Effects of Fat Meals and Heparin on Blood Plasma Composition as Shown by Paper Chromatography.* (18367)

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It has been shown in another study(1) that 6 to 9 hours after a large fat meal, and immediately after intravenous injections of a small amount of heparin the chylomicra and red blood cells have a tendency to aggregate. These changes were frequently attended by a decrease in the hematocrit, and by an increase in the sedimentation rate in non-lipemic plasma and a decrease in the sedimentation rate in lipemic plasma. The maximum aggregation of the chylomicra and red blood cells after a fat meal did not develop 3 hours after the fat meal when the chylomicra were most numerous, but 6 to 9 hours afterwards when the number of chylomicra were rapidly decreasing(1). Because of the possibility that this alteration of the suspension stability of the blood was due to factors which are protein in nature, preliminary studies of the total proteins, albumin, globulin and fibrinogen were carried out. No significant changes in these substances were found apart from a slight, but consistent reduction (about 0.25 g %) in the total proteins.‡

The recent application of the paper chromatographic technic to the study of plasma proteins(2-4) gives us a new tool which is both sensitive and easily applied to a study of blood plasma. With this method it has been possible to show changes in the proteins which correlate with the other changes in the blood described above(1). The application of paper chromatography to the study of proteins has been briefly reviewed in another paper(3).

Materials and methods. Two male and 4 female dogs weighing 10 to 21 kg were studied. A total of 23 experiments were performed in these 6 animals. Cream fat (relatively saturated) and a mixture of cod liver oil and linseed oil (relatively unsaturated) were tested; the test meals consisted of 4 g of fat/kg body weight. The maintenance diet of the dogs was beef heart and fox chow. Prior to the fat tests all dogs were fasted 18 hours unless otherwise indicated. All bloods were withdrawn from one of the leg veins and prevented from clotting by heparin-sodium salt (Connaught Laboratories). Occasionally isotonic ammonium and potassium oxalate were used as anticoagulants to provide controls on the heparin. 0.3 ml heparin (10 mg/ml) was injected intravenously. Observations were made of the number of chylomicra, the hematocrit, sedimentation rates, protein fractions (total protein, albumin, globulin and fibrinogen), and non-protein nitrogen. In the present experiments the hematocrit and sedimentation rates were used to indicate changes in the aggregation tendency of the red blood cells because these measurements are probably more dependable and less subjective than the changes observed in dark field illumination(1). The method of paper chromatography used in this study has been described in detail by Franklin and Quastel(3). Four sets of conditions were used to examine the plasma. One condition did not include the surface active agent, and the others used 0.02, and 0.03 ml Tween 81, and 0.03 Tween 85 per 0.5 ml of the test plasma.§

Results. The effect of intravenous administration of heparin in fasting dogs was

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1. Swank, R. L., *Am. J. Physiol.*, in press, Feb. 1951.

‡ It was also noted that the non-protein nitrogen fell 20% to 60% in 5 of the 6 experiments.

2. Franklin, A. E., and Quastel, J. H., *Science*, 1949, v110, 447.

3. Franklin, A. E., and Quastel, J. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 803.

4. Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, 1950, v111, 605.

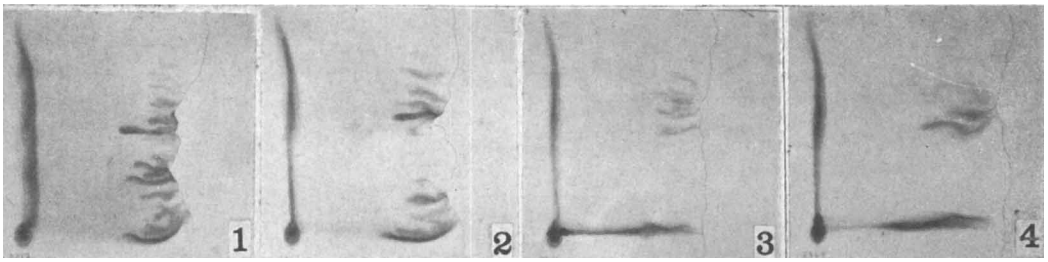

FIG. 1. Comparison between protein chromatograms of fasting dog plasma (No. 1), 6 hr after a high cream fat meal (No. 2) and 10 min. later after intravenous heparin (No. 3). No. 4 shows the protein pattern after intravenous heparin to the same dog after fasting.

