DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date . JAN 24 2000

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From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	D-Ribose
Firm:	Leiner Health Products
Date Received by FDA:	January 21, 2000
90-day Date:	April 17, 2000

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after April 19, 2000.

Robert J. Moore

Robert J. Moore, Ph.D.

95S-0316

RPT 63

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

JAN 24 2000

Hany Farag
Director of Regulatory Affairs
Leiner Health Products
901 East 233rd Street
Carson, California 90745-6204

Dear Mr. Farag:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) dated January 18, 2000, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., D-Ribose) was received by the Food and Drug Administration (FDA) on January 21, 2000. Your submission will be kept confidential for 90 days from the date of receipt, and after April 19, 2000, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

Sincerely,

Robert J. Moore

Robert J. Moore, Ph.D.
Senior Regulatory Scientist
Division of Compliance and Enforcement
Office of Nutritional Products, Labeling,
and Dietary Supplements

LEINER
HEALTH PRODUCTS

901 E. 233rd Street
Carson, California
90745-6204
310/835-8400
Fax 310/835-6615

RECEIVED
JAN 21 2000

January 18, 2000

Office of Special Nutritionals (HFS-450)
Center for Food and Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20204

Re: Submission of 75 – Day Premarket Notification for New Dietary Ingredient.

Dear Sir or Madam:

In accordance with the requirements of section 8 of the Dietary Supplement Health and Education Act, Leiner Health Products is notifying the Food and Drug Administration that it will be marketing d-Ribose as a new dietary ingredient.

We take this action with the understanding that Leiner Health Products will not market this product for a period of at least 75 days after the FDA receipt of this notification.

Company Name and Address:

Leiner Health Products
901 E 233rd Street
Carson, CA 90745

Ingredient Name:

- D-Ribose

Description

There will be two dietary supplements containing the above-mentioned new dietary ingredient.

The first dietary supplement will be in powder form with a suggested use of 4 scoops of powder, (60 g) to be mixed as a drink. This 60 gram dose provides 12 grams of D-Ribose daily.

The second dietary supplement will be in tablet form with a suggested use of 1 tablet, three times daily. This three tablet maximum daily dose provides 3 grams of D-Ribose daily.

68866

January 18, 2000
Office of Special Nutritionals (HFS-450)
Center for Food and Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20204

Product Safety

Please find attached documentation, which establishes that the new dietary ingredient (D-Ribose) when taken under the suggested use is reasonably expected to be safe.

Respectfully Submitted,

Hany Farag,
Director of Regulatory Affairs

THE MERCK INDEX

AN ENCYCLOPEDIA OF
CHEMICALS, DRUGS, AND BIOLOGICALS

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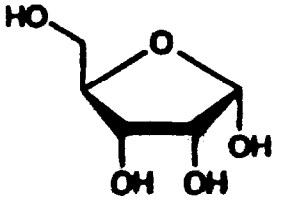
1996

8371. D-Ribose. $C_5H_{10}O_5$; mol wt 150.13. C 40.00%, H 6.71%, O 53.28%. Prepd by hydrolysis of yeast-nucleic acid: Levene, Jacobs, *Ber.* **42**, 1201, 3247 (1909); Levene, Clark, *J. Biol. Chem.* **46**, 19 (1921); Bredereck, *Ber.* **71**, 408 (1938); Bredereck *et al., ibid.* **73**, 956 (1940); Phelps, U.S. pat. **2,152,662** (1939 to U.S. Gov't); by ion-exchange resin chromatography: Cohn, *Science* **109**, 377 (1949); *J. Am. Chem. Soc.* **71**, 2275 (1949); **72**, 1471 (1950). From glucose: Karrer, *Helv. Chim. Acta* **18**, 1435 (1935); Austin, Humoller, *J. Am. Chem. Soc.* **56**, 1152 (1934); Kuhn *et al., Ber.* **68**, 1765 (1935); from nucleosides: Laufer, Charney, U.S. pats. **2,379,913-4** (1945 to Schwarz Labs.); from D-erythrose: Sowden, *J. Am. Chem. Soc.* **72**, 808 (1950); from L-glutamic acid: Koga *et al., Tetrahedron Letters* **1971**, 263. Reduction of D-ribonic acid: van Ekenstein, Blanksma, *Chem. Zentr.* **1913, II**, 1562; Steiger, *Helv. Chim. Acta* **19**, 189 (1936). *Review:* Overend, Stacey, in *Nucleic Acids* **vol. I**, E. Chargaff, J. N. Davidson, Eds. (Academic Press, New York, 1955) pp 1-80.



α-D-Ribofuranose

Plates from abs alcohol, mp 87°. Shows complex mutarotation: Phelps *et al., J. Am. Chem. Soc.* **56**, 748 (1934). Final $[\alpha]_D^{24}$ −25° (water). Sol in water, slightly sol in alc.

Phenylosazone, $C_{17}H_{20}N_4O_3$, yellow needles from pyridine + water, mp 163-164°.

Methyl-D-riboside, crystals from ethyl acetate, mp 83-84°. $[\alpha]_D^{20}$ −113.6° (p = 3), Minsaas, *Ann.* **512**, 286 (1934).

Safety Documentation for Ribose

Side Effects

Oral administration of ribose appears to be well tolerated up to 200 mg per kg body weight (14 g for a 154 pound man) for a single dose. Higher doses result in diarrhea.[1] Ribose consumed orally (1 gm per kg body weight[2]) or given intravenously (15 g over 4 min,[2] 10-20 g over 10-25 min,[3] or 40-50 g over 60 min[4]) to healthy adults and diabetics results in hypoglycemia. The hypoglycemia appears to be more profound in normal subjects than in subjects with impaired glucose tolerance or diabetes,[5,6] is not associated with any clinical symptoms, and appears to be only partially mediated by insulin secretion.[7] Similar hypoglycemic responses have been reported with intravenous administration of ribose in dogs.[8,9,10]

Drug Interactions

In rats, continuous intravenous administration of ribose has been shown to have no effect on the hemodynamic actions of various cardiovascular agents used in conventional cardiac therapy—prazosin (α-blocker), verapamil (Ca-antagonist), and metoprolol (β-blocker).[11,12]

Toxicity

Results from in vitro experiments indicate that ribose inhibits DNA repair synthesis in human lymphocytes (25-50mM)[13] and inhibits cell proliferation and promotes cell death in human peripheral blood mononuclear cells (50mM).[14] A lower amount of ribose (ie, 3.5 mM), which is more consistent with high dose oral administration of 60 g/day, did not inhibit human lymphocyte proliferation in vitro. A 10-fold increase of ribose to 35mM was required to significantly inhibit human lymphocyte proliferation in vitro.[15]

References

1. Gross M, Reiter S, Zollner N. Metabolism of ribose administered continuously to healthy persons and to patients with myoadenylate deaminase deficiency. *Klin Wochenschr.* 1989;67:1205-1213.

2. Ginsburg JG, Boucher B, Beaconsfield P. Hormonal changes during ribose-induced hypoglycemia. *Diabetes.* 1970;19:23-27.

3. Segal S, Foley J, Wyngaarden JB. Hypoglycemic effect of ribose in man. *PSEBM.* 1957;95:551-555.

4. Bierman EL, Baker EM, Plough IC, Hall WH. Metabolism of ribose in diabetes mellitus. *Diabetes.* 1959;8:455-458.

5. Steinberg T, Poucher RL, Sarin RK, Rees RB, Gwinup G. Oral administration of ribose in diabetes mellitus. *Diabetes.* 1970;19:11-16.

6. Beaconsfield P, Ginsburg J. Hypoglycaemia after oral ribose. *Lancet.* 1967;1:153.

7. Gross M, Zollner N. Serum levels of glucose, insulin, and c-peptide during long-term ribose administration in man. *Klin Wochenschr.* 1991;69:31-36.

8. Goetz FC, Greenberg BZ, Maney JW, Wittmers R, Nicoloff D. Indirect mechanism of insulin release from dog pancreas during infusions of ribose. *Am J Physiol.* 1974;227:1158-1065.

9. Ishiwata K, Kuzuya N, Kajinuma H, Tsushima T, Irie M, Hetenyi GJ. Sustained hypoglycemia in response to intravenous infusion of ribose in normal dogs. *Endocrinol Japan.* 1978,25:163-169.

10. Ishiwata K, Hetenyi G Jr, Vranic M. Effect of d-glucose or ribose on the turnover of glucose in pancreatectomized dogs maintained on a matched intraportal infusion of insulin. *Diabetes.* 1969;18:820-827.

11. Lortet S, Zimmer HG. Functional and metabolic effects of ribose in combination with prazosin, verapamil and metroprolol in rats in vivo. *Cardiovasc Res.* 1989;23:702-708.

12. Zimmer H-G, Zierhut W, Marschner G. Combination of ribose with calcium antagonist and β-blocker treatment in closed-chest rats. *J Mol Cell Cardiol.* 1987;19:635-639.

13. Zunica G, Marini M, Brunelli MA, Chiricolo M, Franceschi C. Ribose inhibits DNA repair synthesis in human lymphocytes. *Biochem Biophys Res Comm.* 1986;138:673-678.

14. Barbieri D, Grassilli E, Minti D, et al. Ribose and deoxy-ribose induce apoptosis in human quiescent peripheral blood mononuclear cells. *Biochem Biophys Res Comm.* 1994;201:1109-1116.
15. Pliml W, von Arnim T, Stablein A, Hofmann H, Zimmer HG, Erdmann E. Effects of ribose on exercise-induced ischaemia in stable coronary artery disease. *Lancet.* 1992;340:507-510.

