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The Effect of Oral and Intravenous D-Ribose on Plasma Insulin Levels in Unanesthetized Dogs* (32894)

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Although it is widely acknowledged (1–6) that increased blood glucose levels have a direct stimulating effect on pancreatic β-cells, there has been evidence indicating that secondary, extrapancreatic mechanism(s) for insulin secretion exist. Some of this evidence has indicated that the response to D-ribose may involve such a mechanism. It has been shown that intravenous administration of ribose causes hypoglycemia in both man (7) and anesthetized dogs (3). In addition, intraportal infusion of ribose into anesthetized dogs has resulted in immunoassayable insulin secretion (8) although no insulin secretion was elicited from sliced rabbit pancreas incubated *in vitro* with a ribose-containing medium (6, 9). Since elevated epinephrine levels inhibit insulin secretion despite resulting hyperglycemia (10), it has been suggested that the increased insulin levels observed following intravenous sugar infusion represent, in part, reversal of epinephrine block of the β-cells in a surgically stressed animal (11). It was the purpose of this investigation to clarify further the above questions by studying the effects of oral and intravenous ribose on plasma insulin levels in awake, alert, unstressed dogs.

Procedure. Nine mongrel dogs ranging in weight from 12–22 kg were anesthetized with sodium pentabarbital and prepared for the experimental procedure. Silastic cannulas were placed in the femoral artery for future sampling and in one or two major veins (portal, femoral, or external jugular) for infusing D-ribose. After a minimum recovery period of 72 hours, the dogs were subjected to an 18-hour fast followed by an experiment. A minimum of 48 hours was allowed to elapse between two experiments on a given dog. In

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each experiment D-ribose (0.6 gm or 3.0 gm) was administered via two of four possible routes (orally, intraportally, intrafemorally, or via the external jugular vein). Oral administration was accomplished by inducing the dog to swallow a gastric tube through which 12 ml of 5% or 25% ribose solution could easily be injected directly into the stomach. The intravenous administration was accomplished by constant infusion of a 5% or 25% solution of ribose at 0.76 ml/min for 15 min. Preceding each ribose administration, 0.9% NaCl solution was either infused at 0.76 ml/min for 30 min or fed via gastric tube, depending on the route by which the ribose was to be administered.

The total time elapsed during the experiment was 155 min. All blood samples were taken from the femoral artery, and were collected in graduated centrifuge tubes containing powdered heparin. Three of the 8 ml taken each time were poured into an oxalate–fluoride tube for blood sugar analysis by AutoAnalyzer (ferricyanide method) both before and after incubation with glucose oxidase. Thus, glucose was measured specifically, and ribose was estimated as nonglucose reducing substance. We were able to confirm that the increased nonglucose reducing substance in several blood samples was indeed predominantly ribose by thin-layer chromatography (Gelman Instrument Company's SG-ITLC with an ethyl acetate, pyridine, and water solvent system). The plasma from the remaining blood was frozen for insulin immunoassay (12) at a later date.

Postoperatively and between experiments dogs were maintained on a diet of meat and milk occasionally supplemented with vitamins and oral broad spectrum antibiotics. The tips of the intrafemoral cannulas were found on autopsy to lie in the inferior vena cava; those of the external jugular vein cannulas were found at the junction of the superior vena cava and right atrium; and the tips of the intraportal cannulae (which had been threaded in through a mesenteric vein) were, in fact, within the portal vein.

Results. Figure 1 illustrates the combined data for 16 experiments in 9 dogs. The relative rises of insulin levels following D-ribose administration are quite striking. The mean

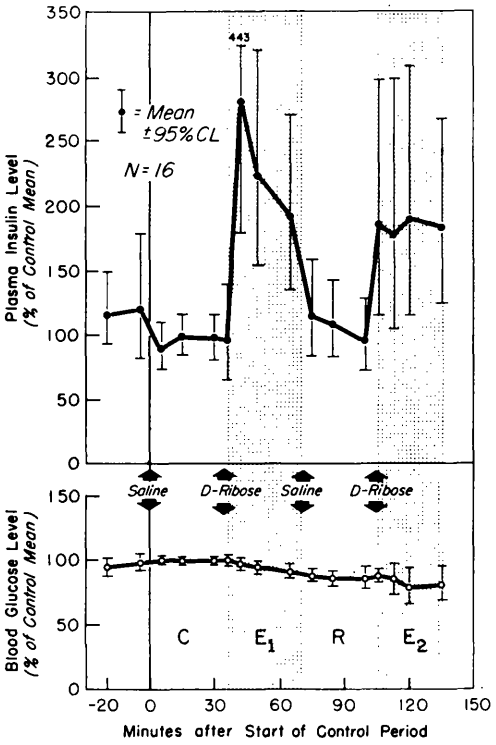

FIG. 1 represents relative plasma insulin levels (above) and relative blood glucose levels (below) for 16 experiments. For each experiment each absolute value of plasma insulin or blood glucose was re-expressed as a percentage of the mean of the three control values of that experiment. Each point on this figure represents the mean of the percentages for all the samples taken at the given time in the 16 experiments ± 95% confidence limits. Samples were taken 5, 15, and 30 min following each administration of saline and 1, 7, 15, and 30 min following each administration of D-ribose.

