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Research Article

RESPONSE OF CITRIC ACID LEVELS TO ORAL ADMINISTRATION OF GLUCOSE. I. NORMAL ADULTS AND CHILDREN [1]

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It has been demonstrated in animal and plant tissue studies that citric acid metabolism is intimately related to carbohydrate metabolism (1–5). A direct relationship between glucose and citric acid metabolism in the human has not been established, hitherto.

A study was therefore undertaken to measure citric acid levels in the human, during enhanced activity in the oxidation of glucose and its conversion to glycogen. Two methods were employed. One method was the study of the changing citric acid levels in the blood serum simultaneously with the changing glucose levels during a glucose tolerance test. The other was to cause a rapid removal of glucose from the blood stream by injecting insulin without glucose administration into the normal adult.

Glucose (1 gm./kilo body weight) was administered orally after a 12-hour fasting period. Blood samples were drawn fasting, and one-half, one, two, three, four, five and six hours after administration of the glucose. All glucose determinations were done on serum by the method of Folin and Wu. Citric acid was determined by the methods previously described (6, 7). Determinations of citric acid by these methods are accurate to within ± 5%. The results obtained may be illustrated by a typical curve obtained on a normal adult (Figure 1).

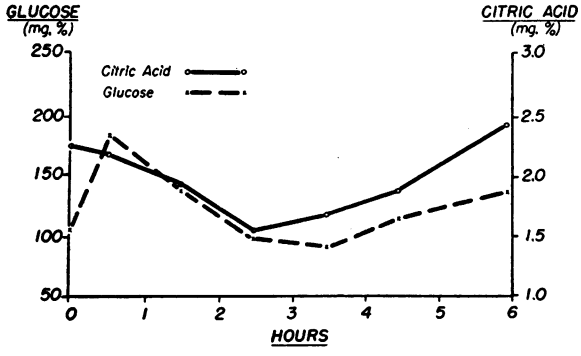

FIG. 1. CITRIC ACID LEVEL RESPONSE TO GLUCOSE ADMINISTRATION

[1] Presented in part at the meeting of the American Chemical Society, New York City, Sept. 15, 1947.

The characteristic glucose tolerance curve is observed in this case with a lowering of the glucose concentration to a value below the fasting level at the end of three hours and a return to normal at the end of six hours. The citric acid level drops slowly for the first 30 minutes and then drops sharply to a minimum point about 140–200 minutes after the administration of glucose. This minimum is from 25 to 35% lower than the fasting level. A gradual return to the fasting level is then observed. It is to be noted that the minimum for citric acid comes later than the glucose peak and corresponds with the time when one would expect most active rate of glucose removal. Table I summarizes the citric acid response for 12 normal adults and 12 normal children.

TABLE I

Adults			Children			
Case No.	Fasting level	Minimum	Case No.	Age	Fasting level	Minimum
	(mg. %)	(mg. %)		(years)	(mg. %)	(mg. %)
1	2.27	1.60	13	6	2.28	1.74
2	1.95	1.10	14	3½	3.66	2.40
3	1.78	1.20	15	4½	2.90	1.80
4	2.50	2.00	16	10½	3.40	2.40
5	2.60	1.70	17	12	3.20	2.05
6	2.23	1.54	18	1	2.47	1.88
7	2.62	1.60	19	2	2.32	0.80
8	2.80	2.30	20	4	2.65	2.08
9	3.03	2.46	21	4	2.67	2.15
10	1.90	0.80	22	14	2.00	1.54
11	3.00	2.45	23	1	3.12	2.47
12	2.47	1.67	24	2½	2.28	1.35
Average % drop = 29.8			Average % drop = 31.2			

When insulin (½ unit/kilo) was injected subcutaneously into six consecutive non-diabetic patients and their blood levels taken one hour later, appreciable lowering of their citric acid level was observed in all cases. As can be seen from Table II, the drop was approximately 29% in citric acid level.

In a similar test, 20 units of insulin were in-

TABLE II

Serum level	Fasting	One hour after injection
	(*mg.* %)	(*mg.* %)
* Glucose	91	36
* Citric acid	2.1	1.5

* Average of six normal adults.

jected subcutaneously into a diabetic whose glucose level was at 400 mg. %. At the end of 45 minutes the glucose level had dropped to 300 mg. %. The citric acid level was lowered from an initial value of 3.4 mg. % to a final value of 2.7 mg. %.

In young children (8, 9) the fasting citric acid levels are generally higher than that found in adults (10–14). Normal infants show a delayed response for a period of up to one hour, but exhibit a normal minimum within two and one-half to three hours. Their recovery to normal levels is quicker, often being complete in four hours. Older children resemble adults in their response.

The citric acid fasting levels in newborns are higher than those observed in older children, ranging from 3 to 6 mg. %. Their citric acid response follows the pattern observed in normal infants but with a steeper initial drop. Simultaneously citric acid and glucose tolerance curves of a newborn infant in whom the initial feeding was the glucose tolerance test is shown in Figure 2.

