Estimation of Clotting Accelerator Activity in Plasma after Ingestion of Fat

By JOHN H. TILDEN AND R. E. SHIPLEY, M.D.

A relatively simple method is presented which permits a quantitative estimation of a "coagulation accelerator activity" which appears in plasma of human and dog blood after oral ingestion of various fats. Accelerator activity and increased plasma turbidity were observed after ingestion of cream, peanut oil, coconut oil, linseed oil, or sodium oleate. Peak responses were seen between 3 and 5 hours after fat intake with gradual return toward control levels in 7 hours.

There have been a number of reports on describing an increase in the coagulability of blood following ingestion of fats. Hypercoagulability has been demonstrated by means of the whole blood coagulation time in colloid or silicone-lined tubes, the recalcified plasma clotting time and the venom "prothrombin time." Of these, the whole blood coagulation time and plasma calcium time are relatively crude tests which do not lend themselves to an investigation of underlying mechanisms. The venom prothrombin time is more accurate and probably more specific, relying as it does on the presence of plasma lipid, but still offers only limited opportunities for further investigations.

The test described in this report is a modification of the standard venom "prothrombin time." It differs from the standard test in two ways: (1) it measures the effect of undiluted BaSO₄-adsorbed test (lipemic) plasma on the venom time of normal "substrate" plasma, and (2) the substrate plasma is diluted with saline to give control clotting times of 44 to 50 sec. We feel that these modifications offer two advantages: (1) dilution of substrate, producing longer prothrombin times, accentuates differences between control and test plasmas and, in this sense, makes the modified test more sensitive than the standard undiluted test, and (2) accelerator activity is measured by the effect of BaSO₄-adsorbed test plasma on normal substrate plasma, thus permitting studies on the nature and properties of the accelerator activity in the test plasma.

In this paper we wish to present observations on the clot accelerating activity observed with this test during alimentary lipemia in humans and dogs following ingestion of cream, peanut oil and other fats.

Materials and Methods

Thromboplastins. Russell viper venom, 1:10,000, ("Stypven" Burroughs-Welshcome and Co.), Permaplastin (Alban and Co.) and Bacto Thromboplastin, (Difco Laboratories).

Test Plasma. Using a no. 20 or 21 needle 9 ml. venous blood was collected into a clean heat sterilized syringe into which had previously been drawn 1 ml. of a 1.34 per cent sodium oxalate solution. Rapid oxalation, as blood is drawn into the syringe, minimizes possible thrombin-fibrin formation. If there is a failure to obtain a satisfactory venipuncture on the first attempt, or if blood is too slowly withdrawn, a second venipuncture should be made with a fresh syringe and needle. Samples were promptly centrifuged at 2500 r.p.m. for 10 min. and plasma was aspirated into clean 13 by 100 mm. test tubes which were heat sterilized.

Substrate Plasma. Oxalated plasma diluted with normal saline.

Barium Sulfate-Treated Test Plasma. One hundred milligrams of powdered barium sulfate, reagent grade were added per milliliter of oxalated plasma, mixed by gentle shaking for 1 min., incubated at 37 C. for 10 min., and centrifuged at 2200 r.p.m. for 10 min. After carefully aspirating the supernatant plasma it was then centrifuged in the same manner and the supernatant plasma placed into another clean tube. The second centrifugation invariably removed visible traces of barium sulfate. If this step was not performed the quantity of barium sulfate remaining was sufficient to prolong the clotting end-points when mixtures were made with diluted non-barium sulfate treated (substrate) plasmas.
Sodium oxalate, 1.34 per cent, sterile11 or freshly prepared and calcium chloride, 0.025 M were also used.

**Test (Lipemic) Plasma.** Cow’s cream, mixed with cocoa and water, or peanut oil or cocoanut oil, mixed in a Waring blender with equal parts of water (to which acacia and cinnamon were added) were given orally after fasting (14–24 hours) in amounts of 0.43, 0.86, 1.29, or 2.15 Gm. fat/Kg, body weight of subject. Bloods were drawn usually at hourly intervals for 5–7 hours after ingestion of fat.

**Lipemia.** The degree of lipemia was estimated on a Hellige-Diller Photo-electric Colorimeter, model no. 500 using a no. 610 filter. Light transmission was measured on the basis of galvanometer readings. Comparisons were expressed as percentage change (decrease) in light transmission between the prefat and postfat plasma samples.

