and of Ca^{47} in saliva, the ratio of saliva concentration to plasma concentration varied from 1/5 to 1/3 for Sr^{85} and from 2/5 to almost unity for Ca^{47}. The average ratios favored secretion of Ca^{47} by a factor of more than 2, the difference in secretion of the 2 isotopes being highly significant. Values for Ca^{47} were in accord with the levels of stable calcium found by us in saliva and previously reported by others. No difference in Sr^{85}/Ca^{47} ratios of saliva in relation to plasma was found between patients on low and on high Ca intake. The high degree of discrimination of the salivary glands against the secretion of Sr^{85} contrasts with the action of the kidneys in favoring secretion of Sr^{85} over that of radiocalcium by a factor of approximately 3 to 1.

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A Plasma Factor Capable of Altering Triglyceride.* (29959)

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A technique for thin-layer chromatography of blood lipids, recently reported from this laboratory, described 2 triglyceride fractions of different mobilities(1). Further studies of the possible origin of these fractions resulted in the finding of a hitherto unknown activity in human plasma capable of altering the Rf-value of added triglyceride on the thin-layer plates.

Materials and methods. Venous blood in the post-absorptive state was collected from 5 normal individuals and 12 patients with disorders of lipid metabolism into heparinized test tubes which were immediately placed in an ice-bath, centrifuged and sampled as rapidly as possible. One ml of each plasma sample was added to a 25 ml volumetric flask, approximately half-filled with Bloor's reagent (ethyl alcohol:ethyl ether, 3:1), following which one drop of triolein† (approximately 12 mg) was added from a capillary pipette. Plasma alone and triolein alone were similarly treated. The mixtures were shaken for 10 minutes at room temperature in a shaking machine, made up to mark and filtered. Ten ml of each filtrate was evaporated to dryness and taken up in 2 ml chloroform. Ten μl of each were sampled for thin-layer chromatography in a system which uses as solvents, first, n-propanol:NH_4 OH for 3 cm, and then, chloroform:benzene to 10 cm, as previously described(1).

In other experiments, the protocol was altered 1) by varying the plasma sample per 25 ml of Bloor's reagent from 0.01 ml to 1 ml, with the difference between the volume used and 1 ml made up with saline, 2) by varying the amount of Bloor's extract derived from the plasma from 0.5 ml to 15 ml with the volume adjusted to 15 ml with Bloor's

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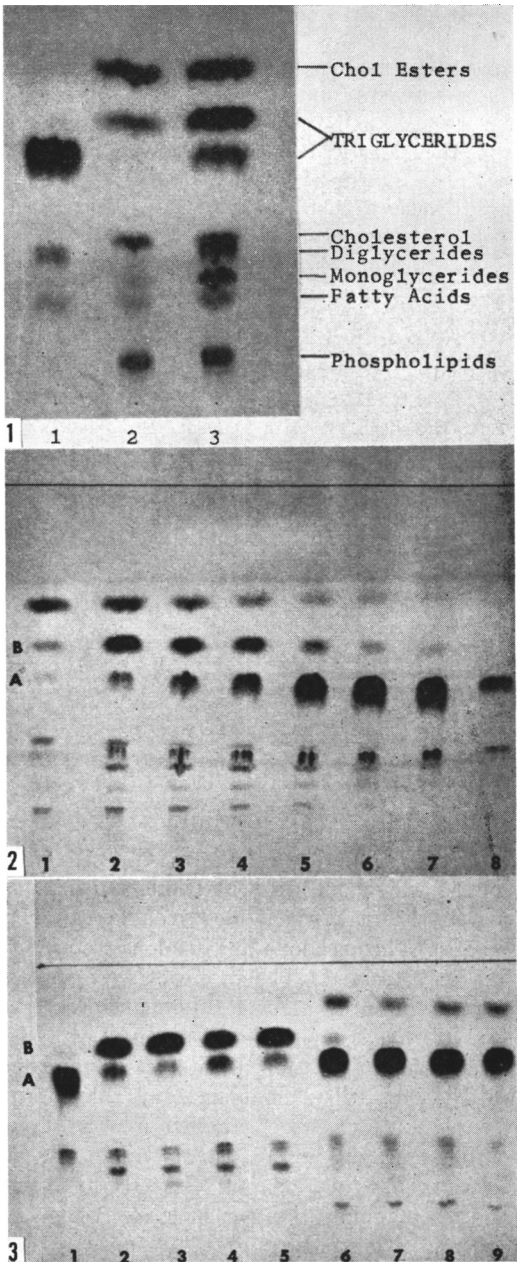

FIG. 1. Effect of Bloor's extract of plasma on Rf value of triolein. (1) triolein, (2) plasma extract, (3) triolein plus plasma extract. Note increase in triglyceride Rf value produced by plasma extract.

