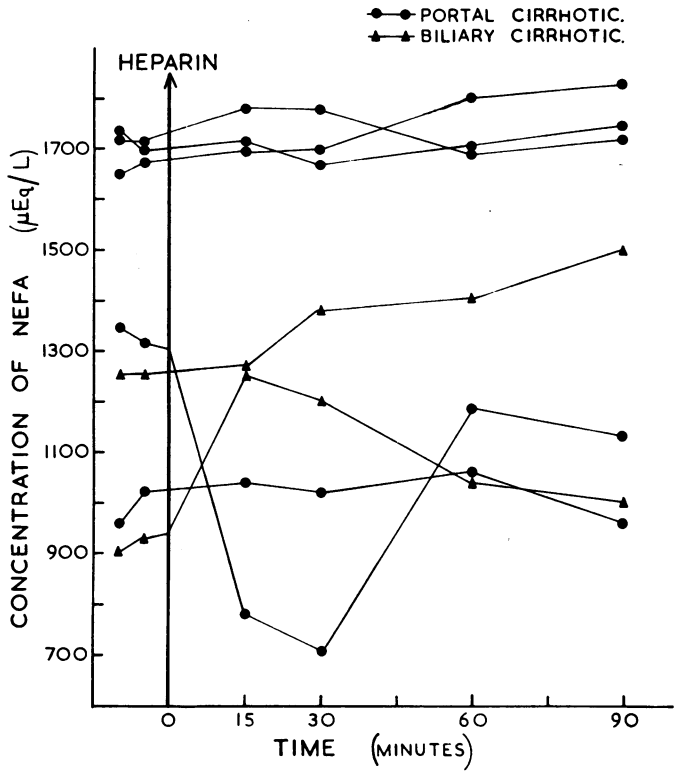

FIG. 2. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of fasting patients with cirrhosis.*

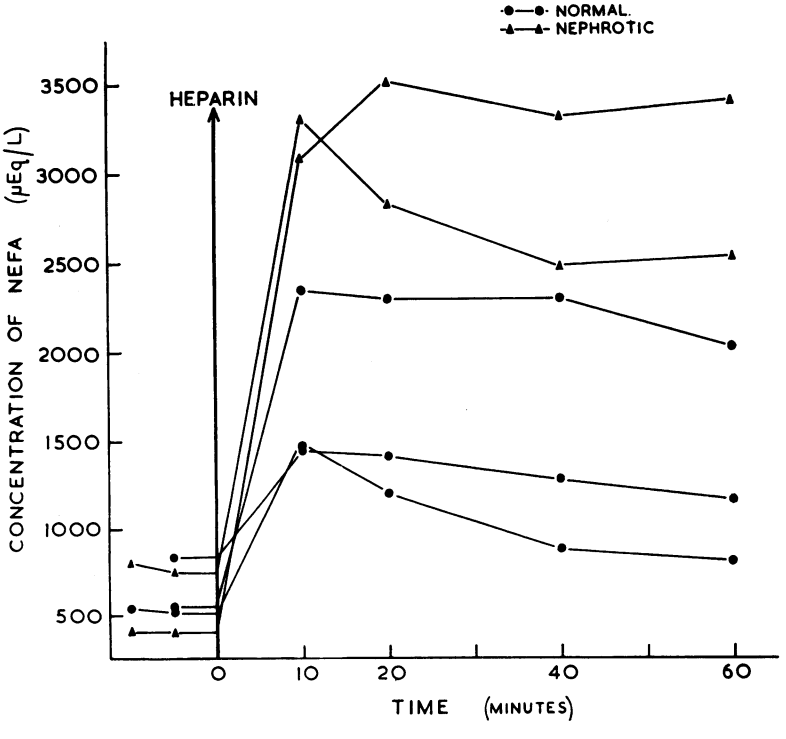

FIG. 3. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of non-cirrhotic subjects in a state of alimentary lipaemia.*

