TABLE II. Differential Staining of Heat-Altered* Collagen by Azo Dyes.

Dye	C.I. No.	No. of chemical groups Azo	$SO_3^=$	Differential stain
Orange I	150	1	1	+
II	151	1	1	+
III	142	1	1	+
IV	143	1	1	+
G	127	1	2	+
Amaranth	184	1	3	+
Sudan III	248	2	0	—
Biebrich scarlet	280	2	2	+
Chlorazol black E	581	3	2	—

* Heat exposure 70°C or more. Specimens excised 90 sec. after heating.

collagen on the sections was deep blue and similar to that seen in the untreated controls. In both portions, all remaining collagen stained blue. These observations served to verify the specificity of the Orange G for staining heat-altered areas of collagen.

In addition, the effect of trypsin further partitions the heat-induced alterations of collagen. In sections failing to stain differentially with Orange G (*e.g.*, 60°C), there was, nevertheless, a loss of collagen substance by enzyme treatment. This effect perhaps demonstrates collagen that has been sufficiently altered by the thermal exposure to render it hydrolyzable by trypsin but still quantitatively not degraded enough to yield the Orange G aqueous soluble products formed at higher temperatures or times of exposure.

Preliminary studies of the specificity of the azo dye uptake in methanol solutions are presented in Table II. These results showed that mono-azo and di-azo dyes with mono, di, and, trisulphonated end-groups all stain heat-altered collagen differentially. The triazo, disulphonated dye, chlorazol black E, failed to stain the heat-altered collagen. In this situation, the heated area was completely unstained. Non-sulphonated azo dyes did not stain collagen at all, while the trisulphonated diazo dye, amaranth, appeared to differentially stain heat-altered collagen, even more intensely than Orange G (di-azo, disulphonated).

These preliminary studies are presented at this time because of their possible usefulness in the understanding of collagen degradation during thermal injury.

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Circulatory Changes in the Hamster's Cheek Pouch Associated with Alimentary Lipemia.* (20123)

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Swank(1) showed that the red blood cells in humans and dogs, when observed in whole blood smears in dark field illumination, have a tendency to aggregate, be adhesive to one another, and be distorted 4 to 9 hours after a large fat meal (2 to 4 g/kg). These changes in the suspension stability of the blood were usually first detected about an hour after the chylomicron response had passed its peak, and they increased, sometimes becoming very marked, as the the visible lipemia lessened. With complete clearing of the visible lipemia 9 to 12 hours after the fat meal the suspension stability of the blood returned to normal. The altered suspension stability of the red blood cells was accompanied by a tendency of the chylomicrons to aggregate, by changes in the sedimentation rate and hematocrit(1), and by alterations in the pattern of paper chromatograms (Swank, Franklin and Quastel)(2).

In the present paper the effects of large fat meals on the intact circulation of the blood

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in the cheek pouch of the golden hamster will be reported.

Material and methods. Golden hamsters weighing 70 to 100 g were fed via stomach tube cream fat meals varying in size from 4 to 15 g kg body weight. At variable intervals after the fat meal the animals were anesthetized with 20% urethane intraperitoneally and one cheek pouch was inverted and prepared for visualization by the method of Fulton, Jackson and Lutz(3). In most cases one membrane of the pouch was removed, but for control purposes a number of pouches were studied with the membranes intact to determine the effect of the increased trauma and exposure occasioned by the removal of one membrane. The pouch was immersed in Ringer's solution and maintained at a temperature of 37.5°C. Observations were made at magnification of 750 X to 1350 X but for cinephotomicrography only the 50 X water immersion objective was used. In a few early studies black and white film was used for cinephotomicrography but usually the circulatory changes were recorded in color. The movies were all taken at 32 frames/second, but they were studied at 16 frames/second to allow better visualization of the blood elements. In a number of hamsters a small polyethylene tube was placed in the femoral vein so that injections could be made and frequent blood samples could be obtained. The blood samples were observed in dark field illumination to determine the chylomicron counts. For control purposes a number of animals were studied without inserting the tube. This study is based on observations in approximately 100 hamsters.