FIG. 2. Dose-response effect of decreasing amounts of plasma on triolein shift. (1) 1 ml plasma, (8) triolein reference spot, (2-7) 1 drop of triolein plus (2) 1 ml, (3) 0.5 ml, (4) 0.25 ml, (5) 0.10 ml, (6) 0.05 ml and (7) 0.01 ml plasma. Note progressive decrease in amount of triolein altered (A→B) as amount of plasma used decreases.

FIG. 3. Effect of plasma ultrafiltrates and residues on triolein shift. (1) triolein reference, (2-5) triolein plus ultrafiltrates of 4 subjects, (6-9) triolein plus plasma residues of the same 4 subjects. Note that shifting activity is exclusively in ultrafiltrates.

reagent or 3) by varying the concentration of triolein added from 1 mg to 20 mg.

Starch block electrophoresis was performed on 4 ml of fresh plasma according to the method of Kunkel(2), after which the separated protein fractions were dialyzed against 25% PVP and the residue (approximately ½ ml) was sampled into the Bloor's reagent as described above. Ultrafiltrates were prepared in the cold by nitrogen pressure filtration (10 lb/sq in) through a cellophane dialysis bag.‡ The residues within the bag and the ultrafiltrates were sampled and triolein added as described.

Results. Fig. 1 illustrates the changes observed. When the triolein was added to the plasma in Bloor's reagent, an increase in Rf-value of a portion of the added triglyceride occurred. The faster moving component migrated with the faster moving of the 2 triglyceride fractions in plasma. The alteration was associated with an increase in diglycerides, monoglycerides and fatty acids. The diglycerides and monoglycerides originally present as a contaminant in the triolein are evident.

In experiments in which the amount of plasma used varied, 1 ml of plasma resulted in almost a complete shift of the added triolein toward the solvent front whereas 0.01 ml failed to produce any triolein change. Between these 2 concentrations were progressive gradations in amount of triolein shifted which were directly proportional to the amount of plasma used. Progressive decreases in the amount of protein-free Bloor's extract added produced similar changes. Changing the concentration of triolein from 1 mg to 20 mg while maintaining a fixed amount (10 ml) of extract clearly showed a gradual progression from a complete shift in the lower concentrations to a point where progressively greater amounts of the added triolein did not shift. It appeared that at a concentration of approximately 12 mg, the

‡ Dialyzing tubing, Visking.

maximum amount of triolein which could be altered by this amount of extract was reached, and increasing the amount of triolein added merely increased the unaltered triolein which could be visualized in the lower of the 2 triglyceride spots. Fig. 2 illustrates the effect of varying the amount of plasma and keeping the triolein concentration constant. Serum obtained after blood clotting produced changes in the added triolein which were similar to plasma.

The triolein "shifting" activity could not be found in any of the protein fractions isolated by starch block electrophoresis and it was assumed that the factor causing the shift either migrated to an electrode bath or diffused into the PVP, either of which would have diluted the activity so that it could not be demonstrated.

Ultrafiltration clearly demonstrated the activity in the ultrafiltrate and its absence in the residue remaining within the cellophane bag (Fig. 3). The factor, therefore, is not protein-bound and is readily diffusible. It is also evident from this experiment that the activity is not present in the plasma lipoproteins, which remain within the bag.

Discussion. The fact that the activity occurs in non-aqueous organic solvents, and is present in protein-free ultrafiltrates suggests that the phenomenon is non-enzymatic. The increase in Rf value of the triglyceride may represent a dehydrogenation reaction, although to our knowledge, this has been described only with fatty acids in the presence of rigidly controlled substrates(3). A more likely mechanism may be the formation of an optical or geometric isomer. Geometric isomers have been described after ozonization of fatty acids(4). Naturally occurring triglycerides possessing optical activity in the glycerol moiety capable of resolution on thin-layer plates have been described recently(5).

Further characterization of the factor producing the shift, the exact nature of the change produced in the triglyceride molecule, and quantitative variations in shifting ability of plasma of different subjects are as yet unknown, but are under study.

Summary. A hitherto unknown factor has been found in human blood plasma capable of producing an increase in the Rf value of triglyceride in thin-layer chromatography. It has been shown that the factor is not protein-bound, appears in ultrafiltrates of plasma, and shows quantitative dose-response relationships. The activity is considered non-enzymatic.

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"Masked" Granulocytosis.* (29960)

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Blood granulocytosis, not reflected by an increased granulocyte concentration in venous blood samples, is described herein.

In 1843, Addison observed leukocytes in a marginal position along the walls of small venules(1). In 1938, Vejlens reported experiments designed to evaluate possible mechanisms leading to this margination(2). The concept that all granulocytes within the confines of the vascular system are not freely circulating was affirmed in this laboratory by labeling granulocytes *in vitro* with radioactive diisopropylfluorophosphate (DFP^{32}) and returning them to the circulation of the donor

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