**Procedure**

Normal oxalated human plasma was diluted with 3 parts of normal saline and used as the “substrate” to which the barium sulfate treated test plasma was added. In the dog studies the normal dog plasma “substrate” was diluted with 7, and occasionally 11, parts of normal saline. Oxalated venous blood was obtained immediately before and, in most instances, at hourly intervals after fat ingestion. The plasmas were promptly treated with barium sulfate powder, as outlined, and tested undiluted as follows:

One-tenth milliliter substrate was placed into a clean, dry 13 by 100 mm. test tube; 0.1 ml. BaSO₄ treated test plasma was added, together with 0.1 ml. 1:10,000 venom. This was mixed gently, incubated for 1 min. at 37 C. in a water bath and 0.1 ml. calcium chloride solution rapidly blown into tube. Clotting end-points were timed with a stopwatch started simultaneously with the calcium chloride addition and ending with the appearance of fibrin threads immediately preceding a solid coagulum.

Determinations should be made within 1 to 2 hours after blood has been drawn to prevent possible artifacts due to partial loss of “labile” factor (proaccelerin, Ac-globulin or factor V). Such loss might minimize or prevent demonstration of the accelerator activity under study. Even slightly hemolyzed plasma samples should not be used and, of course, care should be taken to avoid contamination of the tubes containing thromboplastin, calcium chloride, etc., when pipetting.

Although the test is basically a one-stage prothrombin (or clotting time) test of the diluted plasma substrate, it is modified by appropriate dilution of the substrate so that the addition of the barium sulfate treated control plasma (before fat) together with venom and calcium results in a clotting time in the range of 44 to 50 sec. By this means clotting of the substrate is artificially prolonged by simple dilution. When a postprandial (lipemic) BaSO₄ treated test plasma is substituted for the control BaSO₄ treated plasma the clotting time may be reduced by 0–30 sec. or more depending upon the individual sample.

For convenience, the difference in clotting time between control BaSO₄ treated plasma and postprandial BaSO₄ treated test plasma is expressed as percentage change. For example, with a control (before fat) BaSO₄ treated plasma clotting time of 45 seconds and a postprandial BaSO₄ treated plasma clotting time of 29 sec., the more rapid clotting of the latter is expressed as being 35 per cent faster than the control. This is regarded as a measure of the accelerator activity (AA) appearing in plasma following the feeding of fat. Lipemia (LIP) is also expressed as percentage decrease in light transmission.

**Results**

**Dogs**

Fifty-two studies using 7 normal dogs have been performed. Figure 1 shows the results of 6 experiments in the same dog which received peanut oil, 1.29 Gm./Kg. in each instance. As shown, there is a fairly reproducible increase in accelerator activity and lipemia in this particular dog observed during the 16 months it was used for these experiments. (This is the exception, rather than the rule, in that the response to fat in a given dog may vary from one time to another without obvious reason.) The peak response of both the AA and LIP is usually between 3 and 5 hours. There was no measurable AA or LIP 24 hours after fat feeding.

Figure 2 shows the effects of different dosages of peanut oil in the same dog. In this instance there was a maximum response with the 1.29 Gm./Kg. dose and progressively smaller responses as the quantity of ingested fat was correspondingly decreased. The dose-response relationship illustrated in this dog is not seen in all dogs studied in like manner. In addition, the peak response in some dogs is also variable as to time after fat intake.

Accelerated responses were also seen in dogs following the ingestion of other fats including cocoanut oil, linseed oil and sodium oleate (fig. 3).
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**Fig. 1** Top. Effects of orally administered peanut oil (1.29 Gm./Kg.) observed in 1 dog on 6 different occasions over a 16 month period. Solid lines, acceleration in clotting time as percentage change. Interrupted lines, lipemia as percentage decrease in light transmission. Abscissa, hours after feeding.

**Fig. 2** Bottom. Effects of peanut oil orally administered to the same dog with dosages of (A) 0.43, (B) 0.86, (C) 1.29, (D) 2.15 Gm./Kg. A, A', etc. identify, respectively, changes in clotting time and lipemia in the four individual experiments. Notation as in figure 1.

**Fig. 3** Effects observed in dogs following oral administration of different fats using the same dosage (1.29 Gm./Kg.). Fats given were: (A) cocoa nut oil, (B) linseed oil, (C) sodium oleate. A, A', etc. as in figure 2. Notations as in figure 1.

