15858

A Simple Means to Determine Exact Moment of Clotting in Prothrombin or Thrombin Time Determination.

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The determination of the exact moment of coagulation by direct visual observation is at times difficult, especially if the solutions are water clear as happens for instance in following the action of purified thrombin on a fibrinogen solution. By employing the principle that the fluid in a test tube remains stationary when the tube is slowly revolved, one can accurately time the beginning of solidification since at that moment the contents revolve with the tube. The procedure as applied to the prothrombin time (one-stage method) is as follows: 0.1 cc of oxalated plasma is transferred to a small pyrex test tube (100 x 13 mm) by means of a 1 cc pipette graduated in 0.1 cc cut to 170 mm length (as recommended by Quick[1] for his method) and fitted with a rubber bulb from a medicine dropper. In transferring the plasma the tip of the pipette must touch the bottom of the tube and enough air blown through to produce a few bubbles which gather about the periphery. At least one-half of the circumference should be free of bubbles. The thromboplastin (0.1 cc) is carefully added, and then 0.1 cc of 0.02 M $CaCl_2$ is forcefully blown in to assure prompt mixing. At this moment the stop watch is started. The upper part of the tube is held by the last 2 fingers of the right hand and rotated slowly with the thumb and the first finger. By practice one learns the most advantageous angle at which the test tube is to be held and likewise the best speed of rotation. The bubbles are closely watched and the moment they revolve with the tube, the stop watch is clicked.

The test is performed in a glass water bath (the type used for the Wassermann reaction) kept at 37°C and illuminated by a desk lamp placed opposite the observer. The light must be focused so that the contents of the test tube can be observed while immersed in the bath. All reagents as well as the test tubes used for the determination are kept in the water bath.

[1] Quick, A. J., *Am. J. Physiol.*, 1947, **148**, 211.

15859

Intravenous Carbohydrate Tolerance Tests on Swine.

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Early work[1-4] on carbohydrate tolerance indicated that the rise and fall of blood sugar after the administration of glucose are rapid and reproducible tests of the regulating mechanisms of carbohydrate metabolism. Although the reliability of the method as a diagnostic tool has been questioned because of divergent results obtained after repeated tests on the same normal individual, the procedure is of distinct value if the test is car-

[1] Liefmann, E., and Stern, R., *Biochem. Z.*, 1906, **1**, 299.

[2] Gilbert, A., and Baudouin, A., *C. R. Soc. biol.*, 1908, **65**, 710.

[3] Bang, I., *Biochem. Z.*, 1913, **49**, 19.

[4] Jacobsen, A. Th. B., *Biochem. Z.*, 1913, **56**, 471.

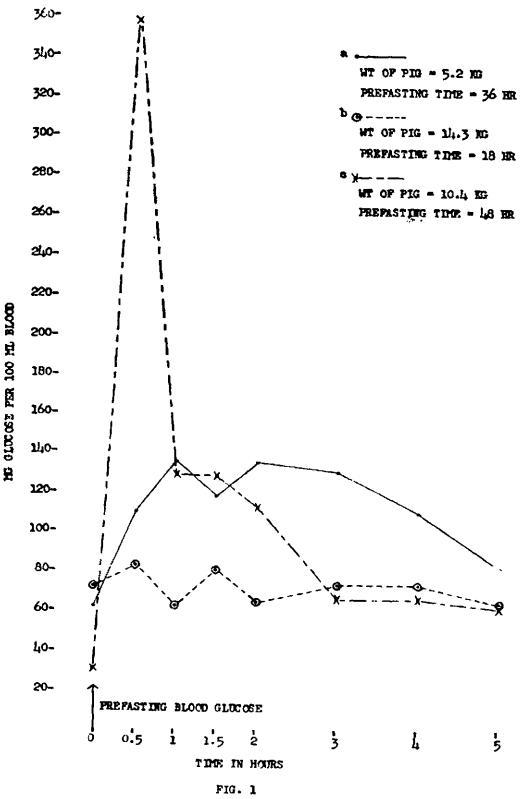

FIG. 1

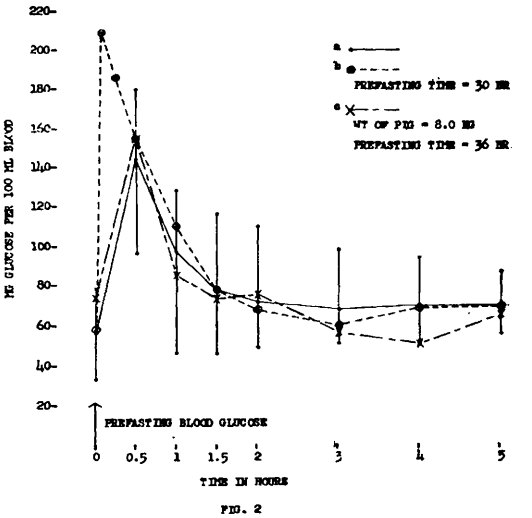

FIG. 2

FIG. 1. a. Oral carbohydrate tolerance test on a pig given 2 g glucose per kilo body weight by stomach tube. b. Intravenous carbohydrate tolerance test on a pig following a prefasting period of 18 hr. c. Intravenous carbohydrate tolerance test on a pig following a prefasting period of 48 hours.