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Klin Wochenschr (1989) 67:1205–1213

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Metabolism of D-Ribose Administered Continuously to Healthy Persons and to Patients with Myoadenylate Deaminase Deficiency

M. Gross, S. Reiter and N. Zöllner
Medizinische Poliklinik der Universität München

Summary. D-ribose was administered orally or intravenously over at least 5 h to eight healthy volunteers and five patients with myoadenylate deaminase deficiency. Intravenous administration rates were 83, 167, and 222 mg/kg/h, which were well tolerated but oral administration of more than 200 mg/kg/h caused diarrhea. The average steady state serum ribose level ranged between 4.8 mg/100 ml (83 mg/kg/h, oral administration) and 81.7 mg/100 ml (222 mg/kg/h, intravenous administration). Serum glucose level decreased during ribose administration. The intestinal absorption rate of orally administered ribose was 87.8%–99.8% of the intake at doses up to 200 mg/kg/h without first pass effect. Urinary losses were 23% of the intravenously administered dose at 222 mg/kg/h. Ribose appeared to be excreted by glomerular filtration without active reabsorption; a renal threshold could not be demonstrated. The amount of ribose transported back from the tubular lumen depended on the serum ribose level. There was no difference in ribose turnover in healthy subjects and patients with MAD deficiency.

Key words: (D-)ribose – Metabolism – Myoadenylate – (adenylate-, AMP-)deaminase deficiency

D-ribose and xylitol are the only substances known to prevent the symptoms of patients with myoadenylate deaminase (MAD) deficiency. An oral administration of 15–20 g ribose per hour can prevent pain and stiffness of the muscles [33, 34].

In experiments with rats, Zimmer [31] showed that after temporary ischemia the myocardium loses its purine nucleotides; their pool has to be recompleted by purine synthesis de novo. The initial cellular purine concentration is regained after 72 h. If ribose is administered, the restoration of cardiac ATP pool takes only 12 h. These results may become important in the therapy of human coronary infarction or chronic coronary heart disease.

The first experiment with ribose in man were done in 1946 [30] but up to today, no investigations were done with long-lasting administration of ribose in man. These early experiments did not show whether a steady state concentration of serum ribose concentration after administering ribose for several hours is obtained.

A hypoglycemic effect of ribose has repeatedly been described [1, 2, 6–8, 21, 22, 24, 25] after short-term administrations. It is not known whether the hypoglycemia induced by ribose will persist during a continuous administration over several hours – an important question for the therapy of patients with MAD deficiency.

Experiments with human lymphocyte cultures revealed cytotoxic effects of ribose after an incubation in concentrations of 20–50 mmol/l after 24 h [15, 23, 28, 35]. It is important to know whether such concentrations may be reached during therapy with this sugar.

The aim of the study was the investigation of basic pharmacological data of D-ribose for the treatment of patients with MAD deficiency. The main topics were intestinal resorption and urinary excretion after oral administration, serum ribose concentration after long-term administration, disappearance of ribose from blood, and side-effects including the known hypoglycemic effect.

Materials and Methods

Subjects and Materials. Tests were performed in eight healthy volunteers between the ages of 23

Abbreviations: MAD = myoadenylate deaminase; i.v. = intravenous(ly)

Table 1. Ribose administration in normal subjects and patients with MAD deficiency

1	2	3	4	5	6	7	8	9
AH	M	24	104	204	2.44	Oral	83.3	6.1
							166.7	29.4
			106	204	2.46	I.V.	83.3	9.2
							166.7	37.2
							222.2	77.7*
JS	M	26	74	188	1.99	Oral	166.7	44.2*
						I.V.	83.3	6.3
							166.7	45.4*
							222.2	85.6*
MW	M	24	84	176.5	2.01	Oral	83.3	7.2
							166.7	34.0
						I.V.	166.7	58.8
SV	M	28	78.5	182	2.00	Oral	200.0	47.3
			79.0		2.00	I.V.	166.7	39.6
MG	M	25	65.5	178	1.82	Oral	83.3	3.6
							166.7	26.8
WB	M	25	71.8	190	1.98	Oral	83.3	2.4
							166.7	24.7
BL	M	37	68.5	178	1.85	Oral	166.7	34.7
JM	F	23	66.3	173	1.79	Oral	166.7	37.4
Patients								
ES	M	56	84.0	178	2.02	Oral	166.7	35.6
GF	M	58	81.5	175	1.97	Oral	166.7	41.9*
HA	M	31	61.5	182	1.80	Oral	166.7	33.9
HW	F	52	58.5	159	1.60	Oral	166.7	33.1
MA	F	45	57.3	164	1.62	Oral	166.7	9.6
			57.0	164	1.62	I.V.	166.7	22.9

1 Initials of the subject
2 Sex
3 Age (years)
4 Body weight (kg)
5 Body length (cm)
6 Body surface (m^2)
7 Route of administration
8 Dose (mg/kg/h)
9 Serum ribose concentration during steady state (mg/100 ml) or during the last hour of administration in cases where the existence of a steady state is uncertain (these values are marked with "*")

and 37, including seven men and one woman (Table 1).

The clinical group consisted of five patients with MAD deficiency, three men and two women. The diagnosis was based on the ischemic forearm exercise test, the histochemical demonstration of absent MAD activity [4], and the biochemical examination of muscle specimens [3, 34]. Most patients had suffered from symptoms of MAD deficiency for several years. Their ages were between 31 and 58 years.

D-Ribose was obtained from Fluca or Sigma. A sterile and pyrogen-free 8.33 g/100 ml D-Ribose solution for i.v. administration was prepared by the pharmacy of our hospital.[1]

Analytical Methods. In urine and blood samples the concentration of D-ribose was determined according to Roe and Rice [20] in the modification of Miller [16]. After centrifugation, the serum sample (0.2 ml) was deproteinized with 1.0 ml 10% trichloroacetic acid. An aliquot of the supernatant (0.2 ml) was heated with 3.0 ml of a solution of 4-bromoaniline (20 g/l) and thiourea (44.7 mmol/l) in 14.8 mol/l acetic acid in a water bath at 70° for 10 min. After that the mixture was kept in the dark at room temperature for another 45 min. The color was measured at 515 nm with a Beckman photometer. The urine samples were treated in essentially the same way, but a deproteination was not necessary; due to high urine concentrations of ribose some of the urine samples had to be diluted with distilled water.

The concentration of glucose was measured in each blood sample by the glucose-oxidase method. In each urine sample the absence of glucose was ascertained with a test strip.

Procedure. All experiments were started in the morning after fasting overnight. Before D-ribose was administered, each subject drank 500 ml of table water (Adelholzener) per hour for a period of 2 h. Thus, a sufficient diuresis was established. Also, after 2 h of water administration, no further changes of the concentration of serum constitutes were expected [10]. Venous blood samples were drawn every 30 min, in some experiments every 5 min.

In experiments with intravenous administration the antecubital veins of each arm were cannulated. The ribose solution was infused into one arm. The contralateral vein was used to draw blood samples. Needles were kept patent by an infusion of saline at a rate of 100 ml/h.

For oral administration the amount of sugar that the person received during 1 h was dissolved in 400 ml water. Every 5 min the subject had to drink 1/12 of this solution.

Intravenous administration was performed using a 8.33 g/100 ml solution of D-ribose (equimolar to a 10 g/100 ml solution of a hexose). Differences in water uptake between the 400 ml the subjects had to drink during oral administration and the infused amount of ribose solution during intravenous administration were equalized by drinking

[1] We are grateful to Dr. Breugst, Apotheke der klinischen Universitätsanstalten München (Dr. V. Mönch)

a corresponding amount of table water every 10 min.

Serum was separated by centrifugation and stored at 4° C. Every 15 or 30 min the subjects emptied their bladders. The determination of glucose was performed within a few hours, the concentration of D-ribose was measured the same day or during the following day after keeping the serum at 4° C.

During the administration of D-ribose heart rate and blood pressure were followed, and the persons were asked to report any unusual sensations.

Results

The administration of ribose was well tolerated. After oral administration of more than 200 mg/kg/h, diarrhea occurred in some cases.

Ribose in Serum. The average value of ribose concentration in serum before administration of ribose was 1.6 mg/100 ml, single values ranging from 0.0 mg/100 ml to 3.5 mg/100 ml.

