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Response of Serum Inorganic Phosphate to Insulin in Normal and Diabetic Subjects. (21270)

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Wigglesworth *et al.*(1) showed that injection of insulin produced a fall in blood inorganic phosphate in rabbits. A number of investigators have confirmed these findings, both in whole blood and in serum(2-11). Similar results were obtained by Harrop and Benedict (2) and by Perlzweig *et al.*(4) after oral administration of glucose to normal subjects. Bolliger and Hartman(8) pointed out that this phenomenon did not take place in pancreatectomized dogs and attempted to apply this finding to diagnosis of diabetes mellitus(12). We worked out a procedure which we termed "Delta G/Delta P test" for the study of carbohydrate metabolism based upon changes in blood sugar and serum inorganic phosphate* from their initial level(13). The test is carried out by injecting intravenously, at a speed of about 10 ml/minute, 1 ml/kilo body weight of a 50% glucose solution, taking samples for blood sugar and phosphate analysis before and 30, 45 and 60 minutes after injection. Subjects are kept at rest for one half hour before and during test. A Delta G at 45 minutes more than + 50 mg/100 ml is taken to indicate decreased glucose removal. In normal subjects Delta P at 45 minutes is greater than —0.30 mg/100 ml. In 45 normal subjects the *averages* were + 16 for Delta G 45′ and —0.67 for Delta P 45′(14). We used this test for classifying 54 diabetic patients into 2 groups: Group I, with a defective glucose-phosphate fall and group II, with a normal fall (15). We assumed that patients from the first group did not produce insulin (69% of patients tested), whereas patients from the second group (31%) were supposed to have a normal or near normal insulin reserve. Until now, only pregnancy(16) and fever and surgical stress(17) have been shown to inhibit at least partially the glucose-phosphate fall in non-diabetic subjects. We do not imply that insulin is the only factor concerned in the glucose phosphate fall. Thus, a glucose phosphate fall has been found to occur in pancreatectomized dogs(18) and in one pancreatectomized human being(19); in both cases, however, the blood sugar had to be raised above 500 mg/100 ml before the glucose phosphate fall took place.

Thus a working hypothesis consistent with available data would seem to be that glucose phosphate fall is mainly dependent upon insulin secretion by the pancreas, although factors other than insulin probably enter also into play in the production of the phenomenon. If this hypothesis were correct, one would expect a group of diabetics showing glucose phosphate fall and a group not showing it, to exhibit roughly equal insulin glucose responses. This would support the theory further, although it would not constitute definite proof for it. We investigated therefore the effect of insulin upon phosphate of diabetic patients previously classified according to their glucose phosphate fall, comparing

* By "phosphate," we mean "serum inorganic phosphate," by "insulin-phosphate fall" the fall in serum inorganic phosphate following injection of insulin and by "glucose-phosphate fall" the fall in serum inorganic phosphate following injection of glucose.

TABLE I. Fall in Blood Sugar after 0.1 U/K Insulin. Means and standard errors, mg/100 ml.

	No. of subjects	Time after inj. of insulin 0′	15′	30′	45′	60′
Normal subjects	42	77 ± 2	−39 ± 2	−43 ± 2	−32 ± 1	−18 ± 3
Diabetics	21	180 ± 10	−24 ± 4	−39 ± 4	−53 ± 6	−53 ± 4
Diabetics, Group I	12	196 ± 13	−23 ± 7	−36 ± 5	−53 ± 9	−54 ± 5
" " II	8	154 ± 10	−26 ± 6	−44 ± 4	−54 ± 6	−51 ± 8

Normal vs. diabetics: Differences statistically significant at 0′, 15′, 45′ and 60′.
Group I vs. Group II: " " " " 0′ only.

results with those obtained in a normal control group.