FIG. 2. a. Average of 17 intravenous carbohydrate tolerance tests on pigs fasted 24 to 36 hr prior to injection of glucose. Vertical lines through each point indicate deviations from the mean. b. Average of 3 intravenous carbohydrate tolerance tests on pigs bled 5 and 15 minutes after injection of glucose. c. Intravenous carbohydrate tolerance curve on pig showing fall in blood glucose below prefasting level.

ried out by experienced investigators and the results evaluated critically.[5]

Carlson and Drennan[6] reported that "the normal pig has a lower tolerance for dextrose, bread or cooked starch given by mouth than any species so far studied." This is contrary to what would be expected because pigs that are full-fed presumably are able to digest and assimilate high carbohydrate diets. Inasmuch as corn, a cereal which contains approximately 70% starch, is usually the chief constituent in rations fed to swine[7] and since only limited data are available regarding carbohydrate metabolism in the pig, further study of carbohydrate tolerance in this species seems advisable.

Methods. In the study reported herein oral carbohydrate tolerance tests were made on 20 healthy weanling pigs. Two grams of glucose per kg of body weight, in 50% solution, were administered by stomach tube. The pigs were fasted for periods of 24, 36, 48 and 96 hours prior to giving the test dose, but in no instance was the typical carbohydrate tolerance curve of the normal human obtained. (Fig. 1a). Since humans and

5 Bodansky, M., and Bodansky, O., *Biochemistry of Disease,* The Macmillan Company, New York, 1940.

6 Carlson, A. J., and Drennan, F. M., *J. Biol. Chem.*, 1912-1913, **13**, 465.

7 Morrison, F. B., *Feeds and Feeding,* 20th Ed., The Morrison Publishing Company, Ithaca, N.Y., 1942.

swine are omnivorous and have simple stomachs, one might expect each of these species to respond in similar fashion to ingestion of glucose. Nevertheless, the results obtained seemed to indicate that this method is unsatisfactory in swine probably because, as pointed out by Dukes,[8] the gastric emptying time is prolonged in the pig. In order to diminish this effect we turned to the intravenous route of administering glucose.

The blood sugar concentration apparently can be raised to almost any level when concentrated solutions of glucose are injected intravenously, but the disposal and subsequent reactions are presumably comparable to those elicited when glucose is given orally.[9] Two preliminary tests suggested that a prefasting period of 18 hours was hardly adequate, since the tolerance curves were irregular with a lag in the rise of the sugar level (Fig. 1b). If, on the other hand, the pigs were fasted 24 hours, a satisfactory curve was obtained, but the blood glucose concentration at the 30-minute interval was not more than 15 to 20 mg higher than the value at the prefasting level. Thus, to increase this span it was necessary to fast the pigs at least 30 hours but not over 36 hours. If the fast was prolonged beyond 36 hours, an exaggerated hyperglycemic response resulted which is the usual consequence of prolonged fasting[5] (Fig. 1c).

Seventeen intravenous carbohydrate tolerance tests were made on healthy pigs ranging in weight from 5.9 kg to 30.8 kg and averaging 11.4 kg. A prefasting sample of blood for glucose determination was withdrawn from the anterior vena cava by the technic of Carl and Dewhirst.[10] Seventy-five hundredths of a gram of glucose per kg of body weight was administered intravenously in 50% solution at a rate of approximately 0.3 g per kg per minute. The concentration of blood glucose was determined by the Shaffer, Hartmann, Somogyi method as described by Koch.[11] Throughout each test the pigs were handled carefully and bled rapidly to eliminate the factor of undue excitement, which in turn might produce a rise in blood glucose.

Average values of blood glucose for 17 healthy pigs during each half-hour interval of the intravenous carbohydrate tolerance test are shown in Fig. 2a. There was a wide variation in the prefasting blood glucose concentration with the levels ranging from 33.90 mg % to 79.10 mg % and an average of 56.62 mg. This divergence may be attributed in part to the age of the pigs and the period of inanition prior to the test. Five minutes after injecting glucose the average concentration on 3 pigs was 209.46 mg glucose per 100 ml blood, whereas in 15 minutes the value dropped to 186.07 mg % (Fig. 2b). Likewise, there was a marked divergence in concentration of blood glucose at the half-hour interval, varying from 97.18 mg % to 179.74 mg % with an average of 144.55 mg per 100 ml blood. During the next half-hour the average drop in glucose concentration was 46.44 mg %, suggesting rapid oxidation and utilization of carbohydrates by the pig. At 1½ and at 2 hours the concentrations fell to an average of 78.96 and 72.99 mg % respectively. In 2 hours, for 6 individual tests, the concentration of glucose dropped to a value below the prefasting level. A similar decline is often observed in oral tests on humans,[5] with the glucose value rising to the prefasting level or slightly above toward the end of the trial (Fig. 2c).

