Lipid-Induced Coagulation Changes in Normal Subjects and in Patients with Atherosclerosis

JOSEPH P. DAILEY, PH.D.,* EMIL A. FULLGRABE, M.D.,† R. L. COLESCOTT, B.S.‡ AND ZDENKA P. TILLOTSON, B.S.‡

In 1951 Swank[1] described the effects of a high triglyceride challenge on clumping of chylomicra and packing and distortion of red cells. In his experiments the chylomicra remained separate until about six hours after the administration of fat. At that time they would tend to aggregate, first as doublets and triplets and then as larger clumps. The red cells, which were largely dissociated in fasting blood samples, formed rouleau and at about six hours after the challenge these cells became more tightly packed and distorted. In some cases the cluster of red cells gave the appearance of an amorphous mass when viewed in dark field illumination at × 1,000 magnification. After nine to twelve hours the serum was clear of clumped chylomicra and the red cells returned to normal. The dose necessary to produce these effects was 2 gm. of fat per kg. body weight in man and double that in dogs. A lesser dose of 0.5 or 1 gm. per kg. did not give as profound a response.

Later, Cullen and Swank[2] administered a similar dose of cream to hamsters and observed the intact circulation in the pouch of the cheek. These investigators were able to observe the same clumping and adhesiveness of red cells *in vivo* and also observed that the rate of circulation was halted for long periods of time in the peripheral vessels. As in the case of the *in vitro* observations, these circulatory effects did not occur until about six hours after the challenge or after the peak of lipemia. The circulation returned to normal after about twelve hours.

As these studies demonstrated an effect of a high fat challenge on integrity of red cells and on rate of circulation in the peripheral vessels, it appeared worth while to investigate the effect of such a lipid challenge on coagulation of whole blood at the times when these circulatory changes were taking place.

Several studies of the effect of fat on coagulation employing less than 2 gm. of lipid per kg. body weight have been carried out. No significant effect on the coagulation system has been established at these dose levels. A number of studies have now been undertaken utilizing a higher lipid challenge and the results are controversial. In none of these studies, however, was the blood coagulation measured at the time demonstrated by Swank to be critical with respect to rate of circulation and packing and distortion of red cells.

Mustard,[3] Buzina,[4] Maclagan[5] and Fullerton[6] are some of the investigators who found that clotting time was shortened following a high dose of fat. Sheehy,[7] Borrero[8] and Sohar[9] were not able to demonstrate a significant shortening of clotting time following a fat meal.

METHOD

Evaluation of these studies revealed a difference in the technics employed to measure

From the Research Department, Armour Pharmaceutical Company, Kankakee, Illinois.

* Director of Research, Armour Pharmaceutical Company. † Present address: Department of Pathology, St. Joseph Hospital, Joliet, Illinois. ‡ Research Chemist, Armour Pharmaceutical Company.

Presented at the Seventh Annual Deuel Conference on Lipids, February 20–22, 1959, Death Valley, California.

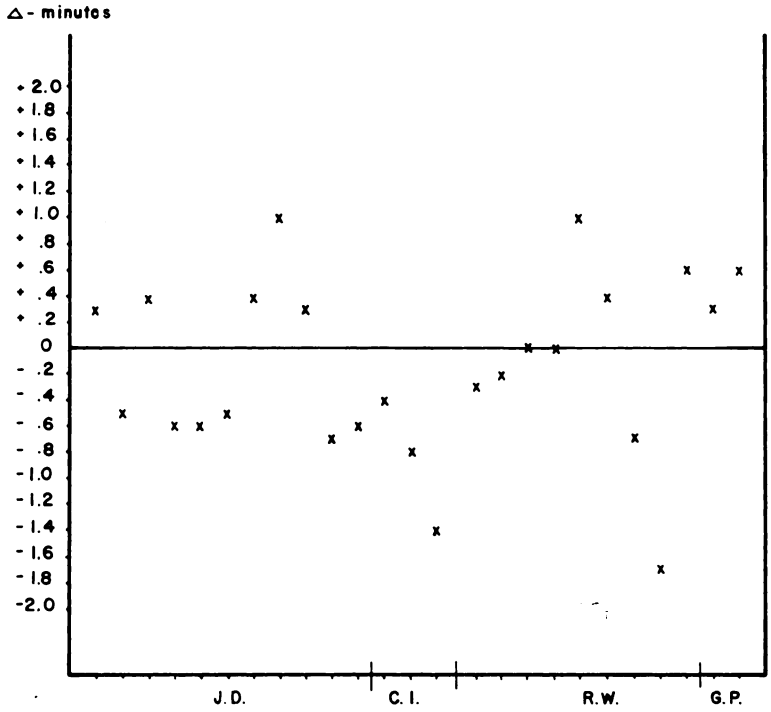

FIG. 1. Reproducibility of method: differences in coagulation time between twenty-five sets of two control samples drawn thirty minutes apart.