Material and methods. Tests are carried out by injecting 0.1 unit of insulin/kilo body weight, intravenously. The subjects, 42 normal young adults and 20 diabetic patients were kept at rest for one half hour before and during test. They were in fasting conditions. Diabetic subjects had been given the Delta G/Delta P test; 8 had a normal glucose-phosphate fall (Group II) and 12 had a defective fall (taking as a limiting value —0.30 mg/100 ml) (Group I). Blood samples were taken from the antecubital vein before and 15, 30, 45 and 60 minutes after injection of insulin, and blood sugar and serum inorganic phosphate determinations were performed in duplicate(20-21). Hemolysis of samples was carefully avoided. The diabetics were all untreated before the test with insulin. Photometric readings were made on a Coleman Model 6 spectrophotometer. The statistical significance of the differences was established with the "t" test(22), the limit of significance being P=0.05.

Results. Results are shown in Tables I and II. Changes in blood sugar were less pronounced at 15 minutes in the diabetic than in the normals, but became more pronounced at 45 and 60 minutes. There was no difference in this respect between the 2 groups of diabetics. Changes in phosphate were more pronounced in normals than in diabetics, a fact which has not been mentioned previously, but there was no significant difference in the response of the 2 diabetic groups.

Discussion. It appears from the results that there are in diabetes mellitus certain factors which are capable of inhibiting the response of phosphate to insulin. Comparing the 45 minute value in the normal and in the diabetic group, shows that insulin phosphate fall in the latter is approximately 50% less than in the former. It is of interest that the diabetic patients classified according to glucose-phosphate fall do not show a difference when insulin is employed, suggesting that the subjects under study are homogeneous with respect to the insulin-phosphate response. This finding is consistent with the idea that the differences found after administration of glucose depend upon the capacity of the pancreas to produce insulin. A fairly numerous group of patients with diabetes show a normal glucose-phosphate fall, but in our experience, all patients who show this phenomenon had an initial blood sugar of less than 250 mg/ml (15), even though the defective phosphate fall may occur with initial levels less than 250 mg/100 ml; in Table I we may note that the average fasting blood sugar for Group I is only 196 mg/100 ml in the present series.

TABLE II. Fall in Serum Inorganic Phosphate after 0.1 U/K Insulin. Means and standard errors, mg/100 ml.

	No. of subjects	Time after inj. of insulin 0′	15′	30′	45′	60′
Normal subjects	42	3.55 ± .07	−.53 ± .07	−.95 ± .07	−.99 ± .07	−.75 ± .06
Diabetics	20	3.52 ± .23	−.29 ± .08	−.39 ± .12	−.51 ± .11	−.33 ± .07
Diabetics, Group I	12	3.53 ± .23	−.31 ± .13	−.37 ± .18	−.51 ± .14	−.25 ± .08
" " II	8	3.49 ± .47	−.27 ± .09	−.41 ± .09	−.51 ± .20	−.43 ± .14

Normal vs. diabetics: Statistically significant differences at 15′, 30′, 45′, 60′.
Group I vs. Group II: " " " " none.

A normal glucose-phosphate fall in persons with changes in glucose tolerance due to hepatic disease has been interpreted by Forsham and Thorn(23) as an indication of "peripheral utilization" of glucose. The only experimental support for this concept comes from the work of Pollack and his collaborators (24). These workers showed that glucose phosphate fall took place in the perfused hind limb of a dog; on the other hand, in a visceral preparation in which all the striated muscles had been removed, the phenomenon did not occur. Although the authors do not state it clearly, the conclusion appears to be based on a single experiment, in which the preparation was kept in good condition. Furthermore, there are grave objections to this type of experiment from the biochemical point of view. It has been shown by others that shock states tended to produce a rise in serum phosphate (25) which could well mask the glucose phosphate fall. Indeed, in Pollack's experiment, the serum phosphate level starts around 5.50 mg/100 ml. Finally, the concept of "peripheral utilization" is in opposition with experimental data indicating that the glucose phosphate fall mirrors an insulin discharge and its effect upon most tissues, including the liver (25-26). From the data available, it appears that the only case in which the glucose-phosphate fall has taken place in the absence of the pancreas has been in the work of Levine *et al.* with blood sugar levels above 500 mg/100 ml during several hours(18), and in one pancreatectomized patient(19). As above mentioned in clinical cases of diabetes we never found a normal glucose-phosphate fall with fasting blood sugar over 250 mg/100 ml.

