Effects of Intravenous and Oral Infusion of Monosaccharides on Serum Insulin Levels in Rabbits

M. S. Nijjar, M.Sc., and W. F. Perry, M.A., M.D., Winnipeg

SUMMARY

The same dose of glucose given intravenously or orally produced a similar increase in serum insulin, although the blood glucose level was much lower after oral administration than after intravenous administration. These findings support the hypothesis that an additional insulinotropic mechanism other than hyperglycemia is associated with the oral administration of glucose. The specificity of this mechanism was investigated by examining the insulin response following oral and intravenous infusions of various monosaccharides other than glucose, e.g., mannose, fructose, galactose, α-methyl glucoside, ribose and xylose. Oral infusion of these monosaccharides, except mannose and fructose, produced a progressive rise in the blood level of the monosaccharides. All monosaccharides examined except α-methyl glucoside led to an increase in serum insulin level when given intravenously. However, after oral administration only glucose, galactose and xylose led to an increase in serum insulin, whereas mannose, fructose and ribose had no effect. These findings suggest that the proposed insulinotropic mechanism elicited by oral administration is not specific to glucose but does require an increase in the blood saccharide level after oral administration. However, oral infusion of the sugar analogue, α-methyl glucoside, did not promote insulin secretion despite rapid absorption and marked increase in its level in the blood. This suggests that a sugar, in addition to being absorbed with subsequent increase in blood level, must possess an active reducing group for the elicitation of insulin release.

The intravenous infusion of readily metabolizable sugars (glucose, mannose and fructose) produced marked insulin response whereas similar infusion of xylose, ribose, and galactose elicited only a moderate insulin response. The intravenous injection of a nonmetabolizable sugar, α-methyl glucoside, had no stimulatory effect. These findings suggest that the rise in blood saccharide level per se after intravenous administration may not be the sole stimulus for insulin secretion but possibly a metabolite of the infused sugar is a stimulus. This inference was supported by the finding that the intravenous injection of pyruvate enhanced the secretion of insulin. DIABETES *19*:155-60, March, 1970.

From the Department of Biochemistry, University of Manitoba, and Department of Clinical Chemistry, Winnipeg General Hospital, Winnipeg, Manitoba, Canada.

It has been shown both in man[1] and in dogs[2] that insulin response to the oral infusion of glucose is greater than if the same amount of glucose is injected intravenously. Since the hyperglycemia following oral infusion of glucose was lower than that found after the intravenous infusion of glucose, an additional stimulus linked with the oral infusion of glucose was proposed.[1,2] McIntyre et al.[1] and Dupré et al.[3] showed that this insulin response to oral infusion of glucose persisted in patients who had the liver excluded by portacaval shunts. These findings led them to postulate that an insulinotropic substance is released from the gut after the ingestion of glucose which stimulates the β cells of pancreas in addition to the hyperglycemia itself. This postulate was supported by further findings of Dupré et al.[4] that the intravenous injection of gut mucosal extract simultaneously with glucose elicited an insulin response which was comparable to that observed after the oral infusion of glucose. The extract was claimed to be biologically distinguishable from pancreozymin, secretin and glucagon.[4]

The purpose of present study was to determine whether the greater insulin response to oral infusion of glucose is specific for glucose or if it can be elicited by other sugars as well. This was done by examining the insulin response to intravenous and oral infusion of the monosaccharides, D-mannose, D-fructose, D-galactose, α-methyl glucoside, D-ribose and D-xylose.

ANIMALS

Healthy rabbits of both sexes, of mixed strain and weighing from 2 to 4 kg., were used. The animals were fed with rabbit chow, cabbage and carrots. On the day of experiment they were anesthetized with an intravenous injection of pentobarbital (30 mg./kg.) after an eight to ten-hour fast. A basal (0 time) blood sample of 2 to 3 ml. was obtained by cardiac puncture, as were the subsequent blood samples at 5, 15, 30, 60 and 120 minutes after the infusion of a test substance.

EXPERIMENTAL PROCEDURES

1. *Intravenous tests*: The sugar being examined was injected rapidly into the marginal ear vein (3 ml./kg. of a 50 per cent solution of the appropriate sugar). Control animals were injected with the same volume of physiological saline. Rapid injection of pyruvate (sodium salt) pH 7.40 (0.91 gm./kg.) was found to be lethal and therefore it was injected slowly over five minutes with no ill effects.

