creases in total enzyme activity simply reflect balanced tissue growth.

Summary. Hexose monophosphate shunt (HMPS) dehydrogenase activity was measured in the remaining kidney at frequent intervals for 4 days after unilateral nephrectomy of rats. Total HMPS dehydrogenase activity in the residual kidney began to increase 18 hr after nephrectomy and was doubled (1.97) at 72 hr, where it remained at 96 hr (1.97). While the total soluble protein (20,000 *g* supernate) showed a continuous increase beginning about 18 hr after nephrectomy, HMPS dehydrogenase specific activity exhibited a biphasic pattern with peaks at 36 hr (25% increment) and 72 hr (40% increment). The data indicate that preferential synthesis of the HMPS dehydrogenase enzymes (G6PD and 6PGD) is an early response of the remaining kidney to unilateral nephrectomy.

1. Addis, T., Poo, L. J., and Lew, W., J. Biol. Chem. 115, 111 (1936); Arataki, M., Am. J. Anat. 36, 437 (1926); Moore, R. A., J. Exptl. Med. 50, 709 (1929).

2. Coe, F. L. and Korty, P., Am. J. Physiol. 213, 1585 (1967).

3. Nowinski, W. W., and Pigon, A., J. Histochem. Cytochem. 15, 32 (1967); Rosenthal, S., Teichmann, I., and Winkelmann, E., Acta Biol. Med. Ger. 8, 530 (1962).

4. Katz, J. and Rognstad, R., Biochemistry 6, 2227 (1967).

5. Sable, H. Z., Advan. Enzymol. 28, 391 (1966).

6. DeLuca, C. and Nitowsky, H. M., Biochim. Biophys. Acta 89, 208 (1964).

7. Gudbjarnason, S., Cowan, C., and Bing, R. J., Life Sci. 6, 1093 (1967).

8. Dies, F., and Lotspeich, W. D., Am. J. Physiol. 212, 61 (1967); Lotspeich, W. D., Science 155, 1066 (1967).

9. Weber, G. and Cantero, A., Cancer Res. 17, 995 (1957); Ostanenko, I. A., Zh. Evol. Biokhim. Fiziol. 2, 541 (1966).

10. Fisher, R. A. and Yates, F., *in* "Statistical Tables for Biological, Agricultural, and Medical Research," 6th Ed. Hafner, New York (1963).

11. Zamecnik, P. C. and Keller, E. B., J. Biol. Chem. 209, 337 (1954).

12. Kornberg, A. and Horecker, B. L., *in* "Methods in Enzymology" (S. P. Colwick and N. O. Kaplan, eds.) Vol. 1, p. 323 Academic Press, New York (1955).

13. Aqvist, S. E. G., Acta Chem. Scand. 5, 1065 (1951).

14. Phillips, T. L. and Leong, G. F., Cancer Res. 27, 286 (1967).

15. Bucher, N. L. R. and Glinos, A. D., Cancer Res. 10, 324 (1950).

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The Stimulation of Insulin Secretion in Dogs by Tris (Hydroxymethyl) Aminomethane* (33431)

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During investigations concerning the metabolism of insulin it was noticed that when Tris[2] was used as a carrier reagent for insulin, a greater amount of insulin appeared in portal vein plasma than could be attributed to the injected insulin. This finding strongly suggested that Tris stimulated the release of endogenous insulin. According to Bennett and Tarail (1), Tris, at pH 10.2, if injected in sufficient quantity produced severe hypoglycemia in normal dogs. They suggested that the hypoglycemia might be due to insulin release and perhaps also to the facilitation of

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[2] Tris (hydroxymethyl) aminomethane also known as THAM: Sigma Chemical Co., St. Louis, Missouri.