In 5 of the trials, urine was collected during the test and only relatively small amounts of sugar were recovered varying from 0.15 to 0.88 g with an average of 0.5 g representing about 5% of the total quantity injected. This is comparable to the amount of glucose recovered in the urine of the bovine following intravenous administration of glucose during carbohydrate tolerance tests in this

8 Dukes, H. H., *The Physiology of Domestic Animals*, 5th Ed., Comstock Publishing Co., Inc., Ithaca, N.Y., 1942.

9 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry. Interpretations*, Vol. I, 2nd Ed., The Williams and Wilkins Company, Baltimore, 1946.

10 Carle, B. N., and Dewhirst, Wm. H., Jr., *J. Am. Vet. Med. Assn.*, 1942, **101**, 495.

11 Koch, F. C., *Practical Methods in Biochemistry*, 3rd Ed., The Williams and Wilkins Company, Baltimore, 1941.

species.[12] It appears, therefore, that intravenous carbohydrate tolerance curves obtained on healthy pigs are quite similar to those procured on normal human subjects.

[12] Bell, F. R., and Jones, E. R., *J. Comp. Path.*, 1945, **55**, 117.

Summary. The form of the carbohydrate tolerance curve obtained after the administration of glucose to normal growing pigs by the intravenous route, apparently closely parallels the type of curve procured when the test is made on normal human subjects.

15860

Thromboplastic Activity of the Urine.

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Hemophilics having hematuria often experience severe attacks of renal colic and pass thin clots obviously representing ureteral casts. Since the clotting time of the blood of one such patient observed in January 1947 was in excess of 2 hours, the formation of such clots within the ureter was difficult to explain, unless blood stagnation existed because of obstructive lesions, or the urine itself exerted a clot accelerating action on the blood. The presence of thromboplastic substances in the tissues of the body, in the saliva from normal[1,2] and hemophilic men[3] and in human milk[4] is known. No mention of the presence of these substances in the urine has been found, and there seems to be no adequate explanation of the mode of formation of clots in the renal pelvis of hemophilics with hematuria.[5-10] The evidence to be presented indicates that urine from normal or hemophilic men contains a thromboplastic substance. Contact with such a substance is probably what causes clotting of the blood extravasated in the renal pelvis.[11]

Testing of clot accelerating activity was done in most instances by adding 0.1 cc of the material to be tested to 1 cc of normal or hemophilic venous blood collected with oiled syringes and kept in paraffin-coated tubes. Unless otherwise stated, collodion-coated glass tubes (13 mm internal diameter) were used for the clotting time determinations. The urine was collected, usually from males, over a period of 24 hours in clean jugs containing a crystal of thymol; it was filtered or centrifuged before use. The usual chemical and microscopical studies were carried out on each urine. Those specimens containing sugar, albumin, casts, blood cells or bacteria were not used.

1. The simple addition of intact urine to normal or hemophilic blood accelerates its coagulation in glass or collodion-coated tubes (Table I). Dilution of the urine reduces its clot accelerating ability. Urine obtained by catheterization from the bladder or the renal pelvis does not differ significantly in its ac-

[1] Bellis, C. J., and Scott, F. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 1373.

[2] Glazko, A. J., and Greenberg, D., *Am. J. Physiol.*, 1939, **125**, 108.

[3] Tocantins, L. M., unpublished observations.

[4] Jacoby, M., and Adler, S., *Enzymologia*, 1937, **1**, 373.

[5] Weil, P. E., *L'hemophilie*, Paris, 1946.

[6] Schlossmann, H., *Die Hämophilie*, Enke, 1930.

[7] Birch, C. L., *Hemophilia*, 1937, Illinois Med. and Dental Monograph, Univ. of Illinois.

[8] Quick, A. J., *Hemorrhagic Diseases*, Thomas, 1942.

[9] Nygaard, K. K., *Hemorrhagic Diseases*, Mosby, 1941; Ferguson, J. H., *Ann. Rev. Physiol.*, 1946, **8**, 231.

[10] Fonio, A., *Ergeb. der Inner. Med. u. Kinder heil.*, 1936, **51**, 443.

[11] Tocantins, L. M., and Lindquist, J. N., *Fed. Proc.*, 1947, **6**, 215.