Results. A. Circulation in the cheek pouch of normal hamsters. Our control hamsters consumed a diet of mixed grain, vegetable greens, and fox chow (approximately 20% of the total calories of fox chow are furnished by fat: the vegetable greens and mixed grain which made up a large part of the diet contain very little fat). In none of our control studies of the cheek pouch of the hamster was stickiness or aggregation of the red blood cells noted. A variable amount of adhesiveness of the white blood cells to the walls of the blood vessels, and an occasional clump of platelets were observed. This usually increased after 3 or more hours of exposure and observation of the pouch, at which time petechial hemorrhages also began to appear. Occasional groups of red blood cells in rouleaux formation were also observed in normal pouches. These usually consisted of no more than 2 to 3 red blood cells, and the individual cells were clearly distinguishable and readily separated from one another when the flow of blood changed direction or was interrupted by a more rapid flow in anastomosing vessels. Even when the flow was greatly slowed and even stopped by the production of shock, by anaphylaxis, or by the injection of the histamine liberator 48/80, no increased tendency to adhesiveness or aggregation of the red blood cells was noted.

B. Circulation in the cheek pouch of lipemic hamsters. It was found that the hamsters usually developed a greater alimentary lipemia if they had been on a high fat diet (approximately 50% of the calories were furnished by fat) for a period of some days. Most of our animals had been on such a diet for one to three weeks prior to observation and frequently this was supplemented by a daily feeding of cream for 2-4 days prior to visualization of their pouches. We have, however, observed a significant lipemia with changes to be described in the circulating red blood cells following a single fat meal without prior high fat feedings. On the *day for visualization,* the pouch was exposed 3 to 6 hours after the fat meal. No change was observed in the red blood cells during the period of increasing visible lipemia. When the chylomicron count began to fall, usually 4 to 7 hours after the fat meal, changes in the suspension stability of the red blood cells developed. First the red blood cells appeared to be slightly adhesive to one another, and here and there a red blood cell stuck momentarily to the wall of a capillary or venule or to a white blood cell which was adherent to a vessel wall. The tendency to rouleaux formation increased; the size of these formations greatly increased and the cells showed a definite tendency to remain together under circumstances which would otherwise have separated them. Simultaneously the speed of the circulation decreased. This

tendency toward aggregation gradually became much greater and in capillaries and venules red blood cells began to aggregate in irregular small clusters of four to 12 or more cells. In many vessels the flow of blood became extremely slow, and in many of these the flow soon stopped completely. These changes became first evident and remained most pronounced in the venules. When the changes just described became pronounced the aggregates of red blood cells assumed an amorphous, homogeneous appearance and the outlines of the individual cells could no longer be distinguished clearly. Frequently the red blood cells had the appearance of being covered by a "doughy" opaque material. In those vessels in which the rate of flow was greatly reduced, particularly in the venules and smaller veins, the blood flowed in great "chunks". When adherent cells separated one could sometimes see them elongated as if stretched out and on a few occasions thin strands could be made out attaching them together (Fig. 6 in paper by Swank(1)). Sometimes clumps of platelets and platelets adhering to red blood cells were observed at this stage. As a rule, however, no increased adhesiveness of the white blood cells was noted unless the pouch had been opened. Petechial hemorrhages usually appeared in those pouches in which adhesiveness of the red blood cells was observed; but they were seen later and were less numerous than in our control hamsters, sometimes being absent after as long as 8 hours of exposure. Some degree of aggregation and adhesiveness of the red blood cells and slowing of the circulation has been observed in all of the hamsters which developed a significant lipemia. These changes have never been seen in any of our control animals.