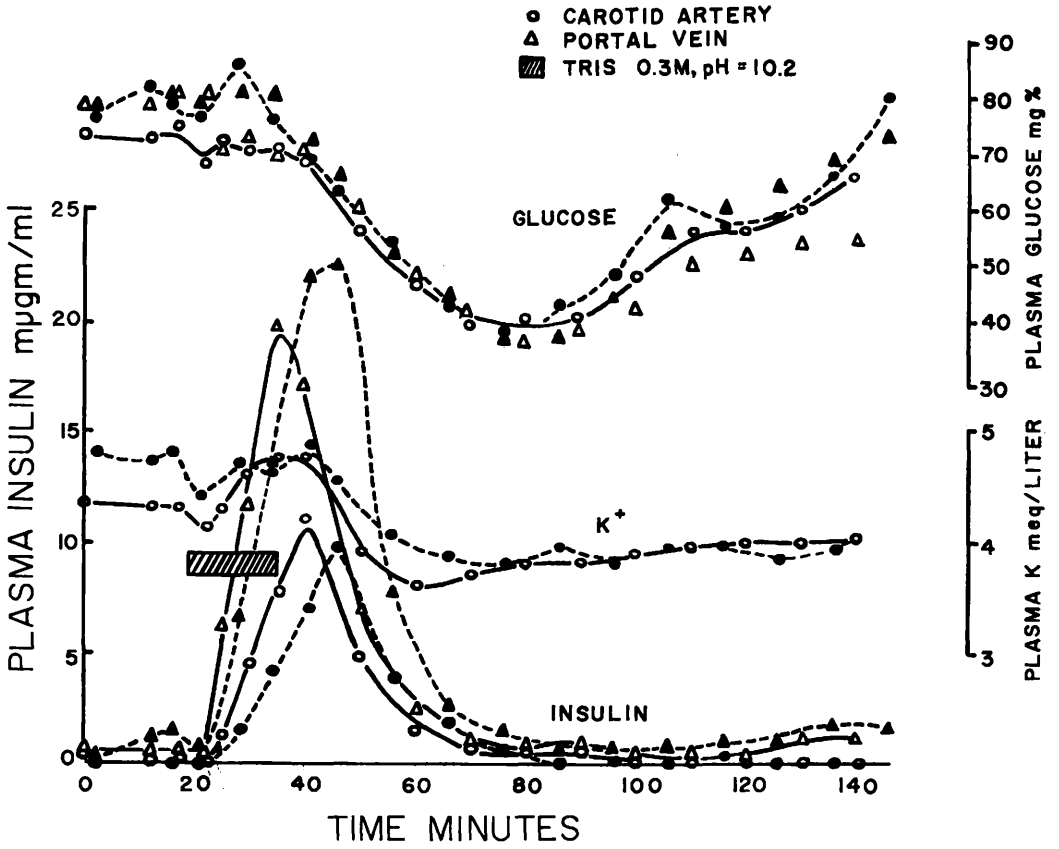

FIG. 1. Plasma responses to Tris, pH 10.2, infusion. Solid and broken lines represent different dogs.

insulin action on glucose uptake. Hartman (2) found that Tris was more effective in inducing hypoglycemia in normal than in pancreatectomized dogs and also attributed this Tris effect to an induced increase in insulin release. However, in neither case were plasma insulin levels estimated. Furthermore, Seltzer (3) failed to observe significant change in insulin-like activity (rat diaphragm assay) of pancreatic vein plasma following Tris administration to dogs, and Buse *et al.* (4) by *in vitro* investigations obtained evidence that Tris directly stimulated utilization of glucose by skeletal muscle.

Since several substances are capable of inducing hypoglycemia independent of insulin release the present study was initiated to determine directly whether Tris stimulates insulin secretion as estimated by immunoassay. After this effect was found, additional work was done to estimate whether the Tris-induced increase in plasma insulin was sufficient to account for Tris-induced hypoglycemia. Studies were also conducted to compare Tris and glucose as to relative effectiveness in inducing insulin secretion in several dogs.

Methods. Mongrel dogs ranging in weight from 9 to 15 kg were fasted overnight and anesthetized with nembutal. A cannula was placed in the carotid artery so the tip was in the brachiocephalic artery. Another cannula was placed in a gastric venous branch so the tip was in the portal vein within 1–2 cm of the liver. Blood samples were withdrawn from these cannulas. For infusion purposes a cannula was placed in the cephalic vein and in some experiments a needle cannula was placed in the cephalic pancreaticoduodenal artery.

