

protein found only in anemic animals or to something not a mucoprotein. Because of the proteolytic destruction of activity(2,9) it is likely that it is of peptide nature or has such material as a necessary cofactor. Association with a different protein could account for the different electrophoretic behavior of the urinary material. Activity might be present in normal plasma but may not be separable by the methods effective with anemic plasma.

Summary. The bulk of erythropoietic activity of anemic plasma can be concentrated in a fraction which represents less than 0.5% of plasma proteins. Yet from the similarity in yield and electrophoretic behavior of the corresponding inactive fraction from normal plasma, it appears likely that the erythropoietic factor constitutes only a small portion of the present concentrates.

1. Rambach, W. A., Alt, H. L., Cooper, J. A. D., *Blood*, 1957, v12, 1101.
2. Lowy, P. H., Keighley, G., Borsook, H., Graybiel, A., *Nature*, 1958, v181, 1802.
3. Lowy, P. H., Keighley, G., Borsook, H., Graybiel, A., *Blood*, 1958.
4. Grant, W. C., Linkenheimer, W. H., Berger, H., *Fed. Proc.*, 1958, v17, 59.
5. Winkert, J., Gordon, A. S., Piliero, S. J., Medici, P. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1958, v98, 351.
6. Van Dyke, D. C., Garcia, J. F., Lawrence, J. H., *ibid.*, 1957, v96, 541.
7. Borsook, H., Graybiel, A., Keighley, G., Windsor, E., *Joint Res. Rep. U. S. N. School Aviat. Med.*, 1953.
8. Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1957, v94, 237.
9. Slaunwhite, W. R., Jr., Mirand, E. A., Prentice, T. C., *ibid.*, 1957, v96, 616.

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Lipoprotein Lipase Response in Laennec's Cirrhosis.* (24458)

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Since the observation that heparin injected *in vivo* cleared alimentary lipemia in dogs(1), much work has been done in elucidating the mechanism of this phenomenon. It has subsequently been demonstrated that heparin-activated clearing factor is a plasma lipoprotein lipase which acts upon the triglyceride moiety of plasma lipoproteins to hydrolyze them to free fatty acid and glycerol(2,3). These studies have evoked increased efforts to relate concentration of this lipase to disorders of lipid metabolism(4), particularly atherosclerosis(4,5). In each instance, emphasis has been placed upon a *decreased* clearing factor or lipoprotein lipase response. Methods used in measuring lipoprotein lipase response or concentration have varied widely. Consequently, until recently, no attempt had been made to determine mean clearing factor response for a given "normal" population. A

standardized *in vitro* method has been developed for determining clearing factor concentration and has been applied to an agewise study of a presumably "normal" population (6). Inasmuch as no significant agewise regression was found in clearing factor response, a mean for this population was established. In this study, it was incidentally observed that patients with primary diagnoses of chronic alcoholism and/or Laennec's cirrhosis demonstrated a significantly *increased* clearing factor response(7). In view of the observation that there is a lesser incidence of atherosclerosis in patients diagnosed as Laennec's cirrhosis, more extensive studies have been made on a larger group of subjects reported herewith. In a small group of patients with recent myocardial infarction (presumptive evidence of coronary atherosclerosis) evaluated in the original study, no significant difference in clearing factor response was observed when tested against the normal popu-

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lation in the same age decades. The fact remains, however, that *none* of the "normal" subjects could be said to be absolutely free of atherosclerosis, particularly in ages 40 to 69 years studied. Consequently, for comparative purposes, and to evaluate their clearing factor response, patients with recent myocardial infarction have also been included in the group evaluated in this study.

Material and methods. Subjects. One hundred patients were selected without regard to age or sex from Medical Services of Cook County Hospital. Basis for selection was hepatic pathology of *any* etiology, jaundice, or recent myocardial infarction. One patient evaluated manifested the nephrotic syndrome. Twenty-nine patients demonstrated clinical and laboratory evidence of Laennec's cirrhosis. Heparin Sodium, 3 mg in 5 ml of isotonic saline[†] injected intravenously, was used as clearing factor (lipoprotein lipase) stimulus. *Lipoprotein lipase response.* The standardized *in vitro* Baker method for determination of clearing factor concentration(7) was employed. *Preparation of samples.* Fifteen milliliter control and 8-minute post-heparin blood samples were obtained from all subjects after overnight fast (at least 12 hours post-prandial). These samples were immediately mixed with dried sodium oxalate (1 mg/ml blood) and cooled to 0°C in ice bath. Plasma was separated by centrifugation at 0°C. It was subsequently kept at 0°C or frozen until used. *Substrate.* One % emulsion of coconut oil (Ediol)[‡] freshly prepared with distilled water was used as substrate. Three-tenths milliliter of this emulsion was added to and thoroughly mixed with a 4.5 ml aliquot of post-heparin plasma, which had been previously incubated for 10 minutes in water bath at 30° ± 1°C. This proportion, in general, gave an initial optical density reading between 0.6 and 0.7 against distilled water on Beckman Model DU Spectrophotometer, with microcuvettes (2.5 x 10 x 25 mm) at 6500 Å. The post-heparin plasma-

TABLE I. Increased Lipoprotein Lipase Response in Laennec's Cirrhosis.