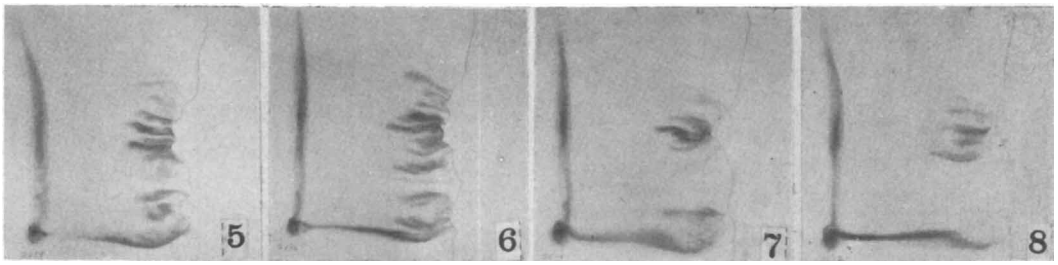

FIG. 2. Differences between fasting plasma (No. 5), 3 hr (No. 6), 6 hr (No. 7) and 9 hr (No. 8) after feeding of a high cream fat meal.

observed in 4 dogs, and 5 tests were made. In every instance a significant change in the protein pattern of the paper chromatograms was observed. The changes under one set of conditions in 1 dog are shown in Fig. 1 (Nos. 1 and 4). In these experiments the hematocrit fell from 2 to 4 points and the sedimentation rate usually increased(1). One additional experiment showed that the changed protein patterns observed in the plasma after intravenous heparin may return to normal in less than one hour. In this experiment the initial changes in the red blood cells and chylomicra were slight. This was frequently the case(1) when the dogs had been fasted for 24 to 36 hours.

Effect of a large cream fat meal with subsequent heparin administration was observed in 6 dogs on 8 occasions. Six hours after the fat meal, 7 plasmas showed definite paper chromatographic changes, presumably due to the fat absorption alone. In all 8 tests, heparin was injected 6 hours after the fat meal was given, and post-heparin samples of plasma were obtained 10 minutes later. All post-heparin bloods exhibited further alterations in the plasma protein patterns. The paper chromatograms for 1 such test are shown in Fig. 1 (Nos. 1, 2, 3). It is interesting to note that the changes produced by fat absorption alone appear to be intermediate between the fasting and post-heparin records in these experiments. The hematocrit showed a progressive decrease of 2 to 5 points during each of these experiments. The maximum change in the sedimentation rate was a reduction from 28 to 3 mm. A reduction of the chylomicron counts was also observed after the administration of heparin (1,5).

The effects of single large fat meals alone were observed 5 times with 2 dogs. Plasmas were taken at 0, 3, 6, and 9 hours after the fat meal. In 2 experiments, cream fat, and in 3 others a mixture of cod liver oil and linseed oil were used. The 3 tests with the mixture of very unsaturated fats all showed changes in the protein patterns at 3 hours, with maximum changes at 6 hours. The

§ We are indebted to the Atlas Powder Co., Wilmington, Del. (per G. F. Sterne & Sons, Brantford, Ont.) for samples of Tween 81 (Polyoxyethylene sorbitan monooleate) and Tween 85 (Polyoxyethylene sorbitan trioleate).

5. Hahn, P. F., *Science*, 1944, v98, 19.

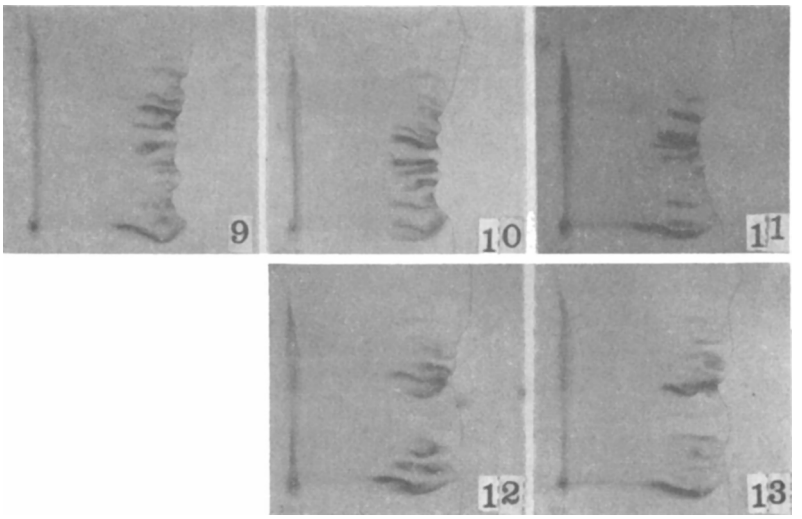

FIG. 3.
Effect of high speed centrifugation on the plasma of dogs which have been fed a high fat meal. No. 9 is the chromatogram of a fasting plasma, No. 10 of the clear fraction of the plasma of a 3 hr lipemia, No. 11 is the same at 6 hr, No. 12 is the lipemic portion at 3 hr and No. 13 is the same at 6 hr.

