5. Hammerman, D., and Hatch, F. T., *ibid.*, 1955, v89, 279.

6. King, J. S. Jr., and Hyder, N., *ibid.*, 1955, v89, 342.

7. Boas, N. F., *J. Biol. Chem.*, 1953, v204, 553.

8. Weiner, R., *et al.*, *J. Clin. Endocrinol. and Metab.*, 1955, v15, 1131.

9. Boas, N. F., submitted for publication.

10. ———, to be published.

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Unesterified Fatty Acids and Lipid Transport in Dogs.* (22406)

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This study was undertaken to investigate the relative importance of two lipid transport mechanisms, lipoproteins and unesterified fatty acids.

Materials and methods. Alimentary lipemia was produced in male, mongrel dogs by feeding 40 ml of cod liver oil. Blood samples were oxalated, promptly refrigerated, and centrifuged in the cold. Unesterified fatty acids were determined by the method of Davis(1) as modified by Grossman(2). Esterified fatty acids were estimated by the procedure of Stern and Shapiro(3). The sugar content of the plasma was determined by the method of Somogyi(4) with Nelson's colorimetric adaptation(5). All analyses were in duplicate.

Results. During absorption, most fats are transported in the blood stream as neutral lipids. It seemed important to know whether under these conditions the dog also uses the other common transport mechanism, unesterified fatty acids. Fig. 1 presents one of the experiments in which esterified and unesterified fatty acids were determined simultaneously during fat absorption. Both esterified and unesterified fatty acids increase in concentration, the latter usually somewhat more slowly. The fasting dog (Fig. 1) fails to show these changes.

It has been shown(6-8) that the injection of heparin raises the level of unesterified fatty acids in the blood. We undertook to learn whether protamine reverses this action. Fig. 2 shows the rise of the unesterified fatty-acid level following the injection of heparin into lipemic dogs, the appearance of clearing factor,† and the lowering of the esterified fatty-acid level of the plasma.‡ All 3 effects can be detected in the blood from the femoral artery and vein, right side of the heart, hepatic, portal, renal, and jugular veins. The administration of protamine to these animals, subsequent to heparin, has the opposite effect, a decrease in the unesterified and a rise in the esterified fatty-acid levels and the disappearance of clearing activity from the plasma. It can also be seen that the complete disappearance of clearing activity from the plasma does not occur immediately after the injection of protamine. A definite, but decreased, clearing activity persists for 2 to 3 minutes. This suggests that the *in vivo* inactivation of clearing factor by protamine may be more complicated than a simple binding of heparin. These experiments imply that the action of heparin and of protamine involves both lipid transport mechanisms, esterified and unesterified fatty acids.

Since unesterified fatty acid transport is probably of secondary importance during fat absorption when lipids of exogenous origin travel in the blood stream, it seemed important to investigate conditions under which endogenous lipids are transported in the blood.

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† These effects can also be demonstrated in nonlipemic dogs and in dogs fasting for 2 to 3 days.

‡ "Clearing activity of the plasma," as shown in the Figure, is the clearing in optical density units of 1 ml of a cottonseed-oil emulsion by 0.5 ml of plasma after 5 hours' incubation at room temperature.

One such condition is prolonged starvation. Fig. 3 is a representative experiment in which 48 to 72 hours of starvation caused a marked elevation of the unesterified fatty-acid level