Both after oral and after intravenous administration (except when diarrhea occurred) a constant ribose concentration in serum was obtained. However, the time to reach this steady state depended on the dose and the route of administration. After oral administration at a dose of 83.3 mg/kg/h the steady state was reached after 45–120 min (in two of four cases after 45 min), at a dose of 166.7 mg/kg/h after 120–150 min. Using intravenous administration, the steady state was reached after 60 min

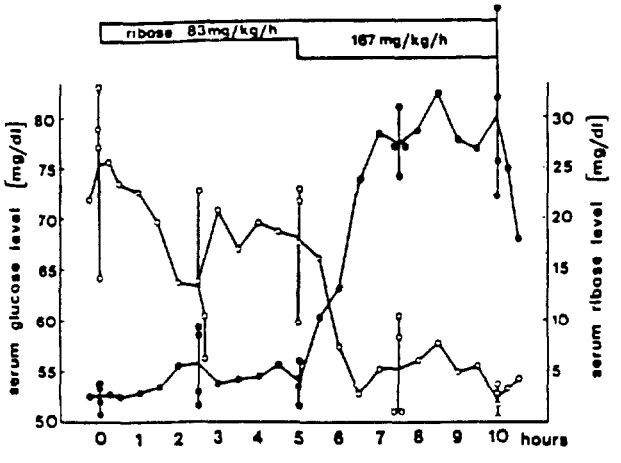

Fig. 1. Average serum ribose and glucose levels during oral ribose administration with rates of 83.3 mg/kg/h (hour 0–5) and 166.7 mg/kg/h (hour 5–10) to four healthy subjects. *Full symbols* indicate serum ribose, *open symbols* indicate serum glucose. At time 0, 2.5, 5, 7.5, and 10, the single values of the four subjects are marked

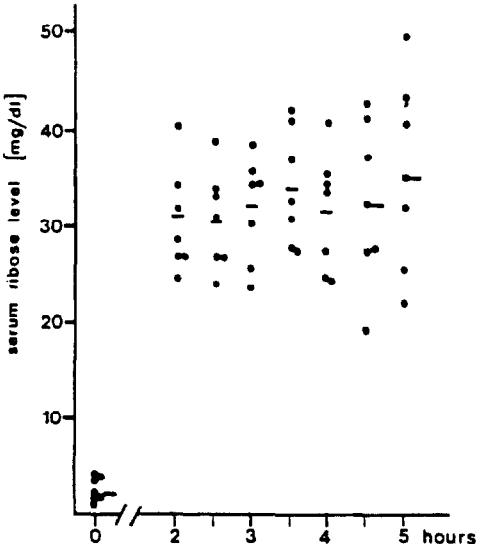

Fig. 2. Serum ribose levels during oral ribose administration of 166.7 mg/kg/h to seven healthy subjects. The *lines* indicate the average values

(83.3 mg/kg/h) or after 120–180 min (166.7 mg/kg/h). In some cases the time of administration was too short to obtain a steady state; these cases are marked by "*" in Table 1.

Observations after oral administration to four subjects are shown in Fig. 1. The subjects received ribose orally at a dose of 83.3 mg/kg/h (dose 1) for the first 5 h. This dose was doubled (dose 2), 166.7 mg/kg/h, for the next 5 h. At the dose level 1, a mean ribose serum concentration of 4.8 mg/100 ml was obtained, the corresponding concentration at dose 2 was 28.7 mg/100 ml.

Three more volunteers started the oral experiments with ribose dose 2. The data, combined with those from Fig. 1, are illustrated in Fig. 2. The average serum ribose concentration was 32.6 mg/100 ml, the individual values of the steady state ribose concentration ranging from 24.7 mg/100 ml to 44.2 mg/100 ml.

One volunteer (SV) received ribose orally at a dose of 200 mg/kg/h leading to a steady state concentration of serum ribose of 47.3 mg/100 ml after 210 min.

During a ribose infusion of 166.7 mg/kg/h in four volunteers, a mean steady state concentration of 45.3 mg/100 ml was observed (Tables 1 and 4).

Two volunteers received an infusion of 83.3 mg/kg/h of ribose, leading to a steady state serum concentration of ribose of 7.8 mg/100 ml. The same two volunteers also received infusions in two other doses (166.7 and 222.2 mg/kg/h). The values from these two subjects are shown in Fig. 3.

Five patients with MAD deficiency had an oral

Table 2. Comparison of oral/iv administration: steady state concentration of serum ribose, decrease of serum glucose, and utilized amount of administered ribose (i.v. experiments)

1	2	3	4	5	6	7	8
AH	Oral	83.3	6.1	8.9%	4.8%	–	–
	I.V.	83.3	9.2	15.3%	9.3%	133.5	1.259
	Oral	166.7	29.4	24.5%	12.6%	–	–
	I.V.	166.7	37.2	26.8%	18.0%	241.3	2.276
JS	Oral	166.7	44.2*	17.8%	18.9%	–	–
	I.V.	166.7	45.4*	21.4%	16.9%	175.4	2.308
MW	Oral	166.7	34.0	29.5%	17.6%	–	–
	I.V.	166.7	58.8	18.2%	25.1%	173.7	2.080
SV	Oral	200.0	47.3	23.2%	16.6%	–	–
	I.V.	166.7	39.6	32.4%	17.2%	181.7	2.300
Patient							
MA	Oral	166.7	9.6	3.1%	3.9%	–	–
	I.V.	166.7	22.9	27.2%	16.1%	132.9	2.332

1 Initials of the subject
2 Route of administration
3 Dose (mg/kg/h)
4 Serum ribose concentration during steady state (mg/100 ml) or during the last hour of administration (*)
5 Average decrease of serum glucose level during steady state compared with the initial level (%)
6 Renal excretion during steady state (% of intake)
7 Within 1 min utilized amount of ribose (intake minus urinary excretion (mg/min))
8 Utilized amount of ribose (intake minus excretion) divided by body weight and time (mg/kg/min)

Table 3. Utilization of ribose and total body clearance

Infusion rate (mg/kg/h)	Subject	Utilization (mg/kg/h)	Total body clearance (ml/kg/min)
83.3	AH	75.6	14.90
	JS	77.9	21.69
	Average	76.8	18.30
166.7	AH	136.7	7.18
	JS	138.5	6.12
	MW	124.9	4.73
	SV	138.0	7.02
	Average	134.5	6.26
	Patient MA	139.9	12.13
222.2	AH	167.5	4.77
	JS	174.2	4.33
	Average	170.9	4.55

ribose intake of 166.7 mg/kg/h. One of them had an intravenous infusion of a ribose solution at the same dosage. The steady state concentrations are shown in Table 1. There is no significant difference of steady state concentrations between the healthy volunteers and the patients, except for patient MA.

Table 4. Comparison of different doses, renal handling

1	2	3	4	5	6	7	8
Oral	83.3	AH	6.1	4.8%	87.9	62.3	54.2%
		MW	7.2	7.9%	128.1	110.3	82.1%
		MG	3.6	3.6%	92.4	87.8	82.9%
		WB	2.4	3.5%	146.4	127.9	101.6%
		Average	4.8	5.0%	113.7	97.1	80.2%
Oral	166.7	AH	29.4	12.6%	106.7	75.7	65.8%
		MW	34.0	17.6%	120.6	103.8	77.3%
		MG	26.8	11.9%	81.3	77.3	73.0%
		WB	24.7	13.0%	104.9	91.7	72.8%
		JS	44.2*	18.9%	94.6	82.2	88.5%
		JM	37.4	17.2%	84.5	81.7	102.6%
		BL	34.7	16.8%	92.3	86.3	64.6%
		Average	32.6	15.4%	97.3	85.5	77.8%
	Patients	ES	35.6	13.9%	91.3	78.2	70.4%
		GF	41.9*	14.5%	91.1	80.0	83.3%
		HA	33.9	17.5%	88.1	84.7	95.2%
		HW	33.1	6.5%	33.1	35.8	45.0%
		MA	9.6	3.9%	68.5	73.2	62.6%
Oral	200.0	SV	47.3	16.6%	95.1	82.3	80.6%
I.V.	83.3	AH	9.2	9.3%	148.3	104.1	97.9%
		JS	6.3	6.5%	107.6	93.5	100.7%
		Average	7.8	7.9%	128.0	98.8	99.3%
I.V.	166.7	AH	37.2	18.0%	142.3	100.1	93.9%
		JS	45.4*	16.9%	78.6	68.3	73.5%
		MW	58.8	25.1%	98.9	85.1	68.7%
		SV	39.6	17.2%	92.0	79.6	78.0%
		Average	45.3	19.3%	103.0	83.3	78.5%
	Patient	MA	22.9	16.1%	115.8	123.7	105.9%
I.V.	222.2	AH	77.7*	24.6%	124.3	87.4	82.0%
		JS	85.6*	21.6%	71.0	61.7	66.4%
		Average	81.7	23.1%	97.7	74.6	74.2%

1 Route of administration
2 Dose (mg/kg/h)
3 Initials of the subject
4 Serum ribose concentration (mg/100 ml) during steady state or during the last hour of administration (*)
5 Renal excretion during steady state (% of intake)
6 Renal ribose clearance (ml/min)
7 Renal ribose clearance (ml/min) related to 1.73 m^2 body surface
8 Relation renal ribose clearance-creatinine clearance

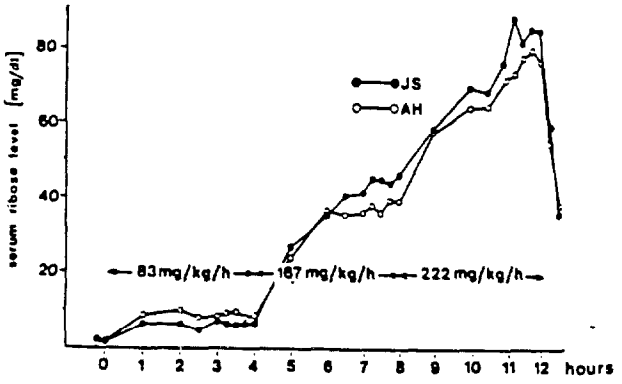

Fig. 3. Serum ribose levels during ribose infusion with three rates to two healthy subjects