2. *Oral tests*: The same dose as above of various test substances was given by a stomach tube. Control animals were given the same volume of physiological saline by stomach tube. Glycine, in amount equimolar to the hexoses, was also administered in the above manner.

METHODS

1. *Blood sugar analysis*: Each blood sample was analyzed for the total reducing sugars by the method of Nelson and Somogyi[5] and for true glucose concentration by a glucose oxidase method.[6] The amount of blood sugar other than glucose was calculated by subtracting true glucose concentration from that of total reducing sugar concentration. To determine the level of total reducing sugars of blood samples containing α-methyl glucoside, the Somogyi filtrates were hydrolyzed in 1 N HCl for two hours at 100° C., cooled, neutralized with sodium hydroxide solution, and analyzed by the Nelson and Somogyi method.

2. *Insulin assay*: Serum insulin concentration was estimated by the immunoassay procedure of Soeldner and Slone.[7] However, the separation of bound insulin-I-125 from free insulin-I-125 was accomplished by the resin absorption technic of Meade et al.[8]

3. *Statistical analysis:* The mean of serum insulin level at 5, 15, 30, 60 and 120 minutes after the infusion of a test substance was compared with the mean fasting level of insulin of the same group. The significance of difference was determined by a *t* test which took into account the pooled variance.[9]

RESULTS

Control experiments:

Both intravenous and oral administration of physiological saline did not elevate serum insulin level significantly ($p > 0.4$), figure 1. Some rise in blood glucose level was noted sixty minutes after both intravenous and oral infusion of saline. This rise was not consistent throughout the group, however, and seemed to be contributed by one animal in each group.

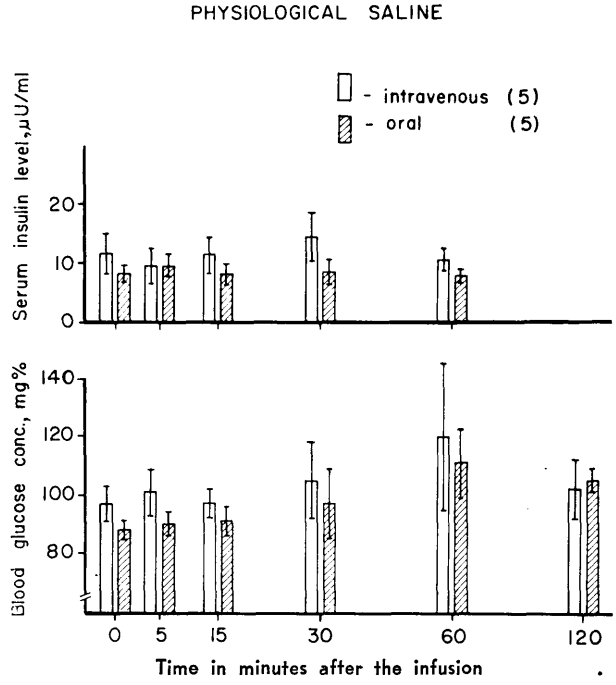

FIG. 1. Serum insulin and blood glucose levels after intravenous and oral saline. Mean values ± S.E.M. Number of animals in parentheses.

Additional control experiments were carried out where saline isomolar with hexose dose was administered. Both the levels of insulin and blood glucose were similar to those observed after the infusion of physiological saline.

Glucose:

The infusion of glucose intravenously and orally produced a marked insulin response ($p < .001$), figure 2a. Despite considerable differences in blood glucose concentrations after intravenous and oral infusion of glucose, the rise of 15.8 μU./ml. in insulin level after oral glucose was not different ($p > .05$) from that of 12.5 μU./ml. following intravenous glucose.

Galactose:

The level of insulin rose from 8.7 μU./ml. to 14.5 μU./ml. five minutes after the intravenous infusion of galactose ($p < .05$), figure 2b. After oral infusion the insulin level rose significantly from 7.9 μU./ml. to 15.3 μU./ml. at thirty minutes ($p < .05$). This rise was similar to that noted with intravenous infusion. There was no significant increase in the blood glucose level after galactose administration ($p > .05$).

