Effect of Glucose on Appearance of Plasma-Clotting Accelerator Activity, Lactescence, and Plasma Lipids in Fat-Fed Dogs

By John H. Tilden, and R. E. Shipley, M.D.

The influence of glucose, given orally and intravenously, on the acceleration of plasma clotting and appearance of lipemia following the feeding of peanut oil was determined in dogs. Oral glucose blocked or masked the appearance of plasma lactescence and clotting accelerator activity, whereas intravenous administration tended to delay their appearance. In other experiments heparin abolished or reduced plasma lactescence without modifying clotting accelerator activity induced by fat feeding.

The ingestion of a high fat meal is usually accompanied by hyperlipemia and an increase in the coagulability of the blood when measured by in vitro tests. There have been several reports that carbohydrate reduces the degree of lipemia caused by the ingestion of fat. In 1918 Bang fed bread with butter or olive oil to dogs and observed that the hyperlipemia was less than that seen when the fat alone was given. Roney and Ching measured total plasma fatty acids in dogs that were fed either olive oil alone or olive oil plus glucose, and concluded that the "glucose inhibited alimentary lipemia." Albrink et al. have reported that in human subjects the usual rise in serum triglycerides was "diminished or absent" when 120 Gm. of extra glucose was fed with a 60 Gm. fat breakfast, and that "the lactescence was less apparent" under these conditions.

Waldron et al. showed that the acceleration in whole blood clotting time induced by fat feeding was less when either lactose or sucrose was fed simultaneously with the fat.

The present report is concerned with the effects observed in dogs following orally and parenterally administered glucose, with and without fat feeding with respect to (1) appearance of coagulation accelerator activity in plasma treated with barium sulfate; (2) plasma lactescence, and (3) changes in plasma concentrations of total lipids, phospholipids and glucose.

Methods

Accelerator Activity (AA). Nine ml. of blood were carefully drawn into a heat-sterilized syringe containing 1 ml. of 1.34 per cent sodium oxalate solution and gently mixed. To the plasma was added barium sulfate powder, 100 mg./ml., mixed 1 min., incubated at 37 C. for 10 min., centrifuged at 2,200 r.p.m. for 10 min., and the supernatant recentrifuged. Clotting times were determined after the treated plasma was combined with equal volumes (0.1 ml.) of a normal "substrate" plasma (previously diluted 1:8 with saline), 1:10,000 Russell viper venom solution ("Stypven" Burroughs-Wellcome and Co.), and 0.025 M calcium chloride. The difference in clotting times between the control (pre-fat) and the post-fat, barium sulfate-treated plasma was regarded as a measure of the clotting accelerator activity (AA) in the plasma caused by the feeding of fat. A more detailed description of the procedure has been previously reported.

Whole Blood Clotting Time. Using a dry sterile syringe and a 20 gage hypodermic needle, 5 ml. of blood were drawn from the femoral vein, the needle removed and, without mixing, 1 ml. was gently delivered into each of 3 plain glass tubes (13 X 100 mm.). A stop watch was started at the first appearance of blood into the syringe. Tubes were kept at room temperature (25 to 28 C.) and the first tube only was gently tilted every 30 seconds until a firm clot was seen, at which time the second and third tubes were successively treated in like manner every 30 seconds. The time required for the third tube to clot was considered as the clotting time.

Lactescence. Optical density of plasma was measured...
Fig. 1. Effect of oral fat only (A), oral fat and oral glucose (B), and oral fat and intravenous glucose (C), in the same 7 dogs.
Plasma Glucose. The photometric adaptation of the Somogyi method was used for the determination of glucose.\textsuperscript{14}

Total Plasma Lipids. The method of Folch, et al.\textsuperscript{15} has been modified by E. R. Diller, Lilly Research Laboratories.

Phospholipids. The method of Fiske and Subbarow\textsuperscript{16} was used.