DISCUSSION

It is apparent from the curves observed in the normal individual that the highest level of citric acid in the blood is the fasting level. Administration of glucose results in a drop in the citric acid level, which returns to the fasting level from five to six hours after administration of glucose. A relationship between glucose and citric acid metabolism is therefore demonstrated in humans.

The observations above coincide with the observations of Stoppani on depancreatinized dogs. When glucose with insulin was injected into these dogs, a drop in the citric acid values was observed (15).

That insulin is the substance responsible for the drop in citric acid levels is not substantiated by our observations reported in the succeeding paper of this series (16).

Among the factors required to degrade glucose to pyruvic acid are phosphate ions, magnesium ions, adenosine triphosphate and coenzyme I (17). These same factors are needed for the formation of citric acid (18–21). One explanation for the dropping of the citric acid levels may therefore be one of inhibition by competition. The large requirements for these elements and coenzymes needed for the conversion of a large mass of glucose suddenly invading the blood stream, as in the glucose tolerance test, would decrease their availability for citric acid production. Phosphate ion is of particular importance for it is needed both for the phosphorylation of glucose and also for citric acid formation (18–21). Inorganic phosphate levels are lowered after insulin administration (22).

An alternative explanation may be based on the evidence that phosphorylation of glucose and glycogen formation require the utilization of the oxidative cycle as an energy source (23). Disappearance of citric acid from the blood stream may reflect this phenomenon.

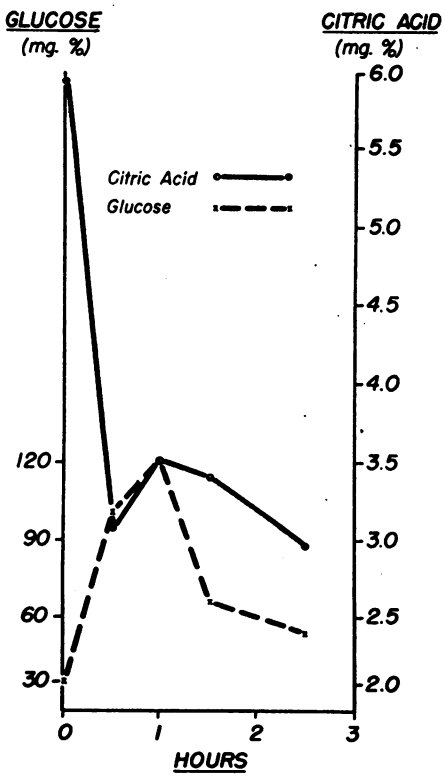

FIG. 2. EFFECT OF FIRST GLUCOSE FEEDING ON CITRIC ACID LEVELS IN NEWBORNS

Some support is found for these proposals in the fact that the citric acid level drops to its lowest level at the point where the glucose is disappearing most rapidly from the blood.

Another explanation which suggested itself as a cause for the lowering of citric acid in the blood on glucose administration to the normal was a lowering of the blood pH. That pH would have an effect was derived from the evidence which has been reported on the effect of the administration of acids and alkalies on citric acid levels.

It has been reported that administration of substances that tend to raise the blood and urine pH will increase the output of citric acid in urine and raise blood citric acid levels. Conversely, administration of substances which tend to lower the blood and urinary pH will cause a diminution in the output of citric acid in the urine and a lowering of the citric acid levels in the blood (24–39).

Hallman (40) in studies on tissue slices found that citric acid is produced best at an alkaline pH (7.5–8.0). Moreover, the activity of the enzyme citrogenase which catalyzes the formation of citric acid from acetoacetic and oxalacetic has its optimum pH in the alkaline range (41–43).

It would therefore appear that production of citric acid should be increased in alkalosis and decreased in acidosis.

In order to test this possibility, namely, that the lowering of citric acid levels during the glucose tolerance test may be an effect due to a lowering of pH, pH and CO_2 measurements were made during a glucose tolerance test. Actually a slight rise in pH (0.02 units) was observed, which was at its peak two hours after the administration of the glucose and corresponding to the minimum for the citric acid level. The pH returned to its original value after three hours.

The change in CO_2 content of the blood was negligible, dropping slightly to a minimum (2% below fasting) at a time corresponding to the minimum of the citric acid level, and returning to its original value at the end of the test. Other observers have reported either no change or a rise in pH after administration of glucose or insulin (44).

These observations eliminate the suggestion that acidosis was responsible for the drop in citric acid level during the glucose tolerance test.

SUMMARY

1. Administration of glucose orally brings about a characteristic changing level of blood citric acid in the normal individual. The citric acid level drops to a minimum (25 to 35% below the fasting level) and returns to the fasting level at the end of from five to six hours; a relationship is therefore indicated, in the human, between glucose and citric acid metabolism.

2. Injection of insulin alone into humans causes a lowering of citric acid levels.

3. The citric acid lowering in blood serum is greatest during the period when glucose is most rapidly disappearing from the blood.

4. In the normal individual the highest level of citric acid in the serum is the fasting level.

5. Various possible mechanisms for the citric acid level response to the oral administration of glucose are discussed.

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