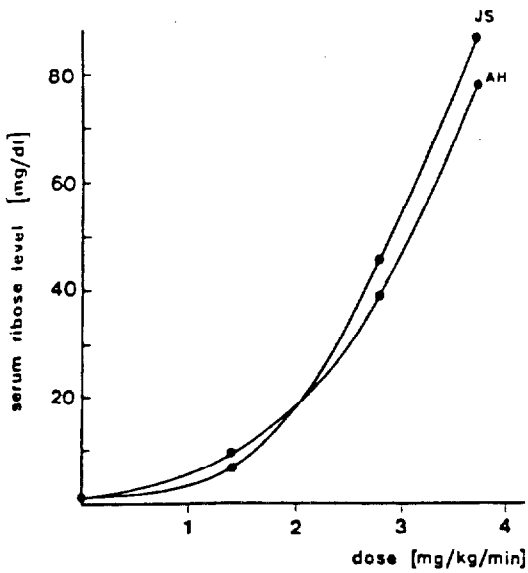

Fig. 4. Average ribose levels (*ordinate*) obtained in two healthy subjects during intravenous ribose administration with three rates (*abscissa*)

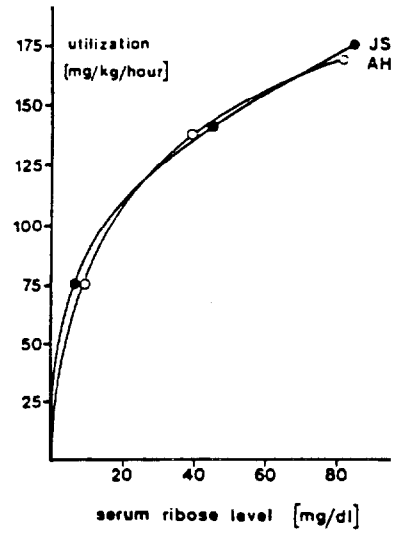

Fig. 5. Serum ribose level (*abscissa*) and utilization (*ordinate*) for two healthy subjects

The ribose concentrations in her serum were low both after oral and intravenous administration.

The single values of the steady state concentrations are listed in Table 1.

Comparison Between Oral and I.V. Administration. Four healthy volunteers and one patient received ribose both orally and intravenously (Table 2). At the same dose the serum ribose concentrations in the i.v. experiments were always higher.

In three of four volunteers, the hypoglycemic effect of ribose was stronger after i.v. administration than after oral administration.

Intestinal Absorption. In Fig. 4 the relation between the i.v. dose (mg/kg/min) and the mean serum ribose level obtained during the last hour of administration of each dose is presented for the two volunteers (JS, AH) who received three doses. For volunteer AH the following parabola was calculated:

$$y = 6.86x^2 - 4.95x + 0.68$$

y: steady state concentration (mg/100 ml)
x: dose (mg/kg/min)

For volunteer JS the following parabola was calculated:

$$y = 7.79x^2 - 5.56x - 0.24$$

Then for the steady state concentrations during oral administration the corresponding i.v. dose was calculated using the second order equation.

In volunteer AH: The steady state concentration of serum ribose during oral administration in a dose of 1.39 mg/kg/min (83.3 mg/kg/h) was 6.1 mg/100 ml. This concentration corresponds to an intravenous administration of 1.32 mg/kg/min. Thus, 95% (1.32/1.39 = 0.950) of the orally supplied amount was adsorbed.

Analogous values for a minimal intestinal absorption at dose 2 (166.7 mg/kg/h) were 87.8% (AH) respectively 99.8% (JS).

Obviously this method does not permit to differentiate between incomplete absorption and catabolism of ribose in the epithelial cells of the gut or in the liver. Nevertheless, the data suggest almost complete absorption at administration rates below 200 mg/kg/h and permit for only minor first pass effects or metabolization by the gut.

Utilization and Total Clearance. During steady state utilization is the difference between intake and excretion. The total body clearance is the quotient of rate of administration and serum concentration during steady state (ml/kg/min) and comprises both the renal excretion and the utilization in the tissues. Calculations from experiments with i.v. administration are listed in Table 3. Figure 5 shows the utilization of ribose and serum ribose concentration for the two subjects who received three doses.

Urinary Ribose Excretion. Both after oral and intravenous administration, a steady state of excretion of ribose in urine was obtained after 60 to

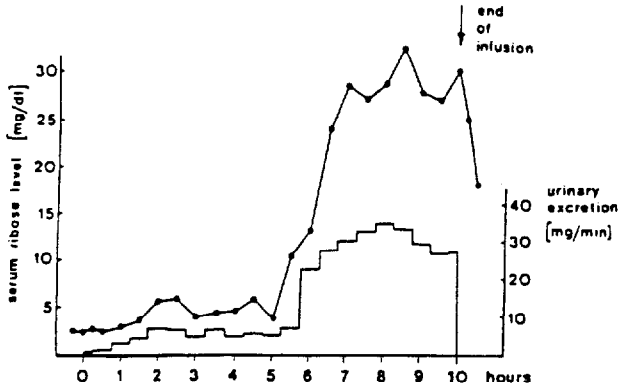

Fig. 6. Average serum ribose level (*points*) and urinary excretion (*bars*) during oral ribose administration in two rates to four healthy subjects

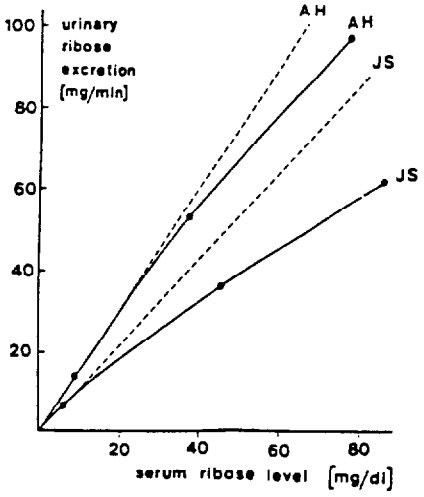

Fig. 7. Average serum ribose level and urinary excretion in two healthy subjects after intravenous administration of ribose with three rates. The *traced lines* connect the measured values, the *dotted lines* indicate the excretion of a substance that is excreted only by glomerular filtration without secretion or reabsorption (calculated from the creatinine clearance of the two subjects)

180 min. This steady state was reached at about the same time as the steady state of the serum concentration of ribose (Fig. 6). In Table 4 column 5 the percentage excretion (excreted amount of ribose divided by the oral or intravenous intake of ribose) during steady state is shown.

In Table 4 the renal clearance for ribose under steady state conditions and the relation to the creatinine clearance are listed. The results of most patients are within the normal range indicating that there is no difference in renal handling between patients and healthy volunteers. As can be seen in Table 4 the percentage of urinary loss of ribose increases with higher doses.

During oral administration ribose could already be detected in the urine when serum ribose concentration was elevated to ≤ 3.5 mg/100 ml or

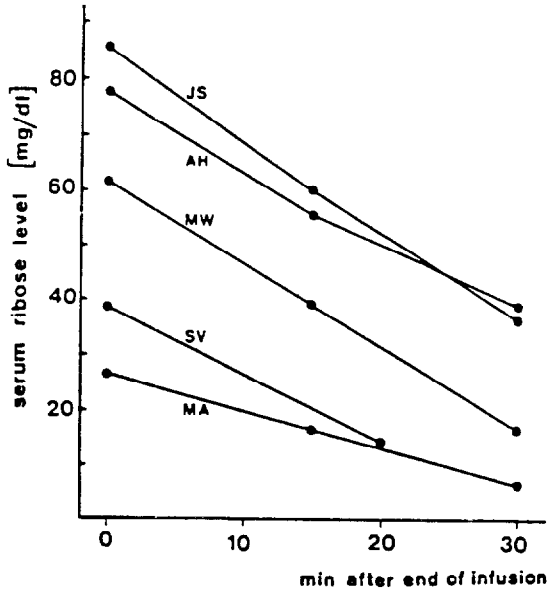

Fig. 8. Disappearance of ribose from serum: time (min) after end of infusion and serum ribose level for five healthy subjects

≤ 2 mg/100 ml higher than the intial level, in some cases even when no elevation could be measured. This indicates that a renal threshold for ribose does not exist or is very low.

In Fig. 7 dotted lines represent renal excretion calculated by the creatinine clearance (JS: 106.9 ml/min, AH 151.5 ml/min) assuming that ribose is only glomerularly filtrated. Traced lines connect the measured points (values during steady state condition). At low serum concentrations the lines are identical indicating a glomerular filtration without important active secretion or reabsorption. The higher the serum ribose concentration the less steep is measured increase in excretion. This indicates a reabsorption of ribose from the filtrated primary urine.

Elimination of Ribose from the Serum. The ribose serum concentrations after the end of infusion are shown in Fig. 8. In a general view, most cases fit a linear relationship as known for the metabolism of ethanol. For subject AH, however, the data correspond to a semilogarithmic relationship.

The linear relationship between time after end of infusion and serum concentration means a reaction of zero order for the elemination of ribose at serum concentrations higher than 10 mg/100 ml. Thus, it is possible to express the disappearance of ribose from blood in terms of mg/100 ml/min (Table 5).