We believe therefore that, as a working hypothesis, it may be admitted that diabetic patients who show a normal glucose-phosphate fall produce insulin, possibly even in quantities greater than are secreted under normal conditions. However, the possibility that other modifying factors might be present during glucose administration which were not present when insulin was given without glucose is not eliminated by the present data.

Summary. Intravenous insulin tests (0.1 U/kg of body weight) were carried out in 42 normal subjects and in 20 diabetics, blood sugar and serum inorganic phosphate being determined before and 15, 30, 45 and 60 minutes after administration of insulin. The blood sugar fall was found to be less in the diabetic patients at 15 minutes, similar in both groups at 30 minutes and more pronounced in the diabetic at 45 and 60 minutes. The phosphate fall was more pronounced in normals than in diabetics. At 45 minutes for instance, the average fall in diabetics was about 50% that found in normals. If diabetics are classified in 2 groups according to their normal or defective phosphate response to glucose no difference is seen between the 2 groups with regard to their response to insulin. It is suggested that the phosphate fall which takes place in a number of diabetics after the injection of glucose may be in part dependent upon an insulin secretion by the pancreas. It is pointed out that the experimental data thus far accumulated do not permit the assumption that the phosphate fall after glucose represents "peripheral utilization" of the carbohydrate.

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Formazan Formation Compared to Phosphatase Activity in Human Serum.* (21271)

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Malignant tissue is stained more intensely with tetrazolium salts than normal tissue according to Straus and his coworkers(1) and Black *et al.*(2). This difference apparently is caused by the higher concentration of the glycolytic enzymes in abnormal tissue(3-7). The question arises whether such enzymatic changes in the tissue are reflected in the blood or serum of the patient. The results of our work indicate no correlation between the reducing power of serum enzymes and malignancy. Instead tetrazolium reduction in serum is apparently correlated with phosphatase activity.

Materials and method. Triphenyltetrazolium reagent: One g of 2,3,5-triphenyl-2-H-tetrazolium chloride (Eastman), 6.7 g potassium dihydrogen phosphate, and 350 ml of 0.1 *N* sodium hydroxide are diluted to one liter with distilled water. The pH of this reagent is 7.2. Two ml of the triphenyltetrazolium reagent, 6.0 ml distilled water, and 0.2 ml of serum are mixed in a test tube, and incubated at 38° C for 24 hours in the dark, because exposure to ultraviolet light itself causes reduction. The tubes are then cooled in an ice-bath and 8.0 ml of acetone is added. This mixture is stoppered and shaken well to dissolve the red formazan. If proteins precipitate and cause cloudiness, the mixture can be centrifuged. A sample of aqueous acetone mixture is pipetted off and placed in a cuvette. The color intensity is measured in a Beckman Model B Spectrophotometer at 485 mμ. Acetone, which has been shaken with an equal volume of water, is used as the reference solution or blank. The alkaline and acid phosphatase analyses were run according to a spectrophotometric modification of the Bodansky(8) and Gutman(9) methods.

Results. Using the method of Straus(1), our results showed that various malignant tumor tissues do not always show a deep red staining, and some normal tissues, such as mucosa of the gastro-intestinal and urinary tracts, hepatic parenchyma, renal cortex, uterine endometrium, and the parenchyma of the testis show a deep staining by formazan.

Sera from patients with cirrhosis, Cushing's syndrome, renal rickets, hypo- and hyperparathyroidism showed as great a reduction in transmission as those patients with neoplasms. However, the lowest per cent transmissions were obtained from patients with cancer of the

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