Diagnosis	No. of subjects	Mean <i>k</i>	Range of <i>k</i>
Laennec's cirrhosis	29	1.16	.37-2.25
"Normal"	84	.74	.13-1.40
Infectious hepatitis	11	.58	.00-2.12
Recent myocardial infarction	12	.58	.06-1.24
Obstructive jaundice	6	.33	.00-.86

k is rate of decrease of optical density/hr. This is directly proportional to lipoprotein lipase (clearing factor) concentration in standardized *in vitro* assay method(6,7).

fat emulsion mixture was kept at 30°C for one hour. During this time, optical densities were read every 5 minutes on 0.3 ml aliquots of the mixture. Optical densities of control plasma-fat emulsion mixtures were similarly determined. All samples were run in duplicate. *Clearing factor (lipoprotein lipase) concentration.* A graph of optical density versus time was then plotted for each sample. To this plot, a straight line was fitted at the section demonstrating greatest rate of change in optical density. The negative slope of this line, *k* (decrease in optical density/hour), was taken to represent rate of clearing of post-heparin plasma, which, in turn, is directly proportional to lipoprotein lipase concentration. *Evaluation of hepatic status.* Comprehensive laboratory tests on aliquots of control blood samples included total and esterified cholesterol, serum bilirubin, icterus index, BSP, total proteins, albumin/globulin ratio, cephalin flocculation, thymol turbidity, gamma globulin turbidity, and alkaline phosphatase. Total serum lipids and lipid phosphorus were also determined on aliquots of control blood samples.

Results. These studies have confirmed the presence of an *increased* clearing factor or lipoprotein lipase response in Laennec's cirrhosis. Table I demonstrates the increased clearing factor or lipoprotein lipase response in patients with Laennec's cirrhosis as compared with "normal" subjects, patients with infectious hepatitis, recent myocardial infarction, and obstructive jaundice. As described in the original study(6,7), *k* (decrease in optical density/hour) is directly proportional to clearing factor or lipoprotein lipase concentration. Clearing factor response was evalu-

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ated in terms of total serum lipids and lipid phosphorus, the former being generally low (below 600 mg/100 ml) in Laennec's cirrhosis. There appeared to be no correlation, however, between clearing factor response and levels of total serum lipids or lipid phosphorus in Laennec's cirrhosis. Serum total cholesterol generally ranged below 200 mg/100 ml. Per cent of serum cholesterol esters was almost uniformly low, being below 50% (below 75 mg/100 ml).

Clearing factor response and serum cholesterol/lipid phosphorus ratio in Laennec's cirrhosis are being more extensively investigated. When compared with Laennec's cirrhotics, a small group of patients with recent myocardial infarction demonstrated a higher cholesterol/lipid phosphorus ratio with a lower lipoprotein lipase response.

It has been observed that the hypoalbuminemia of the nephrotic syndrome markedly *decreases* clearing factor response(8) and that this decrease may be corrected by infusion of serum albumin. A single case of nephrotic syndrome (serum albumin 2.8 g/100 ml) demonstrated an almost negligible clearing factor response, which is in accordance with these observations. In patients with Laennec's cirrhosis, however, hypoalbuminemia produced *no depressant effect* upon clearing factor response, the greatest response being observed with serum albumin below 3.0 g/100 ml.

Lipoprotein lipase response in patients with other types of hepatic pathology such as infectious hepatitis, homologous serum jaundice, and obstructive jaundice has been variable. Observations on relatively small groups of patients to date permit no conclusions at this point. However, it may generally be stated that clearing factor response in these diseases does *not* appear to be elevated as in Laennec's cirrhosis.

Discussion. Perfusion experiments in rats have demonstrated that normal rat liver inactivates or attenuates clearing factor(9). It is, therefore, suggested that the *increased* clearing factor or lipoprotein lipase response in patients with Laennec's cirrhosis is related to their parenchymal liver cell damage. The intriguing possibility that parenchymal liver cell damage, either acute or chronic, may be reflected by concentration of heparin-activated lipoprotein lipase in plasma is currently being investigated. The possible inter-relationship between clearing factor response, cholesterol/lipid phosphorus ratio, lipid metabolism and atherosclerosis is also being explored.

Summary. 1. *Increased* lipoprotein lipase response has been observed in patients with Laennec's cirrhosis. 2. When compared with Laennec's cirrhotics, a small group of patients with recent myocardial infarction (presumptive evidence of coronary atherosclerosis) demonstrated a higher serum cholesterol/lipid phosphorus ratio with a lower lipoprotein lipase response. 3. Hypoalbuminemia in Laennec's cirrhosis produced *no depressant effect* on lipoprotein lipase response. 4. Other types of hepatic pathology and jaundice have demonstrated a variable lipoprotein lipase response, but *no apparent increase*.

1. Hahn, P. F., *Science*, 1943, v98, 19.
2. Korn, E. D., *J. Biol. Chem.*, 1955, v215, 1.
3. Korn, E. D., *ibid.*, 1955, v215, 15.
4. Herzstein, J., Wang, Chun-I, Adlersberg, D., *Ann. Int. Med.*, 1954, v40, 290.
5. Woldow, A., Chapman, J. E., Evans, J. M., *Am. Heart J.*, 1954, v47, 568.
6. Baker, S. P., *Circulation*, 1956, v14, 496.
7. ———, *ibid.*, 1957, v15, 889.
8. Rosenman, R. H., Friedman, M., *J. Clin. Invest.*, 1957, v36, 700.
9. Jeffries, G. H., *Quart. J. Exp. Physiol.*, (London), 1954, v39, 261.

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