Mannose:

Intravenous infusion of mannose elicited a marked insulin response ($p < .001$), figure 3a. The fasting

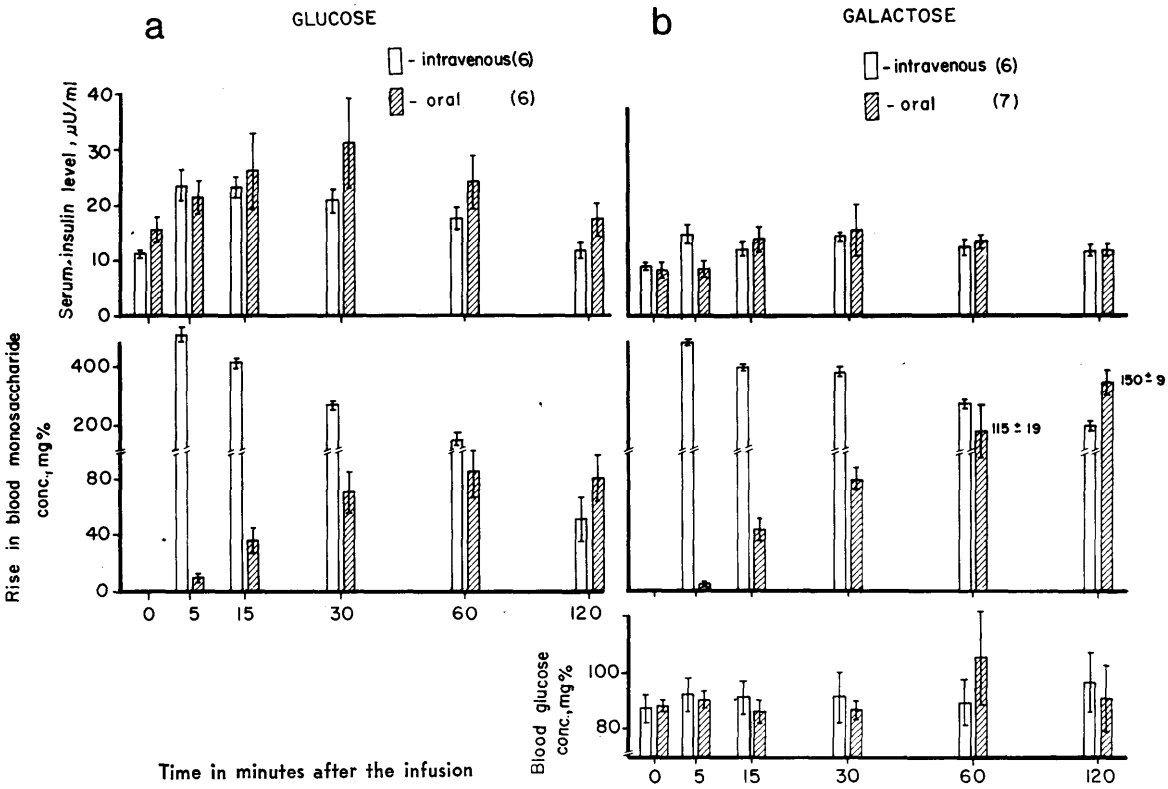

FIG. 2. Serum insulin levels and increase in blood monosaccharide level: a, after intravenous and oral glucose, and b, after intravenous and oral galactose. Mean values ± S.E.M. Number of animals in parentheses.