levels of plasma insulin during the first experimental period (E_1) are significantly higher than those during the preceding control period (C) and during succeeding recovery period (R) ($p<.02$, and $<.001$, respectively). Similarly, mean insulin levels during the second experimental period (E_2) are higher than those during R ($p<.01$). These insulin rises occur despite blood glucose levels which were slowly but steadily falling. Blood ribose levels regularly rose to 25–35 mg/100 ml following the administration of 3 gm of ribose intrafemorally. However, with other routes and/or lower dosage (0.6 gm) insulin levels rose while blood nonglucose reducing substance

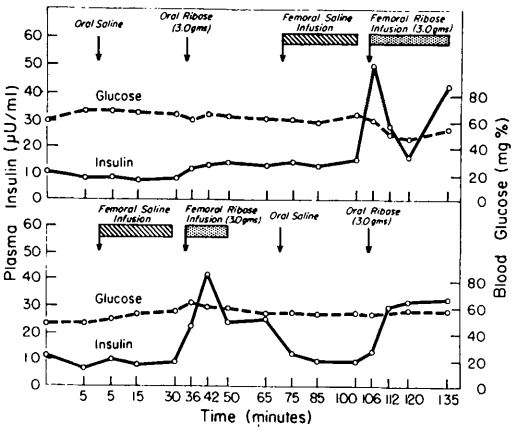

FIG. 2 represents four experiments done on dog CH21B. Each point on the upper graph represents the mean for values of two identical experiments in which D-ribose was first fed and then infused intrafemorally. Each point on the lower graph similarly represents the mean for two experiments in which D-ribose was first infused and then fed.

often remained constant. In fact, the largest insulin responses occurred in two experiments in which the blood ribose levels did not change. Conversely, three of the poorest insulin responses occurred while blood ribose levels were in excess of 50 mg/100 ml.

Figure 2 shows a comparison of the insulin responses of dog CH21B to oral and intravenous doses of 3 gm of D-ribose in four experiments. Insulin levels rose sharply and quickly following all four intrafemoral ribose administrations. Following oral administration, however, insulin levels rose significantly only twice. Also seen in the upper half of Fig. 2 (and to a lesser extent in the lower half) is an apparent bimodal insulin response to femoral ribose infusion. This phenomenon was also observed in two experiments performed on one other dog. Table I shows a further comparison of the insulin response to the two routes of administration in the 10 experiments in which ribose was given both orally and via peripheral vein (femoral or jugular). Again, the insulin responses to intravenous infusion were more consistently elevated than the response to the same dose of ribose given orally.

Discussion. The data summarized in Fig. 1 give further evidence for the stimulating effect of D-ribose on insulin secretion. Small amounts of ribose administered either orally or intravenously resulted in significant increases in plasma insulin levels despite the lack of rise in blood glucose levels. The previously mentioned work by others (6, 9) indicates that these increased plasma insulin levels probably were not the result of direct β-cell

TABLE I. Comparison of Insulin Response Following Oral Versus Intravenous Administration of D-Ribose in 10 Experiments.

Dog no.	Date of expt	Routes[a]	Dose (gm)	iv response[b]	Oral response[b]	Recovery[b]
CH21B	7- 8-66	F–O	3.0	424	396	108
	7-12-66	F–O	3.0	218	212	127
	7-15-66	O–F	3.0	559	233	270
	7-20-66	O–F	3.0	372	122	122
CH25B	8- 1-66	O–F	0.6	430	360	82
CH26B	8-15-66	O–EJ	3.0	90	215	166
CH27B	8-22-66	EJ–O	0.6	1086	39	140
CH28B	8-26-66	O–EJ	3.0	185	223	195
	8-30-66	O–EJ	0.6	1189	393	121
CH29B	9-10-66	O–EJ	0.6	264	148	99
			Mean	482[c]	234[c]	143

[a] Abbrev.: F = femoral infusion; O = oral feeding; and EJ = external jugular infusion. The letters are given in the order in which the procedures were done in each experiment (e.g., F–O = femoral infusion followed by oral feeding).

[b] Each value listed represents the average plasma insulin level during the indicated period expressed as a percentage of the mean control for that experiment.

[c] $p < .05$ when assigning equal importance to all 10 experiments (t test); $p < .10$ when using only one average value for each of the six dogs (t test or Wilcoxon signed rank test).

stimulation by ribose. Also, our results seem to indicate that some dissociation exists between blood ribose levels and insulin response.