The same seven normal mongrel dogs were used for the 6 different types of experimental procedures to be described. The dogs' weights ranged from 11 to 14 Kg. Each dog was starved from 18 to 24 hrs. prior to each study. The 6 groups of experiments were performed as follows:

- **Group A.** Orally (Planters) peanut oil only, 1.29 Gm./Kg. 19 experiments.
- **Group B.** Orally, peanut oil, 1.29 Gm./Kg. and glucose orally, 2 Gm./Kg. (50 per cent solution) given ½ hr. before, with, and at 1, 2, 3, and 4 hrs. after, fat feeding. 17 experiments.
- **Group C.** Orally, peanut oil, 1.29 Gm./Kg. and, in 50 per cent solution, glucose intravenously, 1 Gm./Kg. (dog nos. 3 and 4) or 2 Gm./Kg. (dog nos. 1, 2, 5, 6 and 7) given ½ hr. before, with, and 1, 2, 3 and 4 hrs. after fat feeding. 11 experiments.
- **Group D.** Orally, glucose only, 2 Gm./Kg. (at same times as in group B). 7 experiments.
- **Group E.** Intravenously, glucose only, 1 or 2 Gm./Kg. (at same times as indicated for group C). 5 experiments.
- **Group F.** Orally, water (volume equal to that given in the oral glucose experiments). 7 experiments.

**RESULTS**

Figure 1 shows graphically the results of 21 representative experiments in groups A, B and C from a total of 47 performed on the same 7 dogs.

In the group A experiments, clotting accelerator activity (AA) and plasma lactescence invariably appeared, usually reached a peak in 2 to 4 hours, and gradually returned toward the initial pre-fat levels. Of 19 experiments, one dog exhibited a "double" peak response in one experiment (A-3). Total plasma lipid and phospholipid concentrations usually showed moderate elevations, although not always simultaneously with AA or plasma turbidity. Plasma glucose concentration did not vary appreciably from the control pre-fat level.

Group B of figure 1 illustrates the results observed in the same dogs following the addition of glucose to fat feeding. In marked contrast to the group A experiments, lactescence and AA did not appear. Total plasma lipid and phospholipid concentrations showed little or no elevation. Moderate elevations in plasma glucose levels occurred and usually persisted throughout the 7 hour observation period.

In group C of figure 1 are shown the effects of orally given peanut oil and intravenously

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Effects of oral glucose, intravenous glucose and oral water, respectively, on plasma total lipid, phospholipid, blood glucose, optical density and accelerator activity (AA).}
\end{figure}
given glucose at the times previously specified. In these experiments, AA and increased plasma turbidity were usually seen to appear about 2 hrs. later than those in group A (fat feeding alone).

Typical control experiments illustrating the effects of oral glucose alone (group D), intravenous glucose alone (group E), and oral water alone (group F) are shown in figures 2, 3 and 4 respectively. Except for the expected elevations in plasma glucose concentration, there were no appreciable or consistent alterations in the other plasma measurements.

The influence of different doses of intravenously administered heparin on plasma turbidity, AA and whole blood clotting time was determined in 3 fat-fed dogs, as illustrated in figures 5a, b, and c. The plasma optical density measurements were made approximately 2 hrs. after blood samples were drawn, and the plasma was permitted to stand at room temperature. The smallest dose of heparin (0.2 mg.) had a lipemia clearing effect, but no detectable influence on either whole blood clotting time or AA. Intermediate doses (2.0 mg.) caused lipemia clearing and small but measurable prolongation of whole blood clotting time, but had no effect on AA. Large doses (20 mg.) caused lipemia clearing, rendered whole blood incoagulable, but had no detectable effect on AA.

**Discussion**

The present study extends and enlarges upon an earlier study\(^9\) of the effect of fat feeding in causing an acceleration of the Stypven clotting time of barium sulfate-treated plasma combined with diluted normal plasma "substrate." When fat alone is given, there is a parallelism between AA and plasma turbidity, but in some experiments the correlation is not invariable, and occasionally the plasma may be slightly turbid without AA being present. In other experiments some AA was present without appreciable lactescence. The persistence of AA after "lipemia clearing" with heparin indicates that plasma turbidity itself is not essential to the manifestation of AA and additionally, that the prolongation of whole blood clotting time to the point of incoagulability does not alter the AA effect induced by fat-feeding. The fact that under some conditions AA and increased plasma turbidity are not related does not argue against the possibility that their respective appearances in the plasma may be associated and related to a common mechanism.

