# Comparison Between Changes in Serum Inorganic Phosphorus Induced by Glucose and Glucagon in Diabetics. (21955)

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Kirtley, Waife and Peck(1) first reported the effect of glucagon upon blood sugar and serum inorganic phosphate in diabetics. In 4 "stable" diabetics, blood sugar rose to approximately the same level as in normal subjects, remained elevated longer and the serum inorganic phosphorus fall was impaired. In 4 "unstable" diabetics, there was a lesser blood sugar increase and the return to basal levels was about the same as in normals; phosphorus fall was the same as in normals in this group. Kirtley *et al.*(2) extended these observations and showed with epinephrine results similar to those obtained with glucagon.

In the following investigation, we wished to inquire whether diabetics, classified according to the Delta G/Delta P test (based on magnitude of serum inorganic phosphorus fall after intravenous injection of glucose) presented differences in regard to response to glucagon.

*Material and methods.* The tests were carried out in 35 diabetic patients chosen at random, most of them without previous insulin treatment. A few of the subjects, who were being treated with long acting insulin preparations, were given regular insulin instead for the 2 days preceding the tests. The patients were kept fasting from the night preceding the test and remained at rest for a half hour before and during the test. The Delta G/Delta P test was performed according to a previously described technic, consisting essentially in the intravenous injection of 1 ml/kg body weight of a 50% glucose solution at the rate of approximately 10 ml/m; samples of blood are taken at 0, 30, 45 and 60 minutes. Blood sugar was determined by the Nelson-Somogyi method(3) and serum inorganic phosphorus by the Fiske-Subbarow method(4), modified for the spectrophotometer. According to previous investigations(5,6), an increase in blood sugar at 45 minutes over the initial level (Delta G) of more than 50 mg/100 ml is evidence of a decrease in the subject's capacity to remove glucose from the blood stream, and a decrease in serum inorganic phosphorus below the initial level at 45 minutes (Delta P) of less than 0.30 mg/100 ml is considered defective. A normal Delta P is presumptive evidence of insulin secretion.

TABLE I. Changes in Serum Inorganic Phosphorus (mg/100 ml) after Administration of Glucose in Diabetics. Limiting value between the two groups has been taken as −0.30 mg/100 ml decrease from the initial value at 45 min.

Averages and standard errors.

| Group | No. of cases | Initial values | 30′ | 45′ | 60′ |
|---|---|---|---|---|---|
| Defective △ P | 19 | 3.14 ± .13 | −.07 ± .04 | −.09 ± .01 | −.06 ± .02 |
| Normal △ P | 16 | 3.42 ± .22 | −.31 ± .01 | −.50 ± .04 | −.45 ± .06 |

TABLE II. Changes in Blood Sugar after Administration of Glucose (mg/100 ml) in Diabetics Classified According to Serum Inorganic Phosphorus Fall in the Same Test.

Averages and standard errors.

| Group | No. of cases | Initial values | 30′ | 45′ | 60′ |
|---|---|---|---|---|---|
| Defective △ P | 19 | 236 ± 18 | +143 ± 7 | +112 ± 8 | +87 ± 7 |
| Normal △ P | 16 | 133 ± 12 | +131 ± 9 | + 96 ± 9 | +75 ± 9 |
| "t" values | | 4.8* | 1.1 | 1.3 | 1.1 |

* Statistically significant difference.

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TABLE III. Changes in Serum Inorganic Phosphorus (mg/100 ml) after Glucagon in Diabetics Classified According to Phosphorus Fall after Glucose.

Averages and standard errors.

| Group | No. of cases | Initial values | 15′ | 30′ | 45′ | 60′ |
|---|---|---|---|---|---|---|
| Defective △ P | 19 | 3.08 ± .14 | −.47 ± .07 | −.53 ± .07 | −.40 ± .05 | −.30 ± .04 |
| Normal △ P | 16 | 3.37 ± .13 | −.57 ± .02 | −.72 ± .02 | −.54 ± .09 | −.39 ± .14 |
| "t" values | | 1.5 | 1.3 | 2.8* | 1.4 | .7 |

* Statistically significant difference.

TABLE IV. Changes in Blood Sugar (mg/100 ml) after Glucagon in Diabetics Classified According to Their Phosphorus Fall after Glucose.

Averages and standard errors.

| Group | No. of cases | Initial values | 15′ | 30′ | 45′ | 60′ |
|---|---|---|---|---|---|---|
| Defective △ P | 19 | 232 ± 18 | +32 ± 5 | +46 ± 3 | +48 ± 5 | +42 ± 6 |
| Normal △ P | 16 | 137 ± 14 | +35 ± 2 | +54 ± 3 | +53 ± 4 | +42 ± 5 |
| "t" values | | 4.1* | .5 | 1.8 | .7 | 0 |

* Statistically significant difference.

