THE EFFECT OF ALCOHOLIC EXTRACT OF COMMERCIAL GRANULAR GLUCOSE ON URINARY REDUCING SUBSTANCE.*

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During the course of some experiments by us on commercial glucose, Folin and Berglund1 published an article in which they maintained that the administration of c.p. glucose in amounts of as much as 200 gm. did not produce a glycosuria. We then determined to repeat our previous work, using both c.p. and commercial glucose, and to compare the behavior of the two. Finding a difference in the results with c.p. glucose, we tried to find out what the substance was in the commercial glucose which led to the increased excretion of reducing substance. In the first place, an alcoholic extract of the commercial glucose was prepared, and it was found that most of the coloring matter came down in the extract. This extract was then used in combination with the c.p. glucose. The effect produced by the mixture was compared with a control, the extract used alone, commercial glucose, and c.p. glucose. The work covered the determination of amounts of reducing substance in both blood and urine, and the chemical analysis of the sugars and extracts. The latter is still in progress.

EXPERIMENTAL.

Small weighed portions (about 50 gm.) of commercial granular glucose were ground into a fine powder and extracted repeatedly with small amounts

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1 Folin, O., and Berglund, H., Some new observations and interpretations with reference to transportation, retention, and excretion of carbohydrates, J. Biol. Chem., 1922, li, 213.
of 95 per cent redistilled alcohol until the extract, which was at first dark brown in color, became almost colorless. The combined portions of extract were then evaporated to a syrup and kept in glass-stoppered bottles. The concentration of the extract in terms of the original glucose was carefully noted. The sugar was taken in the form of lemonade and in each case 2 gm. per kilo of body weight were used. When the extract was used either alone or in combination with C.P. glucose the amount was equivalent to that obtained from 2 gm. per kilo of the commercial glucose. The C.P. glucose was Pfanstiehl's C.P. special. Six adult laboratory workers, five women and one man, served as subjects. Five sets of experiments were conducted, as follows: (1) 2 gm. per kilo of body weight of C.P. glucose; (2) 2 gm. per kilo of commercial glucose; (3) 2 gm. per kilo of C.P. glucose plus an equivalent amount of extract; (4) extract alone; (5) control. In the control experiment only water plus lemon was taken.

DISCUSSION.

From Fig. 1 it will be seen that the alcoholic extract when taken alone had little or no effect upon the amount of blood sugar. The very slight rise is accompanied by a speedy return to the basal value or even a further drop. Commercial and C.P. glucose, as well as C.P. glucose plus extract, gave rise to hyperglycemia, showing similar curves. In all cases the peak occurred at the end of the first 30 minute interval and the values returned to very near the basal at the end of the 2½ hour period.

Strikingly different are the figures for urinary reducing substance, shown graphically in Fig. 2. All cases showed a rise at sometime during the 3 hours immediately following the ingestion of C.P. glucose. The peak, as in the case of previous work, did not follow a regular course, but when all figures were averaged it came at the end of 60 minutes, with a value of 21.0 mg.; or 80.4 per cent above the basal figure of 12.4 mg. At the end of 3 hours the average value of reducing substance excreted was still 24.6 per cent above the fasting value. The total amount excreted during the 3 hours averaged 115.3 mg. The average 3 hour increase over the basal was 28.6 mg. The values which Folin and Berglund gave were recomputed in order to compare their results with ours. All their cases showed a very slight increase in urinary sugar, with the exception of one case which rose to 15 mg. or 75 per cent above the basal value at the peak of the curve, coming after 2 hours. At the end of 4 hours this subject was still excreting 50 per cent more sugar than his fasting value.
The ingestion of commercial granular glucose was followed by a rise in urinary reducing substance which was always greater than that brought about by c.p. glucose. The peak with commercial glucose ran from 144.1 to 739.5 per cent above the fasting value and that for c.p. glucose from 4.3 to 196.3 per cent above the fasting value. The average peak for commercial glucose was 220.3 per cent above the fasting value, and occurred also after 60 minutes. The total amount excreted in 3 hours was 57 per cent higher than the corresponding amount for c.p. glucose in the same period. The average 3 hour increase was 101.7 mg. for commercial glucose and 28.6 mg. for c.p. glucose. The amount still being excreted at the end of the period was 108.1 per cent above the fasting value. Thus, no matter what method of comparison is adopted, the commercial glucose invariably leads to a markedly higher urinary sugar content than the c.p. sugar.

The ingestion of c.p. glucose plus extract gave rise to an entirely different situation. The extract contained only 10.4 per cent of sugar, but it was followed by an entirely disproportionate rise in urinary reducing substance. Here the peak values were likewise
scattered, but usually delayed, the average coming after 2 hours, and having a value 283.8 per cent higher than the basal. This is 29 per cent higher than the corresponding value for commercial glucose and 253 per cent higher than that for c.p. glucose alone.