The lack of elevated blood glucose levels indicates that some mechanism other than direct β-cell stimulation by glucose is responsible for the increased plasma insulin levels. Since the animals were used under conditions of minimum stress, catecholamine levels were almost certainly not markedly elevated. Therefore, the mechanism of action of D-ribose in these experiments is not associated with some ameliorating effect on epinephrine block of the β-cells. Previous work with D-ribose in anesthetized dogs in our laboratory has led to the suggestion that a mechanism for insulin release involving the liver may exist (8). The data presented in this paper, while also subject to other interpretations, are still consistent with this hypothesis.

Sites other than the liver, however, have been postulated as being involved in an extrapancreatic mechanism for insulin release. Several workers have observed that glucose given orally results in greater insulin release than glucose given intravenously (13, 14). Thus, McIntyre *et al.* have postulated that some intestinal hormone such as secretin may be involved in a mechanism for insulin release (14). Other workers have suggested that glucagon might be the mediator of such a mechanism (15). Recent evidence has supported this idea. Lawrence has found increased glucagon levels following oral, but not intravenous, administration of glucose (18). Several others have shown that glucagon causes release of insulin from the pancreas *in vitro* (17, 18). However, our data indicate that intravenous administration of D-ribose is more consistently effective in causing insulin release than oral administration. Thus it is difficult for us to associate the mechanism of action of D-ribose with any of the proposed mechanisms for insulin release involving primary stimulation of intestinal mucosa. Since the work reported here was completed (19) Steinberg *et al.* have reported finding increased peripheral plasma insulin levels following intravenous D-ribose infusion into humans (20), in agreement with our present findings in dogs.

Summary. The administration of D-ribose was followed quickly by a sharp rise in plasma insulin levels in 9 awake, alert, unstressed dogs (16 experiments). The elevated insulin levels occurred while blood glucose levels remained constant or dropped. Oral administration of ribose was not as effective as intravenous administration in causing insulin release. Thus the mechanism of action of ribose in these experiments does not appear to be associated with any gastrointestinal factor.

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Effect of Chloramphenicol on the Metabolism and Lethality of Cyclophosphamide in Rats* (32895)

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Cyclophosphamide Cytoxan, *N,N*-bis (β-chloroethyl)-N′, *O*-propylene phosphoric acid ester diamine monohydrate, NSC 26271) is a potent antineoplastic agent of the nitrogen mustard class and has been used in the treatment of patients with many types of neoplastic diseases (1). In contrast to the nitrogen mustards ordinarily used in cancer chemotherapy, cyclophosphamide is inert when placed in direct contact with bacteria, leukocytes, and most tumor cells in culture. Its action depends on *in vivo* activation which occurs in the liver and perhaps in other sites (2,3). The subcellular site of enzymic activation in the liver is the endoplasmic reticulum. The enzyme system which activates cyclophosphamide has been demonstrated to be affected by a number of factors that alter the microsomal metabolism of various drugs. Examples are phenobarbital (4) or chlordane (5) induced stimulation of microsomal enzymes and SKF 525-A (β-diethylaminoethyldiphenylpropylacetate) inhibition of the enzyme system (6).

This paper reports the effect of chloramphenicol (Chloromycetin), an antibiotic which is also an effective inhibitor of microsomal drug metabolism in rodents (7), on the formation of alkylating substance from cyclophosphamide *in vivo* and *in vitro* and toxicity after cyclophosphamide treatment.

Methods. Cyclophosphamide (Cytoxan) and chloramphenicol sodium succinate (Chloromycetin) were obtained commercially.

The active metabolite of cyclophosphamide was estimated by the spontaneous alkylation of γ-(4-nitrobenzyl)-pyridine (NBP reagent) (8, 9). Urine was collected from rats after treatment with cyclophosphamide and chloramphenicol or saline. Plastic metabolism cages were used, the urine was obtained, the cages were rinsed with distilled water, and the urine and rinsings were brought to a final volume of 50 ml with distilled water. Triplicate 3.0-ml aliquots were taken from each total volume for assay of alkylating material. Plasma (1.0 ml) was obtained from blood taken from anesthetized rats by cardiac puncture. The plasma was acidified with 0.2 ml of 1 *N* HCl and heated in a boiling water bath for 3 min. Three ml of distilled water were added, the solutions were mixed, and then centrifuged for 15 min prior to taking a 3.0-ml aliquot for assay of active metabolite.

In the *in vitro* metabolic studies of microsomal metabolism, 5.0 ml of 6.6% trichloroacetic acid was added to incubation beaker to stop the reaction and precipitate the protein at the end of the incubation period. The solutions were mixed and poured into a heavy walled centrifuge tube and centrifuged prior to taking a 3.0-ml aliquot for assay of material which would react with the NBP reagent. Hepatic supernatant fraction (9000*g*) provided the enzymes. The conditions of *in vitro* metabolism provided a NADPH (TPNH) generating system and an atmosphere of oxygen at 37°C. These conditions of incubation, cofactors and concentrations have been previously described (10). The substrate concentration of cyclophosphamide was 10 mg/5.0-ml incubation volume (8.7×10^{-3} *M*).

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