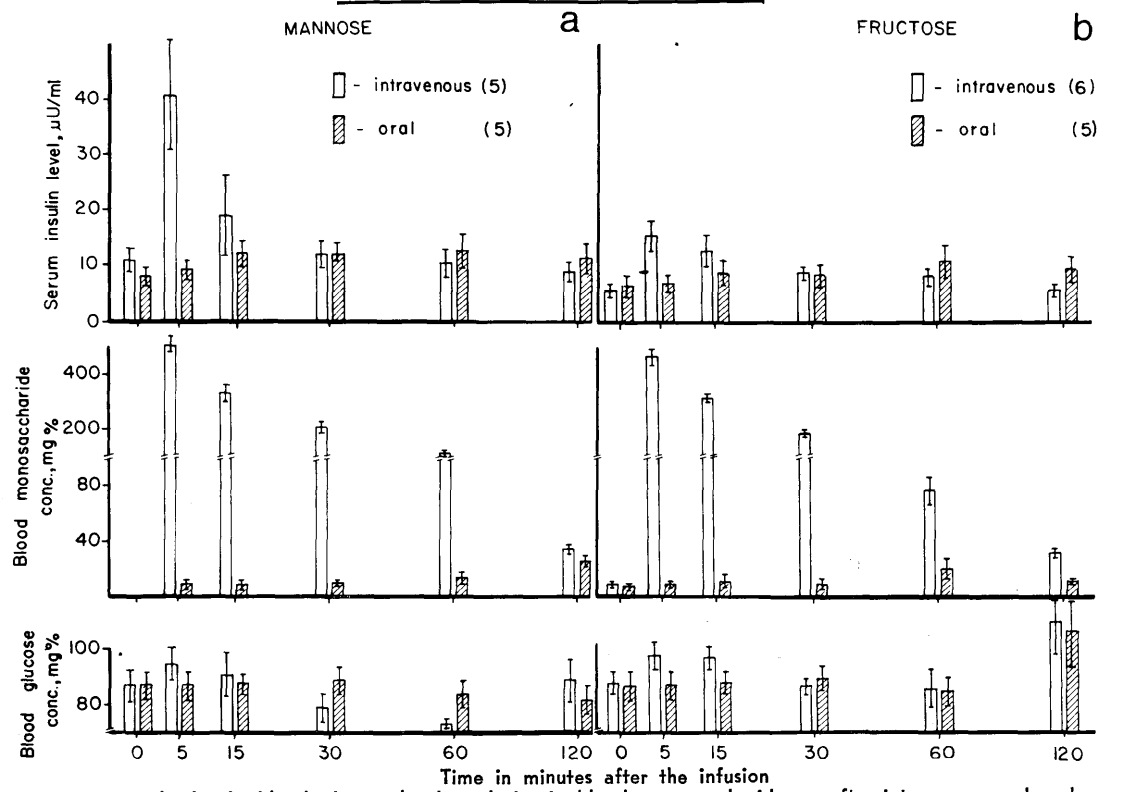

FIG. 3. Serum insulin levels, blood glucose levels and rise in blood monosaccharide: a, after intravenous and oral mannose, and b, after intravenous and oral fructose. Mean values ± S.E.M. Number of animals in parentheses.