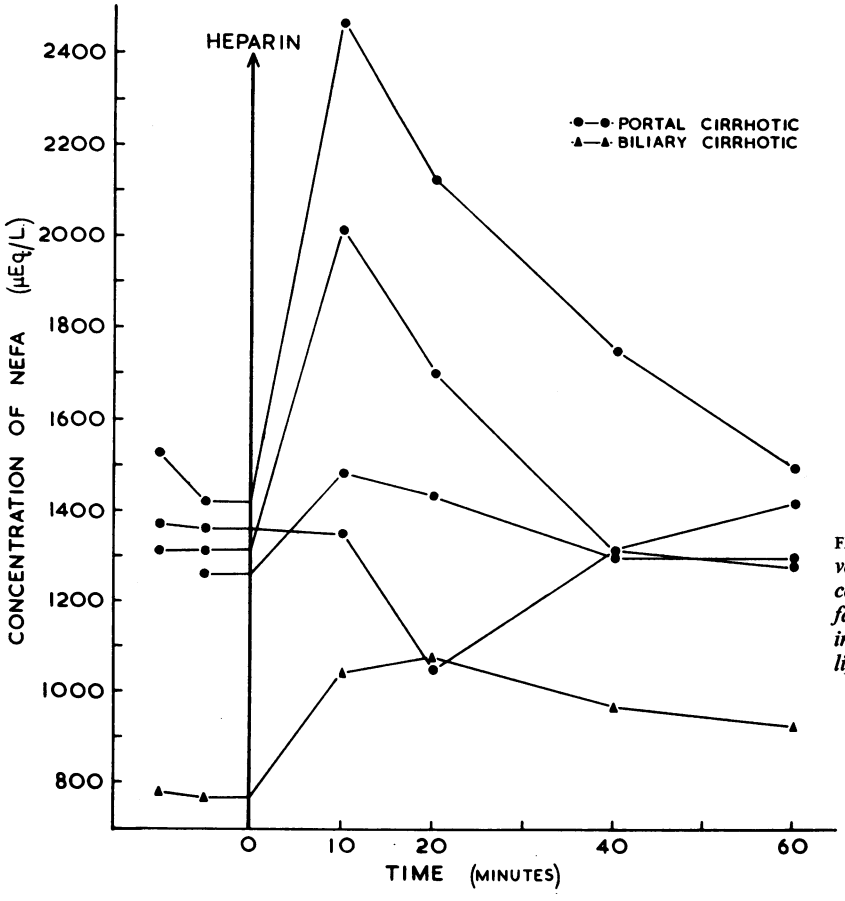

FIG. 4. *The effect of intravenous heparin on the plasma concentration of non-esterified fatty acids of cirrhotic patients in a state of alimentary lipaemia.*

inducing an alimentary lipaemia, it was found that two of the four cirrhotic patients studied had a normal response. This would suggest that although a relatively low level of substrate can be an important factor it does not explain the lack of response to heparin in every cirrhotic patient.

It is conceivable that inhibitors to lipoprotein lipase may be present in the blood of cirrhotic patients. The presence of such substances seems, however, highly unlikely in view of the increased lipolytic activity found in the plasma of most cirrhotics during the present study. This increased activity can be explained by the observation that the liver inactivates lipoprotein lipase (Jeffries, 1954; Spitzer and Spitzer, 1956). Since the liver may be responsible for the destruction or de-activation of heparin (Jaques, 1940; Jaques and Keeri-Szanto, 1952), which is an integral part of the lipoprotein lipase molecule, it is possible that the inactivation of the lipolytic enzyme by the liver may be merely the result of the destruction of heparin. This would result in the presence of increased amounts of heparin in the blood of patients with hepatic damage. Such a prolonged hyperheparinaemia might exhaust the tissue stores of apo-enzyme (Korn, 1955) which could account for the lack of a substantial rise in the plasma non-esterified fatty acids after heparin in two of the four cirrhotic patients in whom an adequate concentration of lipid substrate had been assured by the production of an alimentary lipaemia.

There is also evidence that an excess of heparin can inactivate lipoprotein lipase activity (Korn, 1955; Hollett and Meng, 1956a) and this might explain the fall in the concentration of non-esterified fatty acids which was observed in one cirrhotic patient both under starving and lipaemic conditions.

The high concentration of non-esterified fatty acids in the blood of patients with portal cirrhosis confirms the findings of Stormont and Mackie (1959) and Wajchenberg *et al.* (1960). These high levels could be explained by the increased endogenous lipolytic activity of the plasma found in the present