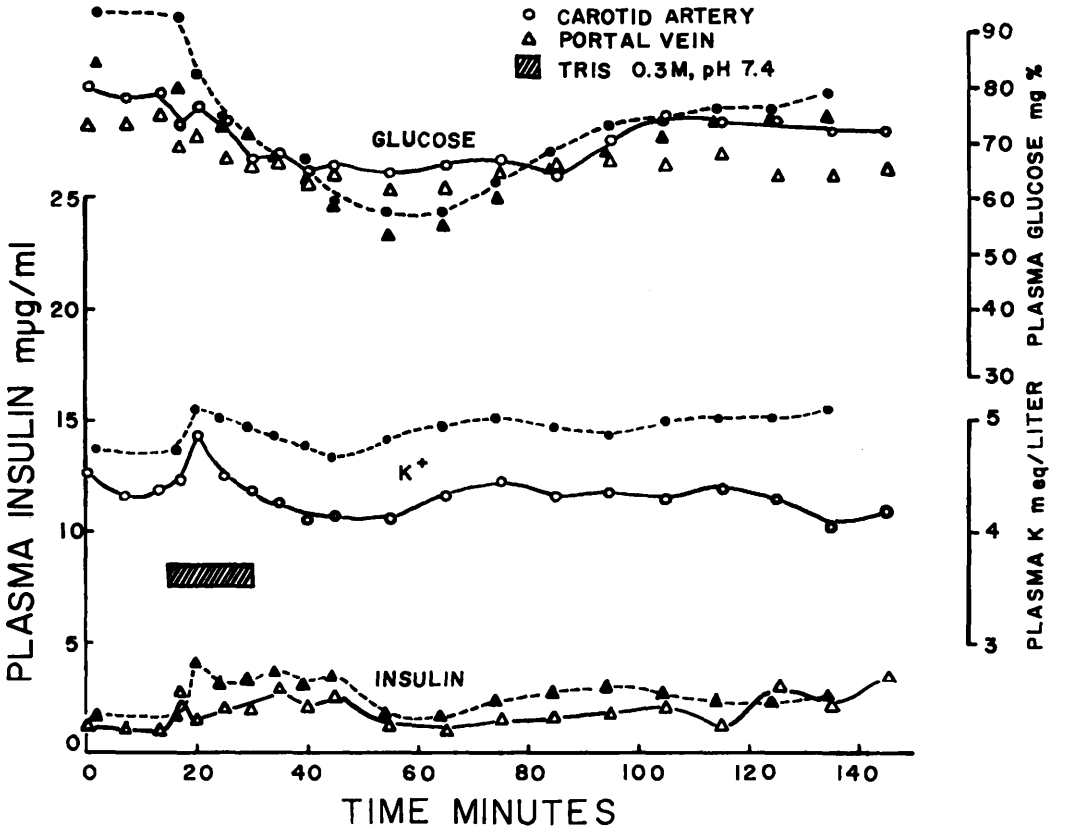

FIG. 2. Plasma responses to Tris, pH 7.4, infusion. Solid and broken lines represent different dogs.

All cannulas for blood collection were heparinized with 1 mg/ml. Samples (2.5 ml) of blood were collected at timed intervals; hematocrit tube was filled; and blood sample was placed immediately in an ice water bath. All samples were centrifuged at the end of the experiment and the plasma was frozen until analysis.

All plasma samples were assayed for glucose by glucose oxidase (5), and for insulin by immunoassay (6). In some experiments plasma potassium was determined by flame photometry (7). All reported values are averages of duplicate determinations.

Experimental and Results. (1) Tris infusion into a peripheral vein. To determine whether hypoglycemia was associated with insulin secretion, two dogs were infused via the cephalic vein with Tris 0.3 *M*, pH 10.2, in distilled water at a rate of 0.5 mmoles/kg/min for approximately 17 min, procedure reported by Bennett and Tarail (1) to result in severe hypoglycemia. Since they also found that a decrease in pH of Tris decreased the hypoglycemia response, two dogs were infused as above but with Tris, pH 7.4.