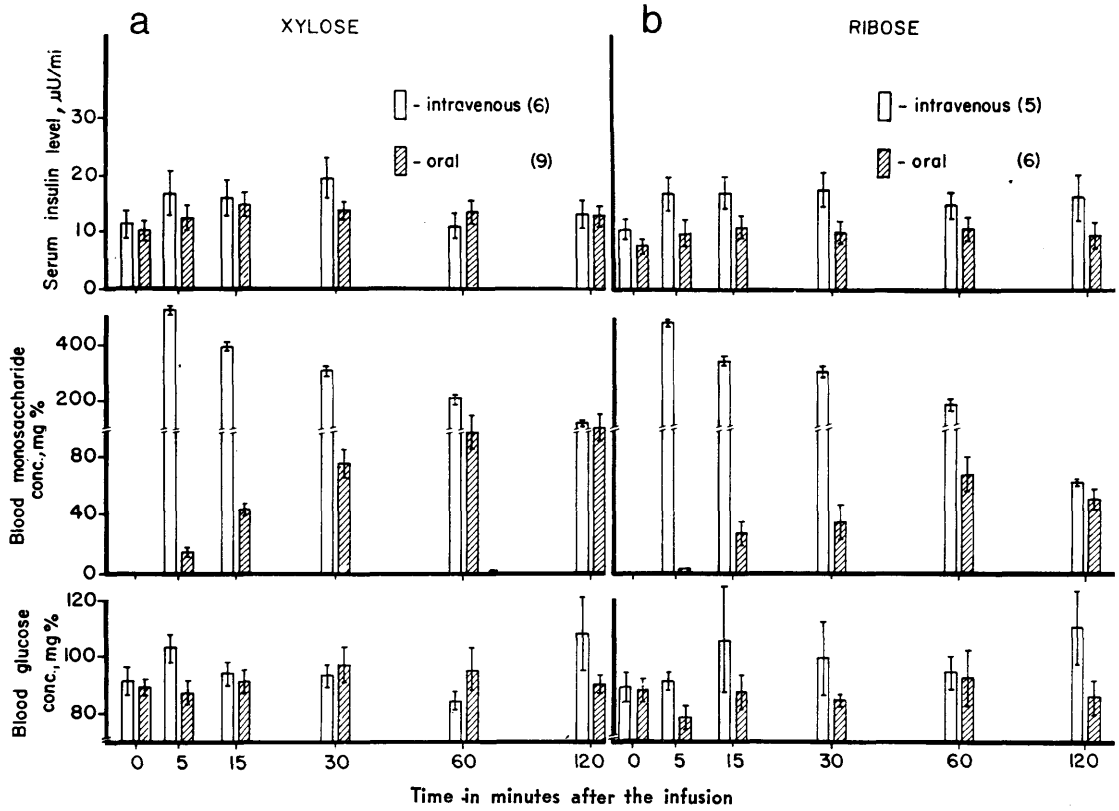

FIG. 4. Serum insulin levels, blood glucose levels and rise in blood monosaccharide: a, after intravenous and oral xylose, and b, after intravenous and oral ribose. Mean values ± S.E.M. Number of animals in parentheses.

level of 10.8 μU./ml. was elevated to 40.7 μU./ml. at five minutes. The insulin response was very transitory as the insulin dropped from the peak level to almost fasting level at thirty minutes. This is probably due to the fact that mannose disappeared from the circulation very rapidly and thus had limited time to stimulate insulin secretion. The oral infusion of mannose failed to stimulate insulin secretion. This failure of oral mannose to stimulate insulin secretion might be explained in terms of the very small rise in blood mannose level after oral insulin. Blood glucose level was not elevated following either intravenous or oral infusion of mannose.

Fructose:

Following intravenous fructose, a rise of 9.9 μU./ml. in insulin level ($p < .01$) at five minutes was noted. Like mannose, oral fructose did not promote insulin secretion. This was perhaps due to the small increase in blood fructose concentration following oral administration. A significant rise ($p < .05$) in blood glucose level was noted at 120 minutes after both intravenous and oral infusions. This was probably due to the conversion of fructose into glucose. The serum insulin level, however, was unaffected at this time interval.

Xylose:

A significant increase in serum insulin level was noted thirty minutes after the intravenous infusion of xylose ($p < .01$) figure 4a. After oral xylose blood xylose increased progressively and a smaller but significant ($p < .05$) increase in insulin was seen at fifteen minutes.

Ribose:

The level of insulin increased from 10.5 μU./ml. to 17.8 μU./ml. thirty minutes after the intravenous iufusion ($p < .02$), figure 4b. The insulin response was well sustained although blood ribose concentration decreased during two hours of experiment. Oral ribose did not elevate insulin level significantly ($p > .05$). Blood glucose level also did not change significantly ($p > .05$) following oral ribose.

α-methyl glucoside:

Since the oral administration of glucose, galactose and xylose elicited an insulin response similar to their intravenous infusions, while mannose, fructose and ribose did not, it was thought that the intestinal transport of a sugar might be associated with the insulinotropic

factor. Hence the effects of intravenous and oral infusion of α-methyl glucoside on insulin levels were studied (figure 5). This sugar analogue is transported in the gut by a mechanism similar to that of glucose.[10] There was no significant change in insulin level after either intravenous or oral infusions, although both infusions of α-methyl glucoside produced very high levels of blood monosaccharide. There were insignificant changes in blood glucose levels ($p > .05$) during these experiments. These findings suggest that in order for a monosaccharide to stimulate insulin secretion, it must have an intact reducing group regardless of the route of administration.

Pyruvate:

The possibility of a metabolite resulting from the infused sugar being responsible for the stimulation of insulin secretion was examined by infusing pyruvate intravenously. The results (figure 6a), showed that the infusion of pyruvate elevated insulin level to 26.8 μU./ml. at sixty minutes ($p < .01$), the increase appearing to be progressive with time. There was a significant rise ($p < .05$) in blood glucose level at 120 minutes. However, this rise in glucose concentration did not correspond with the rise in insulin level.