With clearing of the visible lipemia 7 to 10 hours after the fat meal the circulation became more rapid and the adhesiveness of the red blood cells became less marked. In many vessels in which the circulation had stopped for some hours, the sticky masses of cells were seen slowly oozing out of the vessels into more rapid flowing streams of blood. Occasionally one saw the circulation re-established suddenly in a capillary loop. The circulation has never returned entirely to normal during the same period of observation. This is probably due in part to trauma to the pouch resulting from its long exposure. However, prolonged exposure *per se* causes no change in the adhesiveness of red blood cells. A number of hamsters with circulatory changes as marked as just described were allowed to recover and their pouches were observed several days later. The circulation was then normal.

C. Circulation in cheek pouch after injections of gelatin, egg albumin and dextran. We have seen changes in the suspension stability of the red blood cells similar to those associated with lipemia after intravenous injections of small amounts of high-molecular-weight hydrophilic colloidal solutions of gelatin, egg albumin, and high-molecular-weight dextran. In these experiments the degree of change varied directly with the amount injected and could be reversed toward normal by subsequent injection of low-molecular-weight dextran, as shown previously by Thorsen and Hint(4).

Discussion. The changes in the suspension stability of the blood which we have observed in the cheek pouch of the hamster after large fat meals have some features in common with the changes noted by Lister(5) in the injured wing of the bat, and referred to as "sludging" by Kniseley *et al.*(6) when seen in the monkey in the terminal stage of malaria. Lutz and his coworkers(7) have not observed sludging in infectious disease, in stasis of the circulation and in the many other experimental studies which they have made of the hamster's cheek pouch. Lutz(7) appears to doubt the validity of some of Kniseley's reports because he thought that Kniseley had "extended his concept, without crucial evidence, to include blood flow characteristics which differ from those of the sludged blood he originally defined."

The significance of the adhesiveness and aggregation of the red blood cells which occurs after a large fat meal, and the mechanism of these changes are unknown. They have been discussed elsewhere(1,8). If it can be shown that a significant increase in the viscosity of the blood and hypoxia of the tissues can re-

sult from alimentary lipemia then this biological mechanism is of considerable physiological and probably of pathological significance. One can only speculate concerning the importance of this mechanism in human disease, particularly in atherosclerosis(9), in vascular thrombosis(10), and in multiple sclerosis(8).

Conclusion. Following large fat meals changes occur in the circulation of the cheek pouch of the golden hamster. These changes consist of an increased adhesiveness and aggregation of the red blood cells accompanied by a slowing and even cessation of flow of the blood in the exposed pouch. They appear after the peak of the lipemia has been passed, and develop to their maximum as the lipemia clears. After complete clearing of the lipemia the suspension stability of the blood returns toward normal, and the circulation again assumes a normal appearance.

A 20 minute 16 mm colored cinephotomicrograph with titles has been prepared showing the circulatory changes described in this paper.

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Concerning the Mechanism of Action of Enterokinase.* (20124)

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Lipotropically active fractions from pancreas have been prepared by Dragstedt *et al.* (1) and by Entenman *et al.*(2). Recently Bosshardt *et al.*(3) obtained a fraction possessing lipotropic activity from the residue remaining after extraction of insulin from bovine pancreas. György and coworkers(4, 5) investigated the properties of Bosshardt's preparation and concluded that the lipotropic activity of this fraction depended upon the *in vivo* liberation of a proteolytic enzyme. They established that the lipotropic activity of this fraction was destroyed by boiling and that the fraction itself had no proteolytic activity *in vitro*, but became proteolytic after an inhibitor had been removed. They tentatively identified the inhibitor as pancreatic trypsin inhibitor, the enzyme as trypsin, and the lipotropic fraction as a complex between the enzyme and the inhibitor together with an excess of the free inhibitor. They further showed that the action of the inhibitor could be overcome by a substance present in duodenal juice and presumed to be enterokinase. The latter finding led them to conclude that enterokinase, besides having the well known ability of transforming trypsinogen into trypsin, is capable of overcoming the action of trypsin inhibitor on trypsin.

Since crystalline trypsin, crystalline pan-

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