In confirmation of Bennett and Tarail, infusion of Tris, pH 10.2, resulted in severe hypoglycemia (Fig. 1). Plasma glucose fell from normal to 40 mg/100 ml within an hour after the infusion began and returned to normal within the next hour. Plasma glucose did not start to fall until plasma insulin had risen several fold. Shortly after 5 min of Tris infusion, plasma insulin rose rapidly, and then fell rapidly when the infusion stopped. The rise in portal vein plasma insulin was higher than in the artery which indicates a secretion of insulin from the pancreas. During these two experiments the dogs had to be maintained by artificial respiration.

When the Tris was infused at pH 7.4, the

rise of insulin in the portal vein plasma was much less, 2 to 3 times normal as compared to 20 times normal with Tris, pH 10.2 (Fig. 2). The hypoglycemic effect was also less, reaching a minimal level of around 60 mg/100 ml as compared to 40 mg/100 ml with basic Tris.

The effect of either Tris infusion on plasma potassium was quite small, i.e., no variation greater than 1 meq/liter was measured (Figs 1 and 2). However, in all experiments there was a trend for a rise to occur during the infusion followed by a fall, which agrees with other investigators' findings (8).

(2) Comparison of Tris released endogenous insulin hypoglycemia, to insulin injected hypoglycemia. To investigate whether Tris-induced release of plasma insulin was the main determinant of hypoglycemia or whether Tris itself was independently hypoglycemic, or a potentiator of insulin hypoglycemia, the following comparison was made. Animals infused via a peripheral vein with Tris were compared to animals infused via the portal vein with sufficient insulin to closely simulate that insulin released during Tris infusion. Two dogs were each infused at 1 ml/min for 20 min/infusion via the portal vein with two doses of bovine insulin. Each dog received 40 μg followed 2 hr later by 80 μg. The area above the curve from normoglycemia to minimal hypoglycemia in arterial plasma was measured with a planimeter and plotted against the simultaneous area under the arterial insulin curve starting with the rise from normal insulin values until the curve returned to normal. The data of Fig. 1 and 2 were also analyzed in this manner and the comparison is plotted in Fig. 3. With the limited data available no clear-cut difference is discernable in hypoglycemia resulting from an equivalent arterial hyperinsulinemia when the arterial hyperinsulinemia resulted from: (a) Tris infusion or (b) portal vein bovine insulin infusion. Further corroborating data of this nature would be necessary to statistically settle the contentions that *in vivo* Tris potentiates insulin hypoglycemia and/or that Tris is hypoglycemic independent of insulin action.

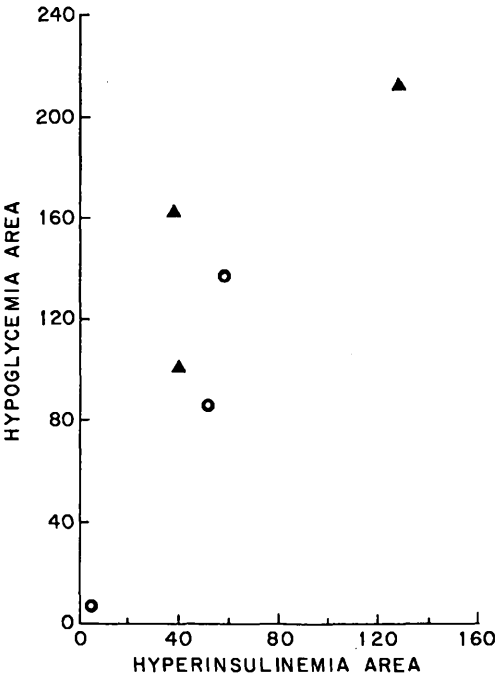

FIG. 3. Comparison of arterial plasma hypoglycemia to hyperinsulinemia: (O), Tris via peripheral vein; (▲), bovine insulin via portal vein.

(3) Comparison of glucose stimulated insulin release to Tris stimulated insulin release. To compare the relative effectiveness of Tris to glucose in the stimulation of insulin secretion, three dogs were each infused via a needle cannula in the pancreaticoduodenal artery with 0.4 *M* glucose at 1 ml/min for 30 min, followed approximately 1 hr later by 0.4 *M* Tris, pH 10.2, 1 ml/min for 30 min.