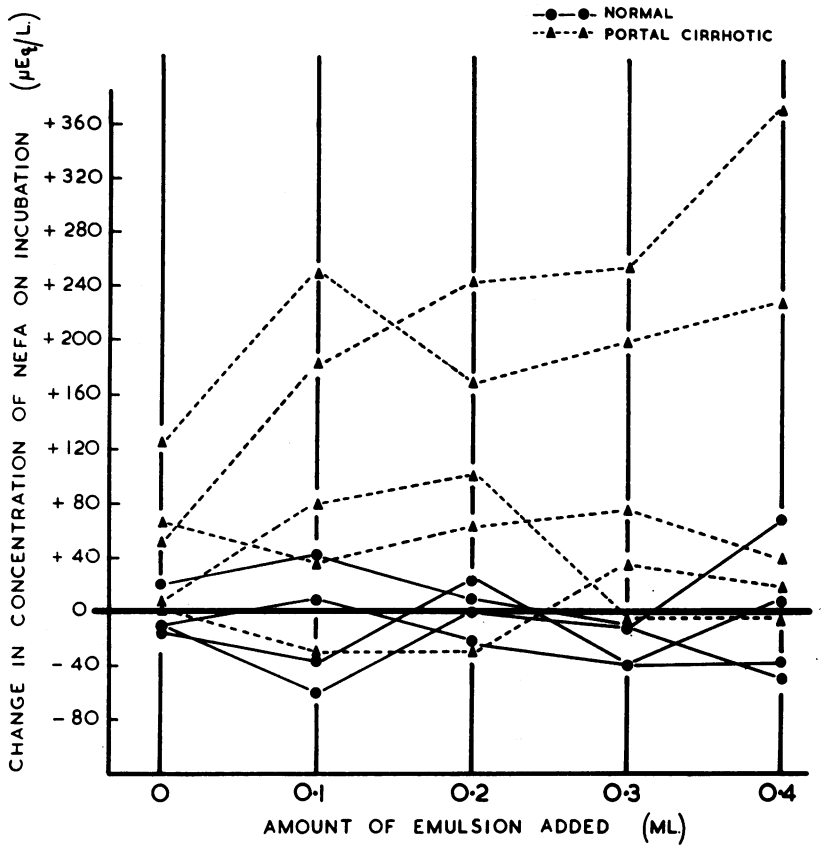

FIG. 5. *The lipolytic activity* in vitro *of normal plasma and of plasma from cirrhotic patients.*

study. It is of interest that the only cirrhotic patient who did not show lipolytic activity had a normal concentration of non-esterified fatty acids (730 μEq./l.). Wajchenberg and his colleagues suggested that the high levels of plasma non-esterified fatty acids in cirrhotics are due to a failure of the damaged liver to withdraw non-esterified fatty acids from the circulation. This suggestion, however, takes no account of the fact that non-esterified fatty acids are also widely extracted and oxidized by other tissues (Gordon and Cherkes, 1956; Gordon *et al.*, 1957; Spitzer and Roheim, 1958; Carlson and Pernow, 1959).

Hollet and Meng (1956b) reported that after an intravenous injection of heparin the lipolytic activity *in vitro* of the plasma was less in diabetics than in normals as measured by a decrease in optical density. Herzstein *et al.* (1954) made a similar observation in nephrotic patients. In the present study, heparin caused a greater rise in plasma non-esterified fatty acids in diabetics and nephrotics than it did in normals. This apparent discrepancy seems to be due to the fact that lipolysis can occur in the absence of any decrease in optical density (Engelberg, 1956b). This is supported by the observation of Herzstein and his co-workers that in all but one of their nephrotic patients there was a decrease in the plasma neutral fat after heparin and by our observation that no visual clearing of the plasma occurred in the two nephrotic patients who showed a great release of non-esterified fatty acids. It seems that the levels of the serum albumin encountered even in patients with severe hypoalbuminaemia do not affect the rate of the lipolytic reaction, a view which is in keeping with that of Baker (1957).

SUMMARY

The intravenous injection of heparin under fasting conditions causes a rise in plasma non-esterified fatty acids in normal subjects and in patients with nephrosis or diabetes. The rise is higher if heparin is given in the presence of an alimentary lipaemia.

In fasting patients with portal cirrhosis, the plasma non-esterified fatty acids fail to rise after heparin and may even fall. In cirrhotic patients with

an alimentary lipaemia, the response of the plasma non-esterified fatty acids to heparin is variable.

No endogenous lipolytic activity could be demonstrated in normal plasma, but it was observed in the plasma of four out of five patients with portal cirrhosis.

It is suggested that the high levels of plasma non-esterified fatty acids found in cirrhotic patients are due to the increased lipolytic activity of the plasma. The lack of effect of heparin in fasting cirrhotic patients is thought to be due partly to a relative deficiency of lipid substrate in the plasma and partly to exhaustion of the stores of lipolytic apo-enzyme in the tissues.

This work was partly supported by a grant from the Endowment Fund of the United Birmingham Hospitals.

REFERENCES

Baker, S. P. (1957). Heparin-activated clearing factor. Standardized test, agewise application, and clinical observations. *Circulation*, **15**, 889-896.

——, Levine, H., Turner, L., and Dubin, A. (1958). Lipoprotein lipase response in Laennec's cirrhosis. *Proc. Soc. exp. Biol. (N.Y.)*, **99**, 670-672.

Block, W. J. Jr., Mann, F. D., and Barker, N. W. (1951). Effect of small doses of heparin in increasing the translucence of plasma during alimentary lipemia: studies in normal individuals and patients with atherosclerosis. *Proc. Mayo Clin.*, **26**, 246-249.