The patients were classified according to their Delta P and were then administered glucagon solution in a fashion similar to the administration of glucose; 0.01 ml of glucagon solution per kg body weight was given intravenously, without dilution, and an additional 15 minute sample of blood was obtained. According to specifications given by the manufacturer, the glucagon solution which was used (lot 208-158B-214) contained 0.95 mg of protein/ml, with 0.25% phenol, pH 3. Purity, as compared to crystalline glucagon was 50%; 0.2 μg per kg injected intravenously to an anesthetised cat caused a maximum increase in blood sugar of 30-40 mg per 100 ml 10 to 15 minutes after the injection. The content of insulin was between 0.005 and 0.05 U/ml of solution. The solution was generously furnished by the Eli Lilly Co.

*Results.* In Table I are shown the changes in serum inorganic phosphorus in the group of diabetics studied, divided according to the fall following glucose.

In Table II may be seen the changes in blood sugar after glucose in both groups.

There is no statistically significant difference with respect to blood sugar rise following glucose between the two groups. The initial level was higher in the group with defective Delta P.

Table III demonstrates serum inorganic phosphorus response to glucagon in the groups classified according to their Delta P after glucose. At 30 minutes, there is a statistically significant difference between the 2 groups,

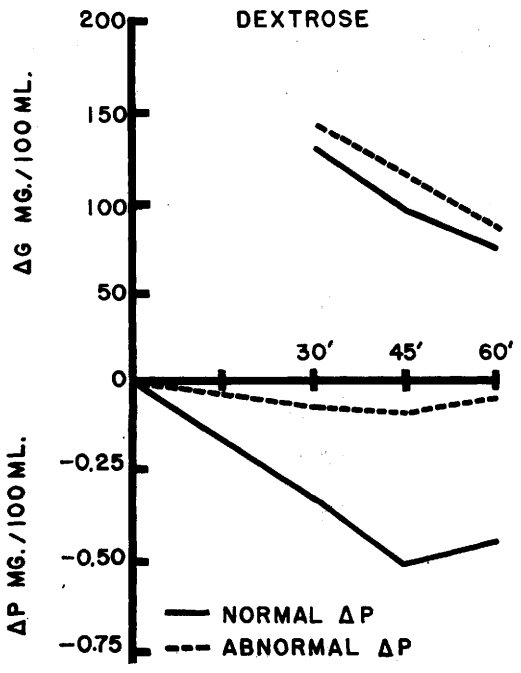


FIG. 1. Changes in blood sugar (△ G) and in serum inorganic phosphorus (△ P) after intravenous administration of dextrose in diabetics classified according to their phosphorus fall in the same test (limiting value between the 2 groups = −0.30 mg/100 ml).

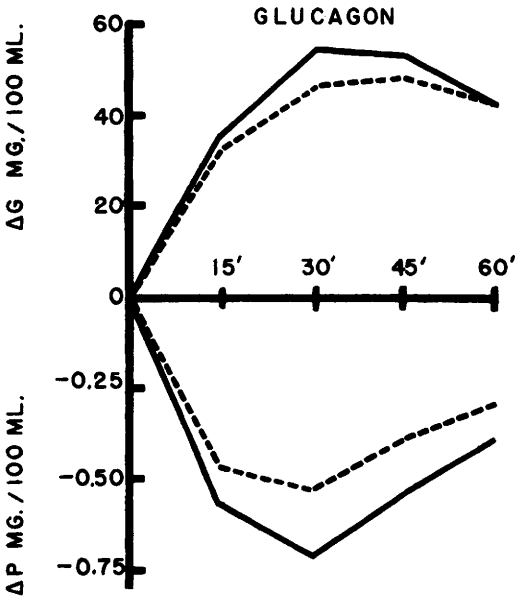


FIG. 2. Changes in blood sugar and serum inorganic phosphorus after intravenous inj. of a glucagon solution in diabetics classified according to their serum inorganic phosphorus response to dextrose. The groups are the same as those indicated in Fig. 1.

the group with "normal" phosphate fall after glucose showing a greater phosphate fall after glucagon. In Table IV the changes of blood sugar after glucagon in both groups are shown. The results are summarized in Fig. 1 and 2.

In Fig. 3 is shown the correlation between maximal Delta P produced by glucagon and maximal Delta P determined by glucose. No definite correlation between the two sets of values appears to exist.

*Discussion.* The serum inorganic phosphorus fall after glucagon is in general agreement with that following glucose; the fall after glucagon is lesser in the abnormal Delta P group, particularly in the 30-minute sample, where the difference is statistically significant.