coagulation values and, in several of the cases, a considerably greater variation in baseline values than is desirable. In order to be in a position to evaluate small changes in coagulation, a modification of the silicone tube whole-blood clotting method was devised. The details of this method will be described elsewhere.[10] The reliability of the method was measured by comparing the fasting clotting times of two samples of blood from the same subject drawn thirty minutes apart. The difference between the clotting times of twenty-five sets of punctures are indicated in Figure 1. The variability of the method in measuring coagulation of samples at two time periods is 0.54 ± 0.15 minutes (P = 0.95 limits). The greatest difference between the coagulation times of the first and second samples in any set of punctures was 1.7 minutes. The baseline clotting time by this method varied with the pretreatment of tubes and was about thirty minutes.

EXPERIMENTAL METHODS

Ten normal men, aged twenty-eight to forty-five years, were given 2 gm. of fat as whipping cream per kg. body weight after a fasting blood sample had been drawn. Additional blood samples were obtained at two, four, six, eight and ten hours after the challenge. Chylomicra, times of packing and distortion of red cells and coagulation of whole blood were measured. No additional lipids were consumed by the subjects during the study. Coffee and tea were allowed throughout the day and crackers and shrimp were eaten after the eight-hour puncture.

The same ten subjects were again challenged with 280 ml. of skimmed milk and 1,260 calories of carbohydrate following a baseline puncture. The carbohydrate was consumed over a one-hour period and consisted of sucrose, jam, toast, bananas and apple sauce. Fasting blood samples were obtained and additional samples were obtained at two-hour intervals. Coffee and tea were permitted and a fat free meal was consumed after the eight-hour puncture.

Through the courtesy of Dr. Eugene Chesrow and with the assistance of Dr. Joseph

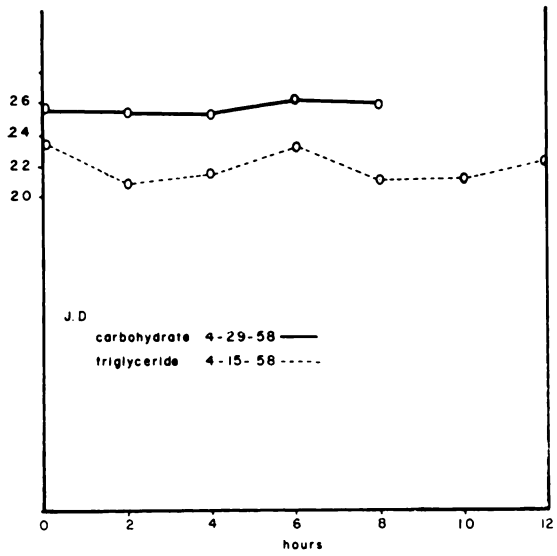

FIG. 2

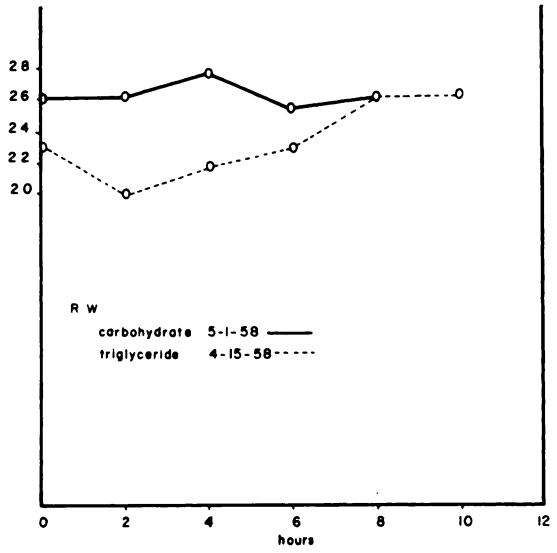

FIG. 3

FIGS. 2 and 3. Coagulation time in minutes following lipid and carbohydrate challenge.