Side-Effects. Diarrhea was noted in some instances during oral administration (JM, dose 167 mg/kg/h,

Table 5. Disappearance of ribose from the blood

1	2	3	4
AH	77.7	1.287	0.01214
MW	61.3	1.489	0.01772
JS	85.5	1.627	0.02141
SV	38.5	1.200	0.01519
MA	26.6	0.660	0.01158

1 Initials of the subject
2 Ribose serum concentration at the end of infusion (mg/100 ml)
3 Disappearance of ribose from the blood (mg/100 ml/min)
4 Disappearance of ribose from the blood, related to the body weight (mg/100 ml/min/kg)

Table 6. Steady state serum levels of different sugars during i.v. infusion

Sugar	Rate of i.v. administration (mg/kg/h)	Steady state serum concentration (mg/100 ml)	Reference
Ribose	83	8	
	167	45	
	222	82	
Galactose	300	28	[9]
Xylitol	500	85–95	[11]
Fructose	500	42	[32]
	1000	87	[32]
	1500	140	[32]

Table 7. Utilization of different sugars during i.v. infusion

Sugar	Rate of i.v. administration (mg/kg/h)	Utilization (mg/kg/h)	Reference
Ribose	83	77	
	167	135	
	222	171	
Galactose	300	290	[12]
Glucose	500	499	[10]
	1000	985	[12]
	1500	1390	[12]
Fructose	500	489	[12]
	1000	947	[12]
	1500	1409	[11]

and in all experiments with doses higher than 200 mg/kg/h). Most of the volunteers reported hyperperistalsis during oral administration. However, it was impossible to differentiate between the effects of ribose and the effects of the long fasting period and the intake of water. Under i.v. infusion hyperperistalsis was reported only occasionally.

Before, during, and after ribose administration no changes in heart rate and blood pressure or in the controlled routine laboratory tests including uric acid concentration in serum, uric acid clearance, and creatinine clearance could be detected, except for volunteer MG: His serum concentration of bilirubin increased from an initial level of 1.3 mg/100 ml to 2.3 mg/100 ml at the end of the experiment, with the major increase being in the unconjugated fraction. Later controls were normal. As MG also showed an increase of serum bilirubin after a 24-h fast, this increase is most likely due to a Gilbert's syndrome.

Discussion

Using the color reaction according to Roe and Rice [20] with the modification of Miller [16], pentose concentrations in serum before administration of ribose were measured with a mean value of 1.6 mg/100 ml. Compared with the values during ribose administration these values are negligible and were not taken into account.

Before administration of ribose a minimal urinary pentose excretion was detected. Part of this may be attributed to various also- and keto-pentoses. In the 24-h urine of normal adults, Tower et al. [27] found mostly arabinose but also xylose and ribose in an amount of 0.54 mg/kg body weight. White and Hess [29] found each of these pentoses in a concentration of 2.5–10 mg/100 ml in the urine of healthy men. Futterman and Roe [5] found 0.1 mg/100 ml ribulose and 0.4 mg/100 ml xylulose in the urine of normal adults. Touster et al. [26] also found l-xylulose in the urine of a healthy person.

Infusions of D-ribose into healthy volunteers were performed by Segal and Foley [22]: After a primer of 4 g, ribose was infused at a dose of 111.9 mg/kg/h or 122.4 mg/kg/h within 100 min. After 40–60 min a steady state concentration of 11 mg/100 ml and 7 mg/100 ml respectively was measured. These concentrations correspond with our results. The early time of the steady state is due to the priming dose. Within 1 h Bierman et al. [2] infused 50 g of ribose at a constant rate into healthy volunteers. The blood pentose concentration increased up to about 90 mg/100 ml without reaching a constant level. Goodman and Goetz [8] infused 500 mg/kg over 35 min leading to a peak level of approximately 80 mg/100 ml at the end of the infusion. In both experiments the time of administration was too short and the dose too high to establish a steady state.

No report was found about an administration of ribose over several hours. Also there is no report on intestinal absorption or mechanisms of renal excretion.

Table 8. Urinary excretion during i.v. infusion (steady state): comparison between ribose and fructose

Sugar	Rate of administration (mg/kg/h)	Urinary excretion (mg/kg/h)	Urinary excretion % of intake	Reference
Ribose	83	6.6	8.0%	
	167	32.2	19.3%	
	222	51.3	23.1%	
Fructose	500	11.2	2.2%	[32]
	1000	52.6	5.3%	[32]
	1500	90.4	6.0%	[32]

Steady state concentrations of ribose related to the intravenous dose are rather high in comparison with other sugars (Table 6). Correspondingly the rate of utilization of ribose is rather low (Table 7).

In accordance with the low utilization rate and the high serum ribose concentration during steady state the urinary excretion is rather high (Table 8).

After a 15-min ribose infusion Segal and Foley [22] measured an average urinary loss of 21% at doses of 5–20 g. They assumed that the urinary excretion decreases if the dose is lowered – this was shown in our experiments. Pizzichini et al. [17] infused 500 mg/kg leading to an urinary loss of 10%–15% of the administered dose. Hiatt [13] measured an average urinary excretion of 8% of the infused amount of ribose (200 or 400 mg/kg).

The decrease of urinary excretion at higher serum concentrations can not be the effect of an active reabsorption for two reasons: First, an active reabsorption would lower the excretion also at low serum concentrations leading to a renal threshold. Second, an active reabsorption would lower the excretion by a constant amount due to saturation, and the traced and dotted lines in Fig. 7 would be parallel. As shown in Fig. 7, the increasing distance between the traced and dotted lines depends on the serum ribose concentration. This can be explained by a concentration-dependent reabsorption as it was shown for fructose [32].

Segal and Foley [22] measured the disappearance of ribose from blood after infusion of various amounts of this sugar. They demonstrated that the removal of ribose from blood is a first order kinetic process only when the ribose blood level is below a critical value of 6 to 20 mg/100 ml. This suggested the saturation of the clearance system at certain blood levels. We found a zero order kinetic process for the first 30 min after end of infusion and for blood levels between 10 and 85 mg/100 ml respectively.

The hypoglycemic effect of ribose administration has been well studied [1, 2, 6–8, 21, 22, 24, 25]. A decrease of serum glucose concentration down to 15 mg/100 ml without clinical symptoms was reported [21]. This hypoglycemic effect is surprising since Hiatt [14] demonstrated that in man ribose is converted to glucose via the pentose phosphate pathway.

No changes in blood level of other metabolites were detected by comparing the concentrations immediately before and after ribose administration. Only subject MG showed a temporary increase of bilirubin serum concentration from 1.3 to 2.2 mg/100 ml. No changes occurred in renal clearance of uric acid and creatinine. It is remarkable that ribose administration does not increase uric acid concentration in serum [18, 19] whereas xylitol [12] and fructose [11] lead to considerable elevations of plasma levels.

There is no significant difference between the results of the patients ES, GF, HA, and HW and those from healthy volunteers. Patient MA, however, differs in the steady state value of blood ribose concentration. After oral administration the average serum ribose concentration was only 9.6 mg/100 ml. This finding and the small urinary excretion (only 3.9% of the oral intake) suggest an incomplete intestinal absorption of ribose. Nevertheless, this cannot explain all findings in patient MA. After ribose infusion the blood level of this sugar was higher than after oral administration but still lower than the average concentrations in healthy volunteers. A possible explanation might be that the utilization of ribose in this patient is higher than in other persons. In contrast the disappearance of ribose from blood is not augmented (Table 5). However, the data suggest that important individual differences in ribose metabolism may occur.

Oral administration of D-ribose is limited by diarrhea occurring at doses higher than 200 mg/kg/h. Up to this dose, the oral intake is well tolerated. D-Ribose is almost completely absorbed without important first pass effect. More than 80% of the intake is metabolized. After long-term oral and i.v. administration a steady state of serum ribose concentration is observed. The only measured biochemical change is an asymptomatic decrease of serum glucose concentration.

These findings are important for the therapy of patients with MAD deficiency. The oral intake should be below 200 mg/kg/h. It is possible to estimate the metabolized amount of orally administered D-ribose. The serum concentrations of the sugar are far below the concentrations leading to cytotoxic effects, and D-ribose disappears very fast

after the end of administration. Thus, the risk of serious side effects is very low. Nevertheless, the measurement of serum ribose concentrations in patients treated with this sugar is advisable due to the individual differences in ribose metabolism.