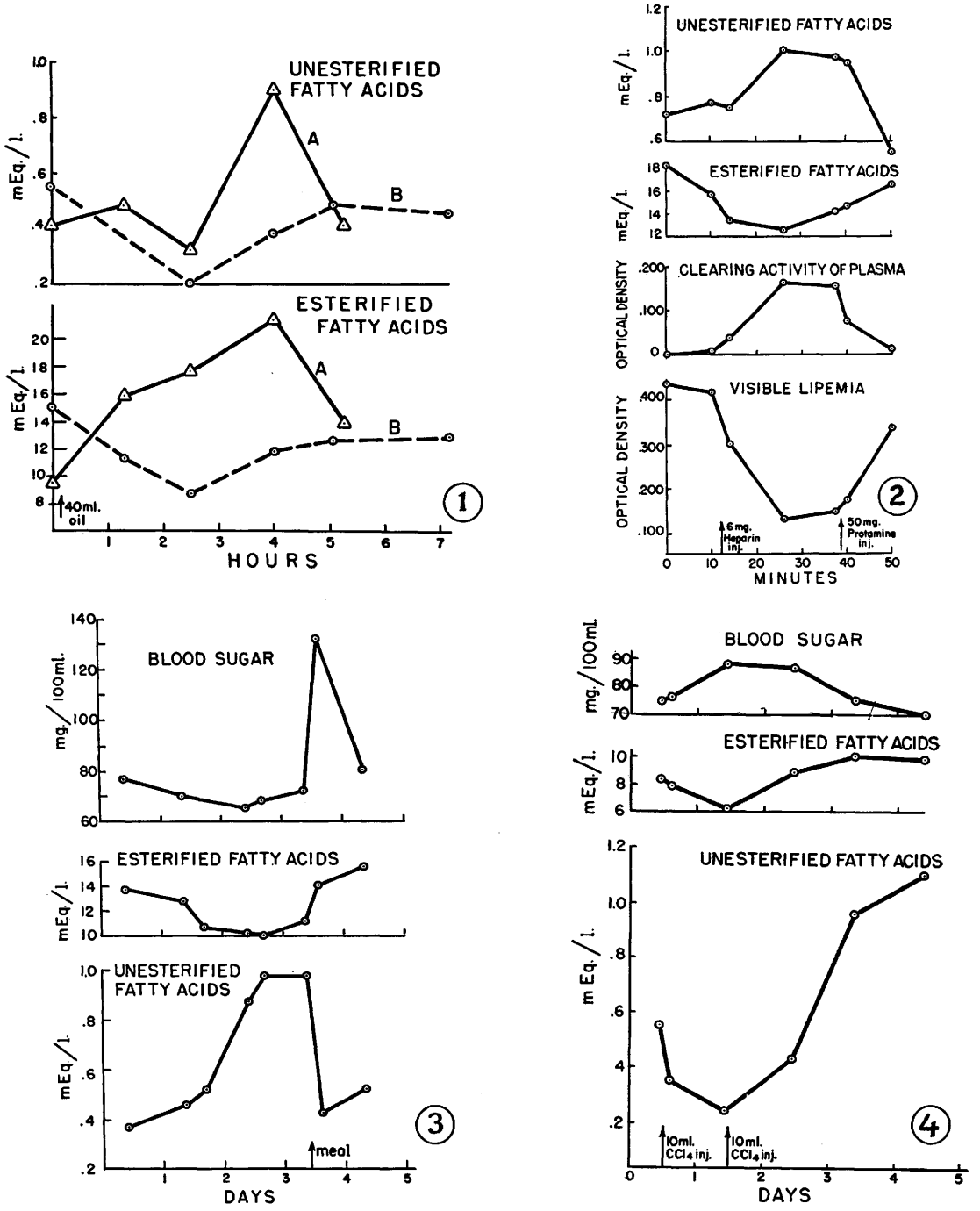

FIG. 1. Changes in unesterified and esterified fatty-acid concentrations of a fat-absorbing (A) and a fasting (B) dog.

FIG. 2. Effects of heparin and protamine in a lipemic dog.

FIG. 3. Sugar, esterified, and unesterified fatty-acid levels in plasma of a fasting dog.

FIG. 4. Sugar and fatty-acid concentration in plasma of a dog injected with CCl_4.

of the plasma, a slight decrease in the amount of the esterified fatty acid, and a very slight hypoglycemia. After feeding, all returned to normal.

Acute carbon tetrachloride poisoning was selected as another condition accompanied by considerable endogenous fat transport. The animals received injections of 10 ml of CCl_4 subcutaneously on two consecutive days, which resulted in an initial drop followed by a marked increase in the unesterified fatty-acid level of the plasma. A small increase in the amount of esterified fatty acids also occurred. Fig. 4 is a representative experiment. There was histologic evidence of typical acute lesions in the liver.

Discussion. During alimentary lipemia, both esterified fats and unesterified fatty acids increase. Conditions may be different in animals absorbing a mixed meal rather than only lipids. Dole(9) and Gordon and Cherkes(10) observed that ingestion of carbohydrates lowers the plasma unesterified fatty-acid level. This is also suggested by our occasional observation that dogs may have very low unesterified fatty-acid levels after the ingestion of a complete meal even though lipemia occurs. Results of the present investigations on the increase in unesterified fatty acids during lipemia are in accord with Grossman's findings for rats and humans(2,11).

During starvation, the total plasma lipid does not change in the dog as it does in some other species(12,13). It has long been suspected that the partition of fats changes under these circumstances(12). Our results show that fasting dogs transport fat primarily as unesterified fatty acids. In dogs poisoned with CCl_4 as in rats(14) a similar transport seems to occur.

An arterio-venous difference in unesterified fatty acids occurred in the majority of animals in the present studies. The lack of a greater consistency in the finding might reflect dynamic changes in unesterified fatty acids, especially of fat-absorbing animals.

These investigations, together with others in progress, indicate that when lipids are used as readily available energy source or are transported in the course of other metabolic functions, a portion of the lipid is transported and utilized as unesterified fatty acids. Alternatively, the esterified fats may undergo lipolysis followed by use in this form. In either case, clearing factor or a similar enzyme may play a decisive role in the metabolic channeling of lipids.

Summary. Plasma neutral fats and unesterified fatty acids were investigated in different conditions of increased lipid transport in the dog. During fat absorption, in addition to the increase in neutral fats, unesterified fatty acid also increases. Heparin and protamine influence both transport mechanisms in the post absorptive state, the former increasing the unesterified fatty acids and decreasing the esterified fatty acids; the latter opposing this effect. Starvation for from 48 to 72 hours and acute CCl_4 poisoning raise the unesterified fatty-acid level markedly. The results indicate the metabolic importance of unesterified fatty acids in lipid transport and the possible role of clearing factor in regulating lipid metabolism.

1. Davis, B. D., *Arch. Biochem.*, 1947, v15, 351.

2. Grossman, M. I., Palm, L., Becker, G. H., and Moeller, H. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 312.

3. Stern, I., and Shapiro, B., *J. Clin. Path.*, 1953, v6, 158.

4. Somogyi, M., *J. Biol. Chem.*, 1945, v160, 61, 69.

5. Nelson, N., *ibid.*, 1944, v153, 375.

6. Shore, B., Nichols, A. V., and Freeman, N. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 216.

7. Robinson, D. S., and French, J. E., *Quart. J. Exp. Physiol.*, 1953, v38, 233.

8. Brown, R. K., Boyle, E., and Anfinsen, C. B., *J. Biol. Chem.*, 1953, v204, 423.

9. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.

10. Gordon, R. S., and Cherkes, A., *ibid.*, 1956, v35, 206.

11. Grossman, M. I., Moeller, H. C., and Palm, L., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 106.

12. Mansfeld, G., *Pflüger's Arch. f. d. ges. Physiol.*, 1909, v129, 46.

13. Deuel, H. J., *Lipids. Their Chemistry and Biochemistry*, Interscience Publishers Inc., New York, 1955, v2, 420.

14. Selden, G. L., and Westphal, U., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 159.

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