The infusion of equimolar concentrations of glucose and Tris base into the pancreaticoduodenal artery caused insulin secretion which could be detected in both the portal venous and arterial plasma (Fig. 4). Great variability existed from dog to dog. In two of the three experiments the insulin response to the glucose infusion appeared greater than the response to a molar equivalent of Tris; in the third experiment the opposite occurred. In all three experiments there were consistent temporal differences in the induced hyperinsulinemias. The insulin rise after glucose was detectible by 2.5 min after the infusion began whereas the rise in response to Tris was never detectible within the first 2.5 min and sometimes not until as late as 10 min after

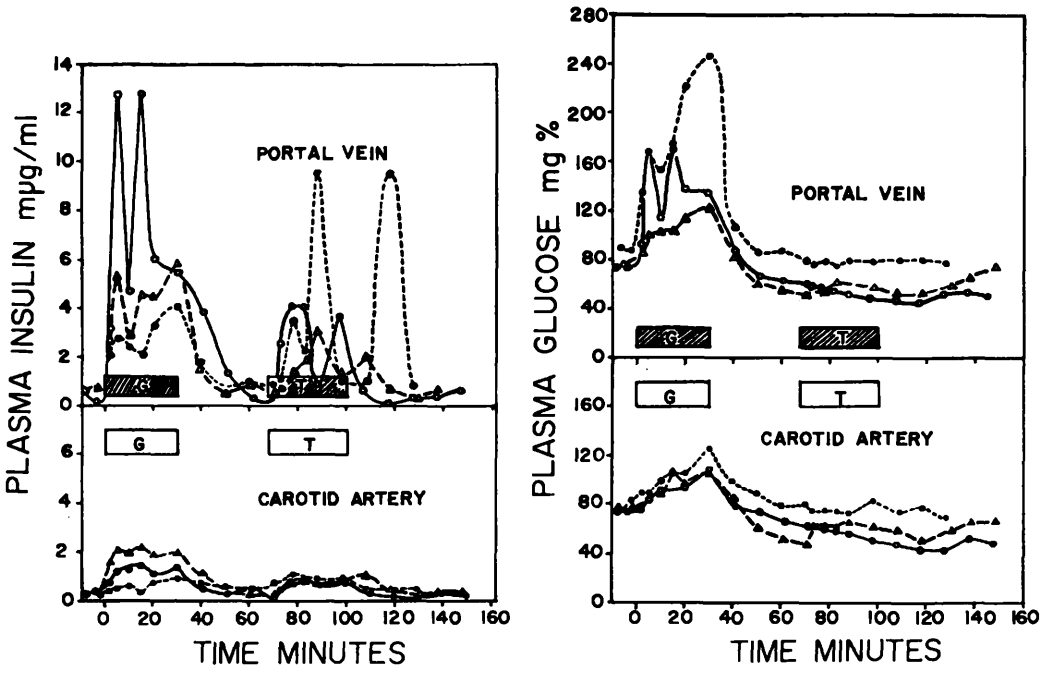

FIG. 4. Comparison of plasma responses to equimolar infusions of glucose and Tris, pH 10.2. Each symbol represents one experiment on one dog.

the infusion had begun. With both substances there were peaks and valleys of insulin plasma levels during the infusion. After the glucose infusion stopped insulin levels always fell, whereas with Tris infusion there was sometimes a rise in insulin even after the infusion stopped.

Arterial glucose levels rose rather constantly with glucose infusion and since the glucose infusion was at a constant rate the change in insulin secretion rates could not be attributed to changes in glucose levels reaching the pancreas unless blood flow rates into the pancreas or through the venous portal system changed. This variable response of insulin secretion to a constant glucose stimulus has also been reported by other investigators when flow rates were constant (9, 10). As yet there is no definite explanation for this phenomenon.

Discussion. The results indicate that Tris acts *in vivo to* cause hypoglycemia by stimulating the release of insulin from the pancreas in the presence of normal or below normal blood glucose levels. This stimulating capacity appears to be pH dependent, a finding which agrees with that of other investigators (8) who have shown that at acid pH Tris either had no effect on blood glucose or was hyperglycemic, whereas at basic pH Tris was always hypoglycemic. It is probably the unionized fraction of Tris predominating at higher pH which penetrates cells to result in a subsequent insulin release. Further studies in this laboratory (11) with isolated pancreatic islets have shown that Tris is capable of releasing insulin from the islets into the incubation medium if proper pH and concentrations of Tris were used. To a degree, the more basic the pH the more effective was the Tris.