References

1. Beaconsfield P, Ginsburg J (1967) Hypoglycaemia after oral ribose. Lancet 2:153
2. Bierman EL, Baker EM, Plough I, Hall WH (1959) Metabolism of D-ribose in diabetes mellitus. Diabetes 8:455–458
3. DiMauro S, Miranda AF, Hays AP, Franck WA, Hoffman GS, Schoenfeldt RS, Singh N (1980) Myoadenylate deaminase deficiency. Muscle biopsy and muscle culture in a patient with gout. J Neurol Sci 47:191–202
4. Fishbein WN, Griffin JL, Armbrustmacher VW (1980) Stain for skeletal muscle adenylate deaminase. An effective tetrazolium stain for frozen biopsy specimens. Arch Pathol Lab Med 104:462–466
5. Futterman S, Roe JH (1955) The identification of ribulose and l-xylulose in human and rat urine. J Biol Chem 215:257
6. Ginsburg J, Boucher B, Beaconsfield P (1969) Mechanism of ribose-induced hypoglycaemia. Acta Endocrinol, S 138–158
7. Ginsburg J, Boucher B, Beaconsfield P (1970) Hormonal changes during ribose-induced hypoglycemia. Diabetes 19:13–27
8. Goodman C, Goetz FC (1970) Oral and intravenous D-ribose and plasma insulin in healthy humans: effects of route of administration and of epinephrine and propanolol. Metabolism 19:1094–1103
9. Heuckenkamp PU, Zöllner N (1970) Über das Verhalten von Galaktose, Glucose, Insulin und der freien Fettsäuren im Plasma sowie der renalen Ausscheidung von Galaktose und Glucose während mehrstündiger Galaktoseinfusion beim Menschen. Z Ges Exp Med 153:136–149
10. Heuckenkamp PU (1973) Zur Bilanz parenteral zugeführter Kohlenhydrate. Verhandlungen der 28. Tagung der Deutschen Gesellschaft für Verdauungs- und Stoffwechselkrankheiten, Erlangen
11. Heuckenkamp PU (1974) Vergleichende Untersuchungen über die Verwertung parenteral zugeführter Kohlenhydrate während mehrstündiger Infusion beim Menschen. Melsunger Medizinische Mitteilungen, Bd 48, Suppl I, 149–160
12. Heuckenkamp PU, Zöllner N (1975) Quantitative comparison and evaluation of utilization of parenteral administered carbohydrates. Nutr Metabol 18 [Suppl 1]:209–226
13. Hiatt HH (1957) The pentose phosphate pathway in human metabolism. J Clin Invest 36:899
14. Hiatt HH (1958) Studies of ribose metabolism. III. The pathway of ribose carbon conversion to glucose in man. J Clin Invest 37:651–654
15. Marini M, Zunica G, Franceschi C (1985) Inhibition of cell proliferation by D-ribose and deoxy-D-ribose. Proc Soc Exp Biol Med 180:246–257
16. Miller B (1974) Der D-Xylose-Test. In: Englhardt A, Lommel H (Hrsg) Malabsorption. Maldigestion. Band 2. Weilheim, S 155–163
17. Pizzichini M, Micheli V, Marcolongo R, Marinello E (1978) Metabolismo del riboso nell'uomo. Boll Soc Biol Sper 54:420–424
18. Pizzichini M, Marcolongo R, Marinello E (1979) Ribose tolerance in gouty patients. J Clin Chem Clin Biochem 17:436
19. Pizzichini M, Marcolongo R, Marinello E (1980) Ribose tolerance in gouty patients. Purine metabolism in man. In: Rapado A, Watts RWE, DeBruyn CHMM (eds) III Biochemical, immunological and cancer research, Part A. Plenum Press, London, pp 81–86
20. Roe JH, Rice EW (1948) A photometric method for the determination of free pentoses in animal tissues. J Biol Chem 173:507–512
21. Segal S, Foley J, Wyngaarden JB (1957) Hypoglycemic effect of d-ribose in man. Proc Soc Exp Biol 95:551–555
22. Segal S, Foley J (1958) The metabolism of d-ribose in man. J Clin Invest 37:719–735
23. Stankova J, Rola-Plesczynski M (1984) Alpha-fucose inhibits human mixed-lymphocyte culture reactions and subsequent suppressor cell generation. Cell Immunol 83:83–91
24. Steinberg T, Ortman P, Poucher R, Cochran B Jr, Gwinup G (1967) The effect of d-ribose infusion on serum immunoreactive insulin and glucose concentration. Metabolism 16:40–46
25. Steinberg T, Poucher RI, Sarin RK, Rees RB, Gwinup G (1970) Oral administration of D-ribose in diabetes mellitus. Diabetes 19:11–16
26. Touster O, Hutcheson RM, Reynolds VH (1954) The formation of l-xylulose in mammals and its utilization by liver preparations. J Am Chem Soc 76:5005–5006
27. Tower DB, Peters EL, Pogorelskin MA (1956) Nature and significance of pentosuria in neuromuscular diseases. Neurology 6. 37:125–142
28. Ulrich F (1983) Inhibition by specific monosaccharides of interleukin 2-induced thymocyte proliferation. Cell Immunol 80:241–256
29. White AA, Hess WC (1956) Paper chromatographic detection of sugars in normal and dystrophic human urines. Arch Biochem Biophys 64:57
30. Wuest HM, Solmssen UV (1946) The urinary excretion of d-ribose in man. Arch Biochem 11:199–201
31. Zimmer HG, Ibel H (1984) Ribose accelerates the repletion of the ATP pool during recovery from reversible ischemia of the rat myocardium. J Mol Cell Cardiol 16:863–866
32. Zöllner N, Heuckenkamp PU, Nechwatal W (1968) Über die Verwertung und renale Ausscheidung von Fructose während ihrer langdauernden intravenösen Zufuhr. Klin Wochenschr 46:1300–1308
33. Zöllner N, Gross M (1986) Myoadenylatedeaminase-Mangel. Erfolgreiche Therapie eines Falles mit Ribose bzw. Xylit. Klin Wochenschr 64 [Suppl V]:34–35
34. Zöllner N, Reiter S, Gross M, Pongratz D, Reimers CD, Gerbitz K, Paetzke I, Deufel T, Hübner G (1986) Myodenylate deaminase deficiency: successful symptomatic therapy by high dose oral administration of ribose. Klin Wochenschr 64:1281–1290
35. Zunica G, Marini M, Brunelli MA, Chiricolo M, Franceschi C (1986) D-Ribose inhibits DNA repair synthesis in human lymphocytes. Biochem Biophys Res Commun 138:6763–6778

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Hormonal Changes During Ribose-induced Hypoglycemia

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SUMMARY

Plasma levels of insulin and human growth hormone (HGH) were measured in healthy adults after oral or intravenous administration of the pentose sugar D-ribose. Increased plasma levels of insulin were recorded after both oral and intravenous ribose; there was no consistent change in plasma levels of HGH after administration of ribose by either route. It is suggested that insulin release occurs at such times and in amounts as may account at least in part for the hypoglycemic effect of this pentose. DIABETES *19*:23-27, 1970.

We have recently reported that the blood glucose concentration falls in man after ingestion of D-ribose,[1] as after intravenous injection of the pentose.[2] How this sugar induces hypoglycemia is not clear, however, since other pentoses such as xylose and arabinose do not lower glucose.[2,3]

As part of a study to elucidate the mechanism of ribose-induced hypoglycemia, the effects of oral and of intravenous ribose on plasma insulin, cortisol and human growth hormone (HGH) were investigated in healthy adults.

MATERIAL AND METHODS

Oral ribose dissolved in water (1 gm. per kg. body weight) or intravenous ribose (15 gm. in a 7½ per cent solution infused over four minutes) was given to ten healthy adult volunteers between the ages of twenty and thirty, tested at rest in the laboratory after an overnight fast. During an initial control period of at least fifteen minutes, two separate blood samples were taken through a needle maintained in an antecubital vein. D-ribose was then given and further samples taken through the same needle. With intravenous ribose, samples were taken at 3, 6, 12, 24, 30, 60, 80, 95 and 110 minutes. After the oral pentose, samples were taken half-hourly for three hours. Four of these subjects underwent similar control studies in which they drank water alone.

Blood glucose was measured by a glucose oxidase technic;[4] in three tests simultaneous determination of total reducing substances was made using a Technicon AutoAnalyzer. Glucose and ribose were also estimated chromatographically in one experiment, the two sugars being separated from deproteinized filtrates of serum as borate complexes on a Dowex column, eluted with borate buffers (0.010 M potassium tetraborate pH 8.5, followed by 0.062 M potassium tetraborate pH 8.5) and then analyzed with a Technicon AutoAnalyzer.[5]

The plasma from heparinized aliquots was rapidly separated by centrifugation and stored at —20° C. Plasma insulin and HGH were subsequently determined in these samples by radioimmunoassay according to the methods described by Morgan and Lazarow[6] and Hartog et al.[7] respectively with a reproducibility of ± 5 per cent.

Plasma cortisol was measured in the first three experiments, but since no change in cortisol levels was recorded after oral or intravenous ribose, further estimations of the hormone were not made.

In view of the reciprocal relationship postulated between the level of free fatty acids and tissue glucose utilization, and the influence of both insulin and human growth hormone on lipolysis, serum free fatty acids were measured after oral ribose in three subjects by the method of Dole.[8]

RESULTS

Oral ribose (table 1, figure 1)

After oral ribose, the blood glucose level fell on average 14 mg./100 ml. over the first hour from a fasting mean value of 80 mg./100 ml. The blood glucose level then rose slightly during the second hour, after

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TABLE 1

Effect of oral D-ribose (1 gm./kg.) on blood glucose (G—mg. per 100 ml.), immunoassayable insulin((I—μU./ml.) and human growth hormone (HGH—mμg./ml.) in five healthy adults

Subject		1			2			3			4			5		
	Time	G	I	HGH	G	I	HGH	G	I	HGH	G	I	HGH	G	I	HGH
Control (mean of two determinations)	0	63	7.75	8.5	101	10.2	4	77	26.0	1.5	67	12.8	11	80	4	6.0
Minutes after ribose	30	57	15	5	85	8.5	2.2	67	20.5	1.5	60	15.5	0.5	63	28	0.5
	60	45	10	12.5	82	10	2.5	75	18.3	1.5	55	40	0.3	65	15	0.5
	90	51	5	20	88	12.5	3	71	37.5	7	61	13	0.3	69	20	3
	120	51	12.5	60	58	13	6	68	42.5	18	57	20.5	1	63	6.5	0.5
	150	48	12	4	52	17.5	2.5	61	36.5	10	45	27.5	9	57	8.5	0.5
	180	48	12	4	48	14.5	4	67	41.5	6						