In some experiments recalcified plasma clotting times were also measured along with AA and LIP. After fat feeding the recalcified plasma clot time varied considerably but ranged from 12 to 46 per cent faster than the control. Although the response was qualitatively similar to those reported by O'Brien and by Poole, the recalcified plasma clotting time method was found to be somewhat less sensitive and reproducible than the method herein described.

**Human Subjects**

Twenty-eight studies were conducted using 13 normal human subjects given either cow's cream, coconut oil or peanut oil at four different dose levels. Table 1 shows the accelerator activity as per cent change in AA and the degree of lipemia expressed as per cent decrease in LIP, using only the 1.29 Gm./Kg. dosage of three different fats. In human beings the AA and LIP responses to fat ingestion are, in general, smaller than those observed in dogs at the same dosage level.
Table 1.—Changes in Clotting Time and Lipemia in Normal Human Subjects, 1.29 Gm. fat/Kg. Dose

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hours Postprandial</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<td>J. T.</td>
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<td>2</td>
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<td>21</td>
<td>75</td>
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<tr>
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<td>5</td>
<td>14</td>
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<tr>
<td></td>
<td>LIP†</td>
<td>2</td>
<td>25</td>
<td>61</td>
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<tr>
<td>C. G.</td>
<td>AA</td>
<td>5</td>
<td>—</td>
<td>24</td>
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<td></td>
<td>LIP†</td>
<td>11</td>
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<td>63</td>
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<td>Peanut Oil</td>
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<tr>
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<tr>
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<tr>
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<td>LIP</td>
<td>5</td>
<td>40</td>
<td>31</td>
<td>19</td>
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</table>

*Accelerator activity as per cent change in clotting time, seconds.
†Per cent decrease in light transmission (lipemia).

Discussion

It is not within the scope of this paper to consider at length the probable mechanisms involved with the effects observed. It is relevant, however, to point out that attempts to demonstrate accelerator activity after fat ingestion with the use of tissue or brain thromboplastin derived from human, dog or rabbit sources, rather than Russell viper venom, have failed to show this activity.

In storage experiments using human post-prandial plasma containing AA the activity was not measurably diminished after the plasma was kept for 6 days at 3 to 5°C. Stored dog plasma containing AA lost little, if any, activity when kept either at 3 to 5°C. for up to 21 days, or when stored at room temperatures (26 to 28°C.) for 3 days.

Summary

Thirteen normal human subjects and seven normal dogs were used in 80 studies in which estimations of plasma coagulation accelerator activity (AA) and increased plasma turbidity were made.

Accelerator activity and increased plasma turbidity were observed after ingestion of cream, peanut oil, coconut oil, linseed oil, or sodium oleate. Peak responses were seen between 3 and 5 hours after fat intake with gradual return toward control levels in 7 hours.

The test described in this report is a modification of the standard venom "prothrombin time." It differs from the standard test in two ways: it measures the effect of undiluted barium sulfate-adsorbed test (lipemic) plasma on the venom time of normal "substrate" plasma, and the substrate plasma is diluted with saline to give control clotting times of 44 to 50 sec.

A possible advantage of the method described is that it may provide a test system whereby the mechanisms of the accelerator activity can be clarified.

Acknowledgments

The author wishes to thank Mr. Thomas Wooten and Miss T. R. Systner, R.N., for technical assistance, and Dr. John B. Miale for his helpful criticism of the manuscript.

Summary in Interlingua

Esseva executate 80 studios in 13 normal subjectos human e 7 normal canes pro estimar le activitate del accelerator de coagulation del plasma e le augmento del turbiditate plasmatic.

Le activitate del accelerator e le augmento del turbiditate esseva observate in le plasma post ingestiones de crema, oelio de arachide, oelio de coco, oelio de lino, o oleato de natrium. Responsas maximal esseva notate 3 a 5 horas.
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post le ingestion de grassia, sequite per le retorno gradual al nivellos de controlo intra 7 horas.

Le test describite in iste reporto es un modification del standard “tempore prothrombinic” a veneno. Illo differe ab le forma standard in duo respectos: Illo mesura le effecto de non-dilute plasma experimental (i.e. lipemic) post adsorption a sulfato de barium super le tempore a veneno de normal plasma “substrato,” e le plasma substrato es diluite in solution salin de manera a resultar in tempores coagulatori de controlo de 44 a 50 secundas.

Un avantage possibile del methodo describite es que illo provide possibilemente un sistema de proba capace a clarificar le mecanismos del activitate del accelerator.

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Circ Res. 1957;5:298-302
doi: 10.1161/01.RES.5.3.298

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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