When glucose was administered orally be-
fore, with, and after peanut oil, AA was not observed and lipemia (increased plasma lipids and increased plasma turbidity) did not appear as when fat alone was given. The possibility was considered that elevation of plasma glucose concentration might have interfered with the detection of AA, but no interference was observed following the in vitro addition of glucose to post-fat plasma having both lactescence and AA properties. Following the administration of glucose intravenously along with fat feeding (group C, figure 1) lactescence and AA were present at times when plasma glucose was elevated (dog nos. 1 and 2), or decreased (dog no. 3), or after return to (dog nos. 5, 6 and 7), or below (dog no. 4), the control level. Plasma glucose concentration per se does not correlate with either lactescence or AA.

The present experiments offer no explanation of the absence of both lactescence and AA effect when glucose was administered orally along with peanut oil. The manner in which glucose blocks or masks the appearance of lactescence and AA is being investigated.

**SUMMARY**

Normal dogs given 1.29 Gm./Kg. of peanut oil orally were bled at hourly intervals for 7 hours and plasma lactescece and concentration of total lipid and phospholipid were measured. A modified Stypven clotting time was determined after pretreatment of plasma with barium sulfate and subsequently combined with dilute normal plasma "substrate." Increases in plasma lactescence were usually paralleled by acceleration of the modified Stypven clotting test. Moderate and irregular increases in total plasma lipids and phospholipids were observed at this level of fat ingestion.

When the same dogs were given glucose, 2.0 Gm./Kg. orally ½ hr. before, with, and hourly for 4 hours after the administration of peanut oil, hyperlipemia was negligible or absent, and there was no acceleration in clotting time.

When the glucose was given intravenously in the same amount and at the same times to fat-fed dogs, the appearance of hyperlipemia and clotting acceleration occurred approximately 2 hrs. later than when fat alone was given. No apparent correlation existed between plasma glucose concentration and lactescence or clotting acceleration. In most experiments moderate elevations in total plasma lipid and phospholipid concentrations were observed.

Heparin given intravenously in amounts sufficient to cause "lipemia clearing" with or without prolongation of whole blood clotting time had no detectable influence on clotting accelerator activity induced by fat feeding.

**ACKNOWLEDGMENT**

The authors wish to thank Mr. O. A. Harvey, Mr. George C. Cokinos and Mr. Thomas Wooten for their valuable technical assistance.

**SUMMARIO IN INTERLINGUA**

Canes normal, recipiente 1.29 g/kg de oleo de arachide per via oral, esseva sanguinate a intervallos horari durante septe horas, e le lactescentia e le concentration de lipido total e de phospholipido del plasma esseva mesurate. Le tempore coagulatori a stypven (in forma modificate) esseva determinate post pretractamento del plasma con sulfato de barium e su combination subsequente con dilute "substrato" de plasma normal. Augmentos del lactescentia plasmatic esseva usualmente accompaniate de acceleration del modificate coagulation a stypven. Moderate e irregular augmentos del lipidis total e del phospholipidos del plasma esseva usualmente observate a iste nivello de ingestion de grassia.

Quando le mesme canes recipeva glucosa in doses oral de 2,0 g/kg, ½ hora ante, con, e 4 vices a intervallos horari post le administracion del oleo de arachide, le hyperlipemia esseva negligibile o absente, e le tempore de coagulation monstrava nulle acceleration.

Quando le glucosa esseva administrate per via intravenose in le mesme doses e al mesme periodos a canes tractate con grassia dietari, le hyperlipemia e le acceleration del coagulation occurreva circa 2 horas plus tarde que quando le grassia esseva administrate sol. Esseva constatate nulle apparente correlation
inter le concentration de glucosa in le plasma e le lactesceultia o acceleration coagulatori de illo. In le majoritate del experimentos, moderate grados de elevation del concentration de total lipido de plasma e del phospholipidos esseva observate.

Heparina in doses intravenose sufficiente a causar un clarification del lipemia con o sin prolongation del tempore coagulatori de sanguine integre non exerceva un influentia detegibile super le activitate de acceleration coagulatori inducite per le alimentation de grassia.

REFERENCES

Effect of Glucose on Appearance of Plasma-Clotting Accelerator Activity, Lactescence, and Plasma Lipids in Fat-Fed Dogs
JOHN H. TILDEN and R. E. SHIPLEY

Circ Res. 1958;6:804-809
doi: 10.1161/01.RES.6.6.804

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1958 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/6/6/804