chromatograms returned to very nearly normal at 9 hours. With the relatively saturated fat one dog exhibited no change; the other exhibited marked changes at 6 and particularly at 9 hours after the fat meal (Fig. 2; Nos. 5, 6, 7, 8). The dog which exhibited marked changes in his plasma proteins exhibited also a drop in the hematocrit (from 50% to 44%) and an increase in sedimentation rate (from 3 mm to 9 mm) at 9 hours, at a time when the chylomicron count had returned about to normal. In the tests with unsaturated fat less marked changes were observed in the hematocrit and sedimentation rate.

The effects of separation of most of the chylomicrons from plasmas by centrifugation at about 13,000 r.p.m. (R.C.F. 20,000 x gravity) were observed in 2 experiments. This gave 2 plasma samples of equal volume, one clear and the other very cloudy, for protein analysis. In Fig. 3 it can be seen that the cloudy portion of plasma (Nos. 12, 13) exhibits definite and similar changes at both 3 and 6 hours, and that lesser changes are to be observed in the clear plasma at 6 hours (No. 11) and none at 3 hours (No. 10).

The effects of intravenous administration of Tween 81 were determined twice in the same dog. On both occasions 5 ml of Tween 81, diluted to 20 ml with distilled water, was injected; once with the dog fasting (Fig. 4; Nos. 14, 17) and subsequently 4 hours after a fat meal (Fig. 4; Nos. 14, 15, 16). During the first test the dog became listless, very weak, and collapsed a few minutes after injection of the Tween, but did not lose consciousness. During the next test, 2 weeks later, the dog was physically unaffected by the Tween injection, which was given at the presumed height of lipemia. In both tests the injection of Tween 81 altered the chromatograms remarkably. Due to the fact that the clustering of chylomicra was much greater in plasma than whole blood(1) red blood cells from 2 normal humans were washed with a volume of normal saline equal to the removed plasma, and chromatograms of this saline were made. A trace of a protein fraction was observed in each experiment, which was believed on the basis of later evidence¶ to be an albumin.

Discussion. The change in the protein pattern in the chromatograms described in this paper is probably not due to an overall reduction in the plasma proteins, since these

¶ Unpublished data (Franklin and Quastel).

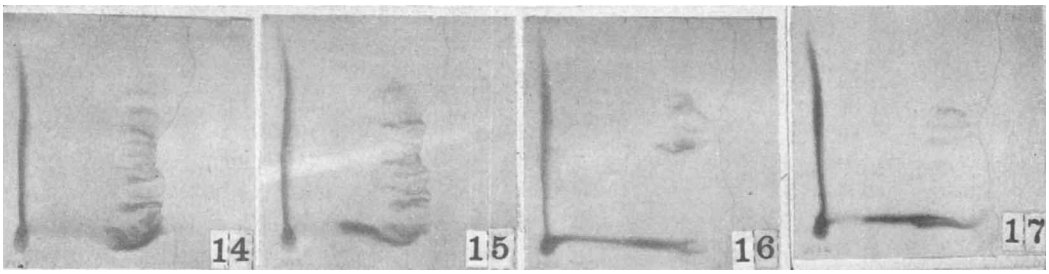

FIG. 4.

No. 14 is the protein chromatogram of a fasting dog plasma. No. 15 is a 6 hr lipemia, No. 16 is 10 min. after the intravenous injection of Tween 81 given after 6 hr of lipemia. No. 17 shows the effect of intravenous Tween 81 on a fasting dog.

NOTE: In all illustrations, 0.03 ml Tween 81 is mixed *in vitro* with 0.5 ml heparinized plasma, and 0.02 ml 0.3% hemin added. 0.02 ml aliquots are applied to the paper.

changed so little. More likely, specific fractions were affected selectively and in a way that altered the protein complex, and consequently its distribution in the chromatograms. The fact that the changes in the chromatograms may be accompanied by increased clumping of the red blood cells and chylomicra, by a decreased hematocrit, and by changes in the sedimentation rate raises the question as to whether the protein complexes in question are associated with the maintenance of the stability condition of the blood. The protein changes were sometimes attended by little or no evidence of physical change in the blood, such as no significant change in the hematocrit and sedimentation rate, indicating that there is a critical level for these protein complexes beyond which the emulsification of the blood elements becomes unstable. The absence of increases in the globulin and fibrinogen is of interest since these factors are increased under certain circumstances in which the sedimentation rate is also increased (6).