Glycine:

Following oral glycine administration there was a significant increase ($p < .05$) in insulin level at thirty minutes only (figure 6b).

DISCUSSION

All the true sugars investigated stimulated insulin secretion when given intravenously, but only glucose, galactose and xylose did so when given orally and only these sugars had appreciable increases in blood level

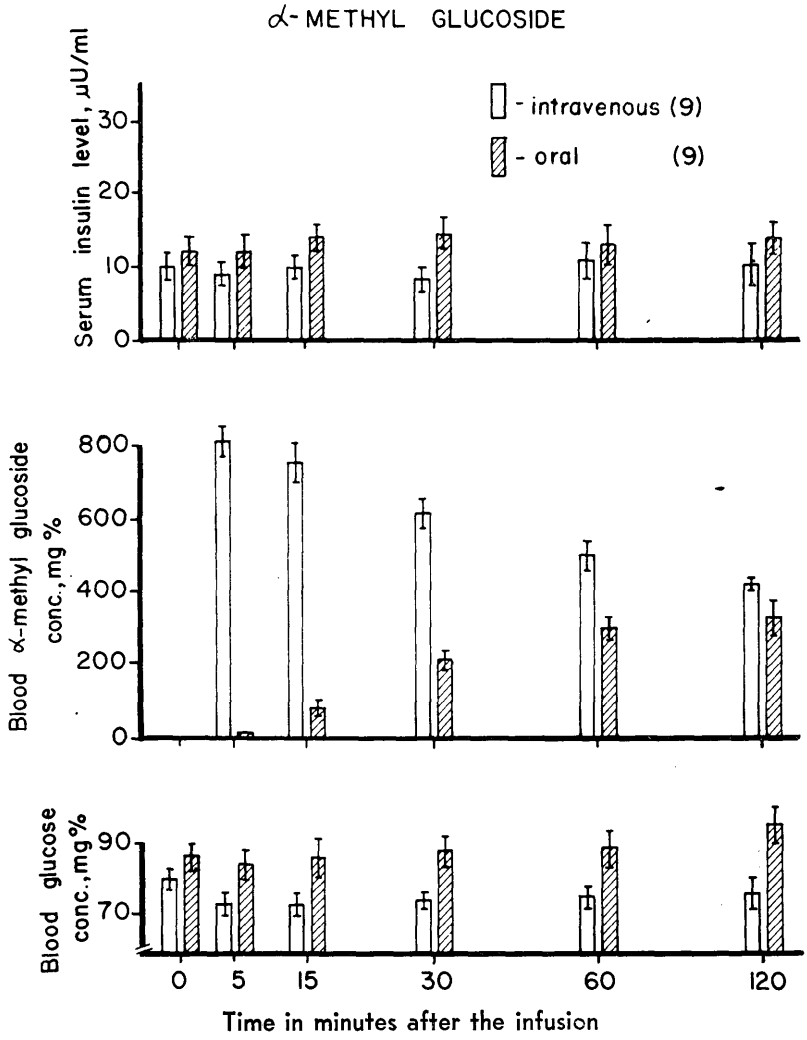

FIG. 5. Serum insulin levels, blood glucose levels and rise in blood α-methyl glucoside level after intravenous and oral α-methyl glucoside. Mean values ± S.E.M. Number of animals in parentheses.