Although the present investigations offer no explanation as to the mechanism of action of Tris, the literature suggests several alternatives: (a) Tris is known to release potassium from cells (8). Increase in plasma potassium has a stimulatory effect on insulin secretion (12). In this study, plasma potassium rose slightly during Tris infusion; perhaps potassium around the islet increased even more so. (b) Tris is a chelator of divalent cations. Chelation of Zn (13, 14) with subse-

quent disaggregation of islet cell insulin–Zn complexes has been suggested as a mechanism for insulin release. (c) Tris is an inhibitor of certain enzymes (1) and may act on glucose metabolism within the islet to allow for the accumulation of a glucose metabolite involved in stimulating insulin secretion. Additionally, cholinesterases are inhibited by Tris (15). Cholinergic mechanisms have been shown to be involved in insulin secretion (16). Investigations of these several alternatives with Tris may prove useful in furthering the understanding of the phenomena involved in insulin release mechanisms.

Summary. Dogs infused with Tris buffer exhibited not only the hypoglycemia reported by others, but also an increase in plasma insulin level as estimated by immunoassay. The induced hyperinsulinemia was much greater in portal vein than in peripheral vein plasma samples. Both hypoglycemia and hyperinsulinemia were much more pronounced when the Tris infused was at pH 10.2 than at pH 7.4. Since Tris molecules are less ionized at higher pH, these findings are in accord with the hypothesis that hypoglycemia induced by Tris is due to this compound penetrating into and inducing release of insulin from the pancreas. The hyperinsulinemia induced by pH 10.2 Tris infusion into the pancreatic artery started later and was more variable than that induced by an equimolar infusion of glucose.

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1. Furness, F. N. (ed.), Ann. N. Y. Acad. Sci. 93, 333 (1961).

2. Hartman, M. C., Nadler, S. H., Ausman, R. K., and Ambrus, J., Federation Proc. 24, 577 (1965).

3. Seltzer, H. S. and Smith, W. L., Clin. Invest. 39, 1027 (1960).

4. Buse, M. G., Buse, J., McMaster, J., and Krech, L. H., Metabolism 13, 339 (1964).

5. Bergmeyer, H. and Bernt, E., "Enzymatic Analysis," p. 123. Academic Press, New York (1963).

6. Zaharko, D. S. and Beck, L. V., Diabetes 17, 444 (1968).

7. Weichselbaum, T. E. and Varney, P. L., Proc. Soc. Exptl. Biol. Med. 71, 570 (1949).

8. Nahas, G., Pharmacol. Rev. 14, 447 (1962).

9. Grodsky, G. M., Bennett, L. L., Smith, D., and Nemechek, K., *in* "Tolbutamide—after 10 years, Proc., Brook Lodge Symposium" (W. J. H. Butterfield, ed.), p. 11. Excerpta Medica Foundation, New York (1967).

10. Kanazawa, Y., Kuzuya, T., Ide, T., and Kosaka, T., Am. J. Physiol. 211, 442 (1966).

11. Fedynskyj, N., Zaharko, D. S., and Beck, L. V., Federation Proc. 27, 496 (1968).

12. Grodsky, G. M. and Bennett, L. L., Diabetes 15, 910 (1966).

13. Maske, H., Diabetes 6, 335 (1957).

14. Ninomiya, R., Miura, Y., Kosaka, K., Nakao, K., and Okinaka, S., Diabetes 15, 44 (1966).

15. Pavlic, M., Biochim. Biophys. Acta Enzymol. 139, 133 (1967).

16. Malaisse, W., Malaisse-Lagae, F., Wright, P. H., and Ashmore, J., Endocrinology 80, 975 (1967).

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Effects of Estrogen on Pituitary Prolactin Levels of Female Rats Bearing Median Eminence Implants of Prolactin* (33432)

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Implants of LH (1), FSH (2), ACTH (3) or GH (4) into the median eminence area of the rat hypothalamus have been reported to specifically reduce anterior pituitary (AP) concentration of each of these hormones. Re-

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