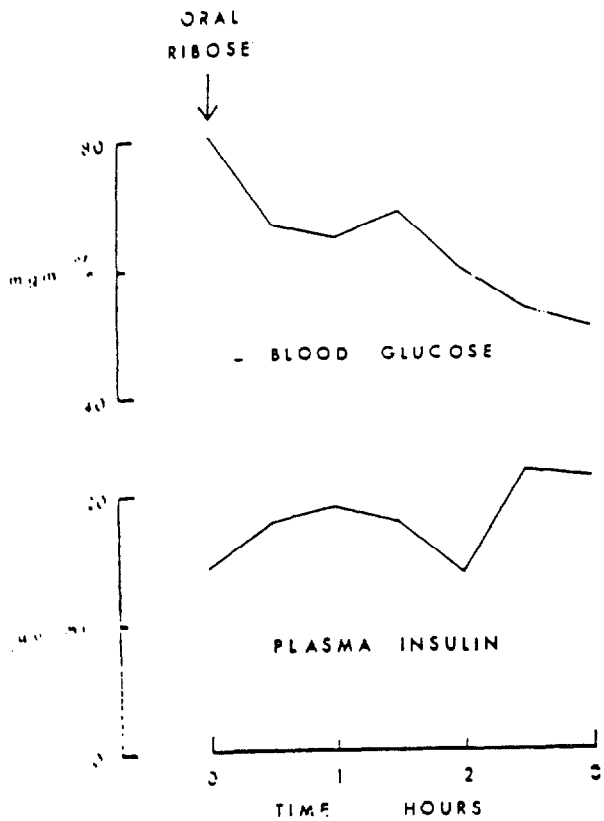

FIG. 1. Mean changes in blood glucose (mg./100 ml.) and plasma insulin (μU./ml.) in five healthy adults after ingestion of D-ribose (1 gm./kg. body weight).

which there was a second drop to a mean of 50 mg./100 ml. at three hours. The levels of blood glucose at one and at three hours were both significantly different (by the t test) from the fasting mean ($p < 0.001$ and $p < 0.01$ respectively) and from each other ($0.05 > p > 0.02$).

The hypoglycemia after oral D-ribose was not accompanied by changes in blood pressure, pulse rate or other evidence of an adrenergic response. One subject complained of a headache at about two-and-a-half hours and another noted marked peristalsis and borborygmi around this time. Diarrhea occurred in two subjects three to four hours after pentose ingestion.

Plasma insulin levels increased within half an hour of giving the oral pentose, from the fasting mean of 13.6 μU./ml. to reach a mean value of 18.7 μU./ml. at one hour. Following a slight fall there was a second rise in serum insulin, to a mean of 21.7 μU./ml., accompanying the secondary fall in circulating glucose and occurring between two and three hours. Both these increases in serum insulin after oral ribose were significant ($0.05 > p > 0.02$).

There was no consistent change in HGH levels during the test. In two patients (cases 1 and 3) there was an increased serum HGH around one to two hours but in each instance the rise occurred in association with the development of either headache or of abdominal symptoms.

Serum free fatty acid levels fell by nearly 50 per cent within half an hour of giving oral ribose (from a fasting mean of 432 mEq./L. to 250 mEq./L. at half an hour); fasting levels were regained after a further two hours.

In the experiments in which blood levels of total reducing substances were determined simultaneously with "true" blood glucose, oral ribose induced an apparent increase in blood sugar from the fasting mean of 75 mg./100 ml. (measured by the AutoAnalyzer-ferricyanide method) to 97 mg./100 ml. at half an hour, 125 mg./100 ml. at one hour, and 122 mg./100 ml. at one-and-a-half hours, fasting levels being approached (67 mg./100 ml.) by three hours. The corresponding mean values for "true" glucose determined by the glu-

cose oxidase method were 65, 55, 50, 54, and 46 mg./100 ml. respectively. That the increase in reducing substances in blood after oral pentose was predominantly ribose was illustrated in the experiment in which the sugars were estimated chromatographically. Ribose was not detected in either of the control samples but one hour after the oral dose, serum ribose was 85.6 mg./100 ml.; two-and-a-half hours after ribose administration, the blood level had fallen to 19.6 mg./100 ml. and was 7.4 mg./100 ml. at three hours. The corresponding values for blood glucose were 72.4, 60.4 and 60.2 mg./100 ml. respectively.

Intravenous ribose (table 2, figure 2)

With the intravenous pentose, blood glucose fell progressively from the fasting mean of 65 mg./100 ml. to 50 mg./100 ml. at one hour; thereafter blood glucose rose and fasting levels were regained within two hours of the injection. There were no changes in pulse rate or blood pressure and no abdominal symptoms after intravenous pentose.

Plasma insulin was more than doubled three minutess after intravenous ribose and then fell progressively over the next half hour. There was no change in blood HGH or cortisol during the period of observation.

Control studies with oral water (table 3)

There were no significant changes in blood glucose or plasma insulin for two-and-a-half hours after water was drunk in these studies. The HGH levels were either low throughout, or fell to low levels with rest as expected.

DISCUSSION

In the present study the fall in blood glucose which occurs after oral as after intravenous D-ribose was accompanied by a rise in serum insulin sustained for at least three hours but with no change in plasma levels of HGH or of cortisol.

In discussing possible causes of ribose-induced hypo-

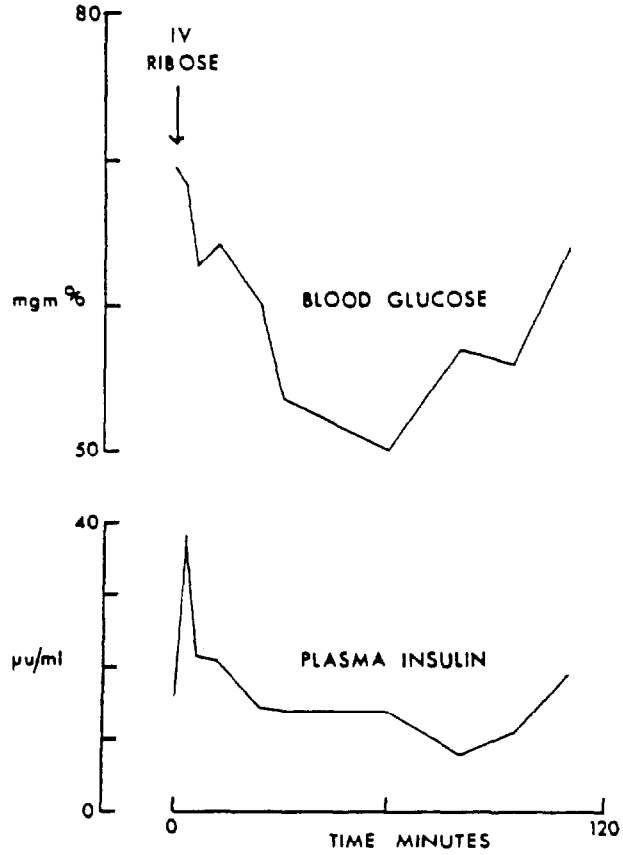

FIG. 2. Mean changes in blood glucose (mg./100 ml.) and plasma insulin (μU./ml.) in five healthy adults after intravenous infusion of 15 gm. D-ribose.

TABLE 2

Effect of 15 gm. intravenous D-ribose on blood glucose (G—mg. per 100 ml.), immunoassayable insulin (I—μU./ml.) and human growth hormone (HGH — mμg./L.) in five healthy adults

Subject		6			7			8			9			10		
	Time	G	I	HGH	G	I	HGH	G	I	HGH	G	I	HGH	G	I	HGH
Control (mean of two determinations)	0	69	14	7.5	69	12.5	2.0	61	20	3.5	59	11.5	9.5	90	21.8	7
	3	69	38	2.5	70	35	2	62	38	2.5	55	28	4.0	86	52.5	8.2
	6	70	17	2.5	67	17	1.5	61	31	2.0	53	21	3.0			
	12	66	28	1.5	63	13	1.5	59	25.5	2.5	51	6	2.5	82	32	9.8
Minutes after ribose	24	54	21	1.5	61	9.5	2.5	57	13.5	2	47	9	6.0	82	46	12.5
	30	52	15	2.0	39	15	1.5	54	12	2	47	12.5	6.0	75	17.5	10.0
	60	72	12	2.5	54	9.5	2	41	22	2	45					
	80				60	8	5.5				43	8	5.0	70		
	95				71	6	8.5	49	17.5	1.5	48	10	2.5			
	110				68	6	4.5	53	14	1.5	54	7.5	2.0	82	49.5	10.5

TABLE 3

Effect of a glass of water on blood glucose (G—mg. per 100 ml.), immunoassayable insulin (I—μU./ml.) and human growth hormone (HGH—mμg./L.) in three healthy adults

Subject		1			4			5		
	Time	G	I	HGH	G	I	HGH	G	I	HGH
Control (mean of two determinations)	0	81	11.8	0	77	7.8	2	79	3.5	23.5
	30	83	9.5	1	86	9.0	1	82	2.0	23.0
	60	79	8.8	1	80	9.5	1.5	80	0	4
Minutes	90	79	9.0	0	83	8.0	3.0	81	0	0
after	120	83	10.0	0	80	7.0	0	79	1.0	0
water	150	81	10.2	3.5	77	7.0	1	80	1.5	0

glycemia, increased secretion of insulin is obviously the first consideration, as originally suggested by Pozza.[9] A rapid rise in plasma insulin was reported after intravenous D-ribose,[10] a finding confirmed in the present study, but Steinberg and his colleagues[10] concluded that the amount of insulin secreted after ribose administration was insufficient to account for the subsequent fall in blood glucose.