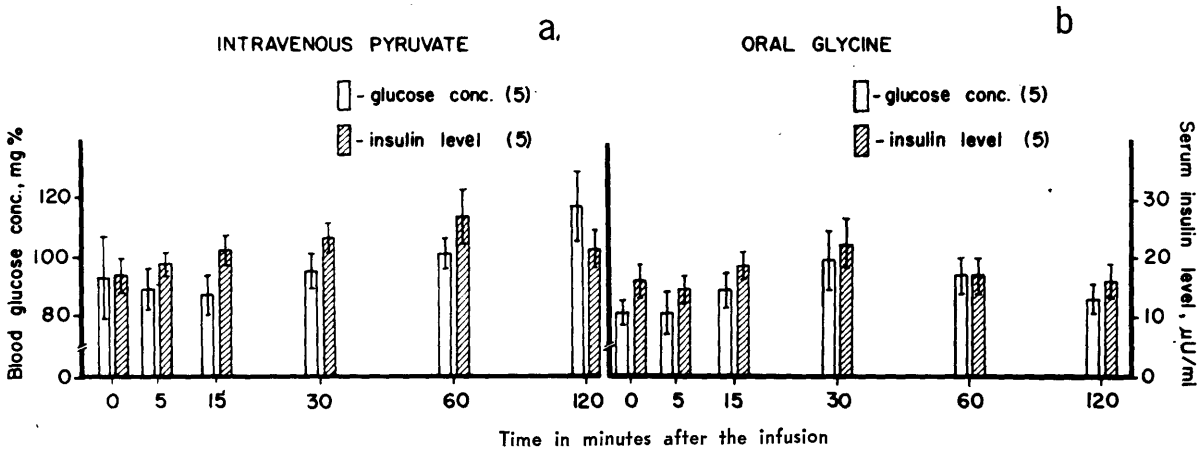

FIG. 6. Serum insulin and blood glucose levels: a, after intravenous pyruvate, and b, after oral glycine. Mean values ± S.E.M. Number of animals in parentheses.

after oral administration. Thus it would seem that the only monosaccharides that stimulated insulin secretion after oral ingestion were the ones that also produced an elevation of the blood monosaccharide level.

Although the blood levels of glucose, galactose and xylose after oral ingestion were lower than after intravenous injection, the increases in insulin level produced by both routes of administration were similar. This suggests that the degree of saccharidemia is not the sole stimulus to insulin secretion. This finding is in accord with the conclusions of McIntyre et al. and Elrick et al.[1,2] from human experiments that glucose in the gut leads to the elevation of an insulinotropic substance from the intestine. These workers found, however, that despite the lower blood glucose level after oral administration, the insulin response was much greater than after intravenous administration. In the present study, as noted, the insulin responses were similar after both intravenous and oral administration. This may be due to species difference.

The findings that oral galactose, xylose and also glycine led to an insulin response indicates that the phenomena of insulin secretion after oral administration is not specific to glucose. For a sugar to stimulate insulin secretion it would appear necessary for it to possess an active reducing group as following both intravenous and oral administration of α-methyl glucoside no insulin response was elicited, despite the high levels obtained after both types of infusions.

The possibility of an intermediary metabolite being involved in insulin secretion is suggested by the finding that the intravenous infusion of pyruvate increased serum insulin levels. However, the insulin response was delayed. This could be due to the slow penetration of pyruvate intracellularly.

ACKNOWLEDGMENT

The work reported in this paper was supported by a grant from the Medical Research Council of Canada.

We wish to thank Dr. F. Chebib, Department of Oral Biology, and Dr. N. Stephens, Department of Physiology, University of Manitoba, for advice regarding statistical analysis of the data.

REFERENCES

1 McIntyre, N., Holdsworth, C. D., and Turner, D. S.: Intestinal factors in the control of insulin secretion. J. Clin. Endocr. *25*:1317, 1965.

2 Elrick, H., Stimmler, L., Hlad, C. J., and Arai, Y.: Plasma insulin response to oral and intravenous glucose administration. J. Clin. Endocr. *24*:1076, 1964.

3 Dupré, J., and Beck, J. C.: Effect of an intestinal mucosal extract on glucose disposal and serum insulin-like activity in man. Diabetes *14*:440, 1965.

4 Dupré, J., and Beck, J. C.: Stimulation of release of insulin by an extract of intestinal mucosa. Diabetes *15*:555, 1966.

5 Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *153*:375, 1944.

6 Huggett, A. St. G., and Nixon, D. A.: Enzymatic determination of blood glucose. Biochem. J. *66*:12P, 1957.

7 Soeldner, J. S., and Slone, D.: Critical variables in the radioimmunoassay of serum insulin using the double antibody technic. Diabetes *14*:771, 1965.

8 Meade, R. C., and Klitgaard, H. M.: A simplified method for immunoassay of human serum insulin. J. Nucl. Med. *3*:407, 1962.

9 Ostle, B.: Statistics in Research. Iowa State College Press, 1956, pp. 272-73.

10 Wilson, T. H., and Landau, B. R.: Specificity of sugar transport by the intestine of the hamster. Amer. J. Physiol. *198*:99, 1960.