Musci of Oak Forest Hospital, Chicago, five male and five female patients, aged forty-one to seventy-two years, who had had a cerebral vascular accident, were challenged with 2 gm. of cream per kg. of body weight in the manner described above. A fasting blood sample was obtained and additional punctures were made at approximately two-hour intervals.

RESULTS

Normal Subjects: The whole-blood coagulation time of either the two- or four-hour sample from each subject was shortened following the triglyceride challenge. By the sixth hour the coagulation time had returned to baseline in eight of the ten subjects. A shortening of coagulation time was demonstrated at either the sixth or eighth hour in eight of the ten subjects, while the coagulation time was at baseline or above in the other two subjects. The maximum drop at the two-or four-hour reading was 18.2 per cent and the maximum drop at the six- or eight-hour reading, coinciding with maximum packing and distortion of red cells, was 17 per cent. Figures 2 and 3 represent examples of the response obtained.

Similar deviations from baseline coagulation values were not obtained when the normal subjects were subjected to an isocaloric carbohydrate challenge. The average of the readings at the two- or four-hour low showed no drop, the average of the readings at the four- or six-hour high was 107 per cent of baseline and the average of the readings at the six- or eight-hour low was essentially the same as baseline.

Patients with Cerebrovascular Disease: These patients responded to the lipid challenge with coagulation times of 58.3 to 95.6 per cent of baseline at either the second- or fourth-hour puncture. The rebound values at either the fourth- or sixth-hour puncture ranged from

TABLE I
Average Coagulation Values for Each Group of Ten Subjects

Challenge and Subject	Postchallenge Coagulation Values (Per Cent of Baseline)		
	2–4 Hour Low	4–6 Hour High	6–8 Hour Low
Triglyceride, normal subjects	89.7	96.6	95.7
Carbohydrate, normal subjects	100.0	107.2	99.0
Triglyceride, patients	81.6	96.9	78.1

87 to 108 per cent of baseline. A second drop in coagulation time at either the sixth- or eighth-hour puncture was evidenced by coagulation values ranging from 62.8 to 87.6 per cent of baseline. This second depression in coagulation time coincided with maximum packing and distortion or red cells. The averages of these values compared to the normal group are indicated in Table I.

CONCLUSIONS

The whole-blood coagulation time following a lipid challenge was shortened in both normal subjects and patients with cerebrovascular disease. This effect was measured utilizing a coagulation method of low inherent variability. The coagulation time curve had two depressions, one at two to four hours and one at six to eight hours after the challenge. The shortening in coagulation time at two to four hours after the ingestion of cream confirms earlier studies. The drop in coagulation time at six to eight hours after the challenge corresponds to the time of maximum packing of red cells and decreased rate of circulation reported by Swank. This second shortening in coagulation time was greater in the older patient group than in the normal subjects. An isocaloric carbohydrate challenge to the normal group did not produce a similar alteration in coagulation time of whole blood.

REFERENCES

1. Swank, R. L. Changes in blood produced by a fat meal and by intravenous heparin. *Am. J. Physiol.*, 164: 798, 1951.
2. Cullen, C. F. and Swank, R. L. Intravascular aggregation and adhesiveness of the blood elements associated with alimentary lipemia and injections of large molecular substances. *Circulation*, 9: 335, 1954.
3. Mustard, J. F. Increased activity of the coagulation mechanism during alimentary lipemia: its significance with regard to thrombosis and atherosclerosis. *Canad. M. A. J.*, 77: 308, 1957.
4. Buzina, R. and Keys, A. Blood coagulation after a fat meal. *Circulation*, 14: 854, 1956.
5. Maclagan, N. F. and Billimoria, J. D. Food lipids and blood coagulation. *Lancet*, 271: 235, 1956.
6. Fullerton, H. W., Davie, W. J. A. and Anastasopoulos, G. Relation of alimentary lipemia to blood coagulability. *Brit. M. J.*, 2: 250, 1953.
7. Sheehy, T. W. and Eichelberger, J. W. Alimentary lipemia and the coagulability of blood. *Circulation*, 17: 927, 1958.
8. Borrero, J., Sheppard, E. and Wright, I. S. Further experiences with blood coagulation after fat meals and carbohydrate meals. *Circulation*, 17: 936, 1958.
9. Sohar, E., Rosenthal, M. C. and Adlersberg, D. Plasma lipids and coagulation of blood. *Am. J. Clin. Path.*, 27: 503, 1957.
10. Dailey, J. P., Fullgrabe, E. A., Tillotson, Z. P. and Colescott, R. L. Determination of effect of lipemia on whole blood coagulation. To be published.