Fig. 2. Average percentage increase in reducing substance over the fasting values of urine after the ingestion of various grades of glucose.

The total amount of reducing substance in 3 hours ranged from 112.4 to 334.7 mg. with an average of 178.7 mg. The average 3 hour increase was 109.8 mg. These figures are closely similar to those of commercial glucose. At the end of the period, however, the average amount excreted was still 201.0 per cent above the
fasting value, which is nearly twice as high as the figure for commercial glucose in the corresponding interval, and somewhat more than eight times as high as the c.p. glucose.

The reducing substance excreted in 3 hours with c.p. glucose represents practically the total excess caused by the ingestion of 2 gm. per kilo of body weight, since the value returned almost to the basal in this time. With the commercial glucose, however, the excretion was still marked after 3 hours, so that an excess of this sugar probably continued to be excreted for some time afterward. When extract was added to the c.p. glucose the elimination of the excess of reducing substance was even more prolonged, and the 3 hour value represents considerably less of the total amount than in either of the two previous cases.

The ingestion of extract alone in 80 per cent of the cases was followed by a slight rise in the excretion of reducing substance. The peak occurred at the end of 1 hour in most cases, and the average was 41.3 per cent above the fasting value, but in every case it was followed by a decrease to below the fasting value. At the end of 3 hours the average excretion was 30 per cent below the basal. The average total excretion during the 3 hours was 90.0 mg. As a control experiment, lemon and water were used, and the effect was not essentially different from that of extract, although not so pronounced. The average peak, occurring also at 60 minutes, was 27 per cent above the fasting value, and at the end of 3 hours the reducing substance excreted was 10.1 per cent below the basal. The average total amount excreted during the 3 hours was 71.2 mg., slightly lower than with extract. Possibly Constam's\(^2\) theory of a "rinsing out" process might account for some increased sugar excretion dependent upon the diuresis following a water intake.

The work done leads to the conclusion that there is some substance which may be extracted from commercial glucose with alcohol, which in itself has little effect on the amount of blood sugar and on the excretion of reducing substance, but which, in addition to c.p. glucose, invariably raises the amount of reducing substance in the urine. The blood sugar is only slightly affected. Although the extract contained 10.4 per cent of glucose, this

\(^2\) Constam, G., Über den Einfluss peroraler Einnahme von Glucose auf Blutzucker und Glucurone beim Gesunden, Biochem. Z., 1923, cxliii, 73.
amount added to the total amount of c.p. glucose ingested could not possibly account for the total increase in the excretion. Thus, in the case of the heaviest subject, weighing 67 kilos, the whole dose of extract taken contained only 2.2 gm. of glucose, which is 1.6 per cent of the total amount ingested, whereas the total increase in excretion of reducing substance was 284 per cent higher when the extract was added than when c.p. glucose was used alone. It is worthy of note that the extract added to the c.p. sugar had very little effect on the blood sugar curve, but very marked effect on the excretion of reducing substance even after the blood sugar had returned to the fasting value. This fact seems to indicate that the extract of commercial sugar acting in connection with c.p. glucose has a negative effect on the glycogen-forming process or the mechanism of tissue absorption. Benedict's theory on the glycuressis following the ingestion of pure glucose is based on the assumption that the cells select from the mixture of α- and β-glucose more of one form than of the other, and the rest is eliminated. Possibly the extract has the power to change the chemical structure of the c.p. glucose ingested so that the less easily absorbed form predominates, and the elimination is increased. Further work on this subject is in progress in our laboratory.

SUMMARY.

1. A comparison was made of the reducing substance in blood and urine after the ingestion of 2 gm. per kilo of body weight of commercial granular glucose, c.p. glucose, c.p. glucose plus an alcoholic extract of commercial glucose, and extract alone.

2. Similar blood sugar curves occurred in the first three series. Extract alone gave a very slight rise in blood sugar.

3. All cases showed a rise in urinary reducing substance. The c.p. glucose showed the least rise and the quickest recovery; commercial glucose led to a much greater increase in urinary reducing substance. c.p. glucose plus extract gave a prolonged urinary excretion with the peak coming an hour later than in

*Benedict, S. R., and Osterberg, E., Sugar elimination after the subcutaneous injection of glucose in the dog. Including a discussion of the paper on observations on carbohydrates by Folin and Berglund, J. Biol. Chem., 1923, lv, 760.
the other series. Extract alone, like the control, produced very little rise in urinary sugar.

4. The work leads to the conclusion that there is some substance which may be extracted from commercial glucose with alcohol, which in itself has little effect on the amount of blood sugar and on the excretion of reducing substance, but which, in addition to c.p. glucose, invariably raises the amount of reducing substance in the urine. The active principle in the extract seems to have a negative effect on the glycogen-forming process or the mechanism of tissue absorption.
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