The fact that glucagon is able to produce a serum inorganic phosphorus fall in the group where such a fall does not occur after glucose is interesting. It is not likely that this effect is due to insulin contamination, since each cc of the solution contains only between 0.05 and 0.005 U/ml and the amount injected is 0.01 ml per kg. The phosphorus falls found with glucagon are comparable or greater than those found in diabetics after 0.1 U of insulin per kg(7). It would then seem that glucagon has an action of its own upon

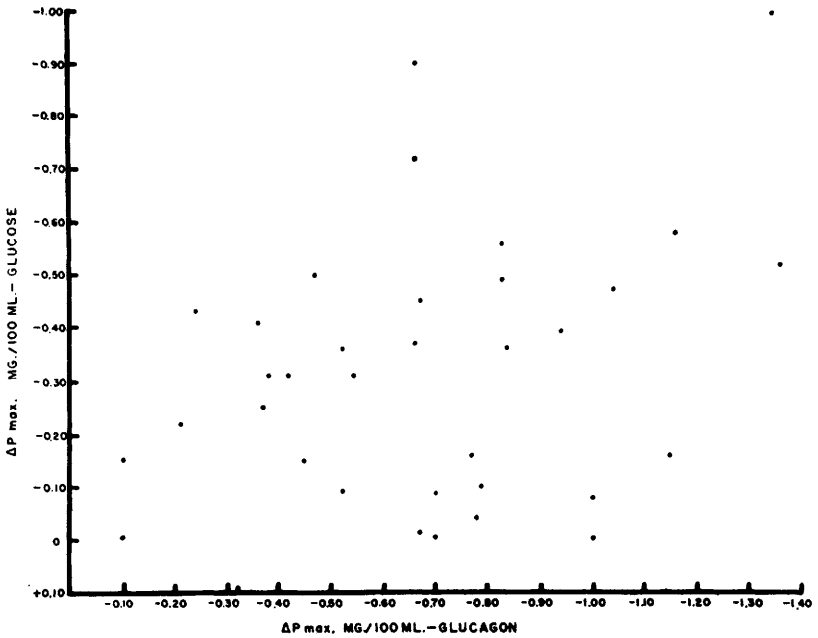


FIG. 3. Correlation graph between maximal serum inorganic phosphorus fall induced by dextrose and by glucagon.

serum inorganic phosphorus, independent from that determined by secondary insulin liberation. Speculatively, it may be thought that this effect depends upon the utilization of phosphate in the disintegration of glycogen by phosphorylase.

*Summary.* The effect of intravenous glucose upon serum inorganic phosphorus was compared to that of glucagon in 35 diabetic patients taken at random.

The subjects were classified in two groups according to their serum phosphate fall following glucose. In a group of 19 patients, the phosphate fall after glucose was less than 0.30 mg/100 ml, and in another group of 16 patients, more than 0.30 mg/100 ml similar to that found in non-diabetic individuals. As a working hypothesis, it has been assumed that diabetics with a normal phosphate fall are producing insulin. It was found that the group with a normal phosphate fall after glucose showed a greater fall after glucagon than the group with defective phosphate fall. On the other hand, the group with defective or absent phosphate fall after glucose also showed a very definite fall after glucagon. It would seem then that the latter substance exerts a direct effect upon serum inorganic phosphate, independent of the reactive insulin secretion produced by the glucose load. This effect is possibly related to the activation of phosphorylase which acts in the process of disintegration of glycogen through the introduction of phosphate in the bonds existing between the hexose molecules.

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## Anti-Inflammatory Activity of $\Delta^1$-9$\alpha$-Fluorohydrocortisone Acetate. (21956)

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There have been many important advances in the use of steroids as a method of treatment of the inflammatory diseases during the past few years. Since the introduction of hydrocortisone as one of the most efficacious treatments, it has been continually modified with the hopes of increasing its antiphlogistic activity while diminishing its undesirable side effects. It has been found that halogenation at the C-9 position greatly increases the glycogen deposition potency(1,2) and the anti-inflammatory effects(3) but causes a relatively greater increase in mineralocorticoid activity (4,5,6). A second analogue of hydrocortisone, 1-dehydrohydrocortisone, has been reported to possess greater antiarthritic properties in man (7) than the parent compound. It has also been shown that this compound is a more potent glucocorticoid in animals(8,9) and that this increase is not associated with an increase in mineralocorticoid potency(8).

It was, therefore, of interest to study the effects of both of these modifications when introduced into the same molecule. Accordingly, the anti-inflammatory activity of $\Delta^1$-9$\alpha$-fluorohydrocortisone acetate was compared with that of hydrocortisone. Data are also included showing the relative potencies of $\Delta^1$-hydrocortisone acetate, 9 $\alpha$-fluorohydrocortisone acetate, and hydrocortisone for comparative purposes.

*Materials and methods. Animals.* Male rats obtained from the Upjohn colony (Sprague-Dawley ancestry) were used throughout these experiments. The body weights ranged from 150-170 g. All animals