The similarity of the changes in the protein patterns, and in the physical characteristics of the blood, both after a high fat meal and after intravenous heparin, and the apparent cumulative nature of these changes suggest that their mechanisms are similar. It has already been suggested (1) that the chylomicra which appear in the blood stream after a fat meal may, by competing with the red blood cells, produce a deficiency in the so-called emulsification factors. This deficiency may last no longer than 3 to 6 hours as indicated by changes in the physical state of the blood (1), and in the protein pattern. The action of heparin is not clear, but it may be that this substance, either directly or indirectly, causes changes in the plasma by surface active forces resulting in alterations in the protein patterns. The duration of this change may be less than one hour. Anderson and Fawcett (7) have shown that the surface tension of plasma decreases after injection of heparin effective in clearing the lipemia. These authors ascribe this to the formation of a surface active heparin-phospholipid complex.

The paper chromatogram observations on lipemic plasma, subjected to very fast centrifugation, indicate that the changes in the protein patterns are not due to the presence of the chylomicra alone. It is also evident that a very marked lipemia produces additional changes in the paper chromatogram resulting in protein patterns similar to those seen after intravenous heparin. As further evidence that the presence of chylomicra is not the sole cause of the protein changes, it is to be noted that the maximum changes in the proteins and physical characteristics of the blood were observed in the cream fat test in one dog (Fig. 2) 9 hours after the fat meal, when the number of chylomicra had returned nearly to normal; at 3 hours when the lipemia was maximum no such changes in the protein

6. Fahraeus, H., *Physiol. Rev.*, 1929, v9, 241.

7. Anderson, N. G., and Fawcett, B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 768.

patterns were observed.

The evidence would indicate that the administration of heparin, as well as a high fat meal, modifies the surface active substances in the plasma with the result that a marked change occurs in the protein pattern seen in the paper chromatograms. This is noted also in the effects due to administration of a surface active agent such as Tween 81. It has been shown recently(8) that heparin inhibits the action of hyaluronidase, possibly by competition with hyaluronic acid for the enzyme. Conceivably the change in the protein chromatogram of a plasma after heparin administration is due to some similar association with a protein fraction.

8. Astrup, T., and Alkjaersy, N., *Nature*, 1950, v166, 568.

Summary. 1. Changes in the plasma protein patterns of dogs after high fat meals, and after the intravenous administration of heparin or Tween 81, have been demonstrated, using the paper chromatographic technic of Franklin and Quastel. 2. Concurrent reductions in the hematocrit, and changes in the sedimentation rate were observed after these procedures. The chylomicron counts were decreased after intravenous heparin. 3. The evidence indicates that the surface active substances present in the blood are modified after the above administrations, thus affecting the protein patterns of the paper chromatograms.

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Role of Cortisone in Regulation of Inflammation.*† (18368)

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It is well established that certain generalized reactions such as lymphocytolysis(1), lymphocytopenia(2) and eosinopenia(3,4), which occur following the application of noxious stimuli, are mediated by the secretion of the adrenal cortex. However, the role of the secretion of the adrenal cortex in localized host reactions elicited by topically applied stimuli has not yet been evaluated. Outstanding among these local reactions is the process of inflammation. The term "inflammation" is used here to denote only the local reactions that occur when an injurious agent acts topically upon tissues. It is the object of this study to secure information on the influence of the secretions of the adrenal cortex upon each of the various component events composing the inflammatory response. A stimulating agent was chosen which was not only known from prior studies to produce typical inflammatory alterations, but which has also been implicated in the etiology of connective tissue disease. To eliminate the influence of endogenous hormone secretion, which is difficult to estimate, adrenalectomized animals were used. The involvement of secretion of the adrenal cortex was then studied by comparing the inflammatory reaction of untreated adrenalectomized animals with that of adrenalectomized animals treated with a standard dose of cortisone acetate (Cortone acetate, Merck & Co.).

Materials and methods. Approximately 150 CBA mice 16 weeks of age, of both sexes,

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1. Dougherty, T. F., and White, A., *Am. J. Anat.*, 1945, v77, 81.

2. Dougherty, T. F., and White, A., *J. Lab. and Clin. Med.*, 1947, v32, 584.

3. Hills, A. G., Forsham, P. H., and Finch, C. A., *Blood*, 1948, v3, 755.

4. Thorn, G. W., *The Diagnosis and Treatment of Adrenal Insufficiency*, Charles C. Thomas, 1940.