Carlson, L. A., and Pernow, B. (1959). Studies on blood lipids during exercise. I: arterial and venous plasma concentrations of unesterified fatty acids. *J. Lab. clin. Med.*, **53**, 833-841.

Chlouverakis, C., and Harris, P. (1960). Composition of the free fatty acid fraction in the plasma of human arterial blood. *Nature (Lond.)*, **188**, 1111-1112.

Cleland, W. W., and Iacono, J. M. (1957). Role of lipoprotein lipase in intravascular clearing of fat in human subjects. *Fed. Proc.*, **16**, 383.

Engelberg, H. (1956a). Human endogenous lipemia clearing activity. Studies of lipolysis and effects of inhibitors. *J. biol. Chem.*, **222**, 601-610.

—— (1956b). *Studies of circulating human endogenous lipoprotein lipase (heparin lipaemia clearing factor)*. Third Int. Conf. on Biochem. Problems of Lipids, pp. 367-378.

—— (1958). Human endogenous plasma lipemia clearing activity. Observations in 482 individuals. *J. appl. Physiol.*, **13**, 375-380.

Gates, H. S. Jr., and Gordon, R. S. Jr. (1958). Demonstration of lipoprotein lipase in fasting human serum. *Fed. Proc.*, **17**, 437.

Gordon, R. S. Jr., and Cherkes, A. (1956). Unesterified fatty acid in human blood plasma. *J. clin. Invest.*, **35**, 206-212.

——, ——, and Gates, H. (1957). Unesterified fatty acid in human blood plasma. II: The transport function of unesterified fatty acid. *Ibid.*, **36**, 810-815.

Grossman, M. I., Palm, L., Becker, G. H., and Moeller, H. C. (1954). Effect of lipemia and heparin on free fatty acid content of rat plasma. *Proc. Soc. exp. Biol. (N.Y.)*, **87**, 312-315.

Hahn, P. F. (1943). Abolishment of alimentary lipemia following injection of heparin. *Science*, **98**, 19-20.

Herzstein, J., Chun-I Wang, and Adlersberg, D. (1954). Effect of heparin on plasma lipid partition in man: studies in normal persons and in patients with coronary atherosclerosis, nephrosis and primary hyperlipemia. *Ann. intern. Med.*, **40**, 290-306.

Hollett, C., and Meng, H. C. (1956a). Comparison of fat emulsion clearing activities of postheparin plasma and pancreatic lipase. *Amer. J. Physiol.*, **184**, 428-432.

——, —— (1956b). *Purification and characterization of a lipaemic clearing factor inhibitor from normal plasma*. Third Int. Conf. on Biochem. Problems of Lipids, pp. 190-199.

Jaques, L. B. (1940). Heparinase. *J. biol. Chem.*, **133**, 445-451.

——, and Keeri-Szanto, E. (1952). Heparinase. II. Distribution of enzyme in various tissues and its action on natural heparins and certain synthetic anticoagulants. *Canad. J. med. Sci.*, **30**, 353-359.

Jeffries, G. H. (1954). The sites at which plasma clearing activity is produced and destroyed in the rat. *Quart. J. exp. Physiol.*, **39**, 261-270.

Korn, E. D. (1955). Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. *J. biol. Chem.*, **215**, 1-14.

Rosenman, R. H., and Byers, S. O. (1959). Study of possible causal role of lipoprotein lipase deficiency in nephrotic hyperlipemia. *J. clin. Invest.*, **38**, 1036.

——, and Friedman, M. (1957). *In vivo* studies of the role of albumin in endogenous and heparin-activated lipemia-clearing in nephrotic rats. *Ibid*, **36**, 700-705.

——, ——, and Byers, S. O. (1956). The causal role of plasma albumin deficiency in experimental nephrotic hyperlipemia and hypercholesteremia. *Ibid.*, **35**, 522-532.

Spitzer, J. A., and Spitzer, J. J. (1956). Effect of liver on lipolysis by normal and postheparin sera in the rat. *Amer. J. Physiol.*, **185**, 18-22.

Spitzer, J. J., and Roheim, P. S. (1958). Uptake and release of plasma unesterified fatty acids in the dog. *Fed. Proc.*, **17**, 155.

Stormont, J. M., and Mackie, J. E. (1959). An abnormality of non-esterified fatty acid metabolism in alcoholic patients with cirrhosis. *J. clin. Invest.*, **38**, 1047.

Wajchenberg, B. L., Hoxter, G., Mello, E. H. L., and Ulhoa Cintra, A. B. (1960). Non-esterified and esterified fatty acids in hepatocellular disease. *Lancet*, **1**, 1218-1219.