This conclusion can be questioned since their estimate of insulin secretion was based solely on the peak serum insulin level recorded after ribose injection. In the present study, after oral ribose there was a sustained rise in insulin, and the biphasic fall in blood glucose was accompanied in each instance by a significant rise in blood insulin above control levels. Conversely, with intravenous ribose the onset of recovery from hypoglycemia was associated with the return of insulin to basal levels. No such rises in plasma insulin were found in the studies in which no D-ribose was given and the absence of marked fluctuations over a three-hour morning period of rest and fasting is similar to that noted by Schalch.[11] Furthermore, with more frequent blood sampling (as in a repeat test in subject 1), serum insulin was found to double within fifteen minutes of oral ribose and to remain elevated at this level for over half an hour. And since in normal adults the half time of intravenously injected insulin disappearance from plasma is two to three minutes[12] the sustained rise of serum insulin demonstrated in the present study reflects a considerable increase in insulin secretion. Hence release of insulin in response to oral ingestion of the pentose D-ribose apparently occurs at times and in amounts sufficient to explain at least partially the fall in blood glucose. The fall in serum free fatty acids after ribose administration is also in accordance with this view, for the hormone inhibits lipolysis.

The fact that the blood sugar level did not decrease in two diabetic patients maintained on insulin[1] even with large doses of oral ribose (2 mg./kg.), and that there was no fall in blood glucose after ribose infusion in the pancreatectomized dog,[13] provides further evidence of an association between insulin release and the fall in circulating glucose observed after administration of D-ribose.

The diphasic fall in blood glucose was associated in each instance with a concomitant rise in plasma insulin. It is unlikely that this "cycling" resulted from release of HGH, for no related changes in plasma levels of HGH were observed in the recent study. Increased glucagon secretion in response to falling levels of blood glucose, as reported after oral glucose,[14] might account, however, for the results. Our findings may also imply that the homeostatic mechanics concerned with the regulation of blood sugar cannot distinguish between total blood sugar and blood glucose levels after D-ribose. Accordingly, hypoglycemia after D-ribose administration might result partly from insulin release and also partly from the influence of this pentose on a receptor stimulated by glucose and which normally induces increased hepatic glucose output after cessation of insulin action. Studies of the effect of D-ribose on glucose turnover in dogs[15] support the possibility that the pentose interferes with the homeostatic mechanism maintaining normoglycemia by substituting for glucose at some receptor site.

The mechanism by which ribose stimulates insulin secretion is not, however, clear. The rapid rise of plasma insulin after intravenous ribose argues against the involvement of an intestinal hormone such as secretin. That the pentose influences insulin production directly is suggested by the increased insulin liberated when ribose was incubated with isolated rat islets.[16] Since the amount of insulin released after intraportal ribose was found to be much greater than after infusion of equivalent amounts of glucose,[17] a carbohydrate sensitive regu

lator of insulin secretion, located outside the pancreas and acting independently of the actual level of blood glucose, was postulated.[17]

The level of arterial glucose has been considered the primary stimulus determining the release of the glucoregulatory hormones—particularly of insulin and HGH—plasma insulin increasing after a glucose load and falling when blood glucose fell[15] while blood levels of HGH were suppressed by glucose and increased after insulin-induced hypoglycemia.[19] That this concept is inadequate to explain all aspects of blood glucose homeostasis is illustrated both by the glucose and hormonal responses to ribose and by many recent studies which have shown that the correlation of plasma levels of HGH and insulin with arterial blood glucose is not always close. Striking changes in HGH and insulin may occur, for example, after injection of certain amino acids which evoke only minor changes in blood glucose;[20] in subjects with spontaneous hypoglycemia there is poor correlation between blood levels of glucose and of HGH[21] and fluctuations in HGH unrelated to changes in circulating glucose have been observed during a twenty-four-hour fast.[22] Similarly, the inconsistent changes in HGH and the progressive fall in free fatty acids after oral ribose in the present study support the view that the release of HGH may relate more to the symptoms associated with hypoglycemia[21] than to the absolute or relative level of blood glucose, secretion of HGH being increased in "stressful" situations regardless of the fall in blood glucose.

The hypoglycemic action of D-ribose and its stimulant effect on insulin secretion may also have therapeutic implications, and the lowering of blood glucose after oral ribose observed in a patient with maturity-onset diabetes[1] suggests that this possibility merits further investigation.

ACKNOWLEDGMENT

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REFERENCES

1 Beaconsfield, P., and Ginsburg, J.: Hypoglycemia after oral ribose. Lancet 2,153, 1967.

2 Segal, S., Foley, J., and Wyngaarden, J. B.: Hypoglycemic effect of D-ribose in man. Proc. Soc. Exp. Biol. Med. 95:551-55, 1957.

3 Beaconsfield, P., and Ginsburg, J.: Unpublished observations, 1969.

4 Middleton, J. E., and Griffiths, W. J.: Rapid colorimetric micro method for estimating glucose in blood and CSF using glucose oxidase. Brit. Med. J. 2:1525-27, 1957.

5 Wood, D. J., and Cousins, A. R.: Automation in Analytical Chemistry, Vol. 2. Technicon Symposia, 1966. New York, Mediad, Inc., 1967, pp. 97-102.

6 Morgan, C. R., and Lazarow, A.: Immunoassay of insulin using a two-antibody system. Proc. Soc. Exp. Biol. Med. 110: 29-32, 1962.

7 Hartog, M., Cafaar, M.A., Meisser, B., and Fraser, R.: Immunoassay of serum growth hormone in acromegaly. Brit. Med. J. 2:1229-33, 1964.

8 Dole, V. P.: Relation between non-esterified fatty acids in plasma and metabolism of glucose. J. Clin. Invest. 35:150-54, 1956.

9 Pozza, G., Galansino, G., Hoffeld, H., and Foa, P. P.: Stimulation of insulin output by monosaccharides and monosaccharide derivatives. Amer. J. Physiol. 192:97-161, 1954.

10 Steinberg, T. T., Ortman, P., Poucher, R., Cochran, B., and Gwinup, G.: The effect of D-ribose infusion on serum immunoreactive insulin and glucose concentration. Metabolism 16:40-46, 1967.

11 Schalch, D. S.: The influence of physical stress and exercise on growth hormone and insulin secretion in man. J. Lab. Clin. Med. 69:256-69, 1967.

12 Stimmler, L.: Disappearance of immunoreactive insulin in normal and adult onset diabetic subjects. Diabetes 16:652-55, 1967.

13 Bierman, E. L., Baker, E. M., Plough, I. C., and Hall, W. H.: Metabolism of D-ribose in diabetes mellitus. Diabetes 8:455-58, 1959.

14 Young, J. D., and Jenkinson, I. S.: Serum insulin and glucagon during glucose tolerance test. Lancet 1:1321, 1966.

15 Hetenyi, G., and Ishiwata, K.: Effect of D-ribose on the turnover of glucose in dogs. Amer. J. Physiol. 214:1333-39, 1968.

16 Montague, W., Howell, S. L., and Taylor, K.: Pentitols and the mechanism of isulin release. Nature 215:1088-89, 1967.

17 Goetz, F. C., Maney, J. W., and Greenberg, B. Z.: The regulation of insulin secretion: Effects of the infusion of glucose, ribose and other sugars into the portal veins of dogs. J. Lab. Clin. Med. 69:537-57, 1967.

18 Welborn, T. A., Rubenstein, A. H., Halflam, R., and Fraser, R.: Normal insulin response to glucose. Lancet 1:280-83, 1966.

19 Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A.: The influence of blood glucose on the plasma concentration of growth hormone. Diabetes 13:355-61, 1964.

20 Merimee, T. J., Lillicrap, D. A., and Rabinowitz, D.: Effect of arginine on serum levels of human growth hormone. Lancet 2:668-70, 1965.

21 Marks, V., Greenwood, F. C., Howorth, P. J. N., and Samols, E.: Plasma growth hormone levels in spontaneous hypoglycemia. J. Clin. Endocr. 27:523-28, 1967.

22 Quabbe, H. J., Schilling, E., and Helge, H.: Pattern of growth hormone secretion during a 24-hour fast in normal adults. J. Clin. Endocr. 26:1173-77, 1966.

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THE METABOLISM OF D-RIBOSE IN MAN

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Previously thought to be primarily a structural component of nucleic acids, the pentose, D-ribose, in the form of the ester, ribose 5-phosphate, has now been shown to be an important intermediate in the pentose phosphate pathway of glucose metabolism. The sequence of the reactions of this pathway has been summarized by Horecker and Mehler (1) and Wood (2), and surveys of its significance in various mammalian tissues have been reported (3–6).

Naito (7) has investigated the glycogenic effect of ribose after its injection into mice and rabbits, and Herrmann and Hickman (8) have described the utilization of ribose by beef cornea. ecker, Gibbs, Klenow, and Smyrniatis (9) ....e demonstrated the conversion of ribose to hexose with rat liver homogenates and Katz, Abraham, Hill, and Chaikoff (10), incubating ribose 1-C^{14} with rat liver slices, observed the formation of glucose with a pattern of labeling that was predictable from the reactions of the pentose phosphate pathway. Similar findings have been reported by Hiatt (11), studying the *in vivo* conversion of labeled ribose to glucose in the mouse. Recently Agranoff and Brady (12) have isolated and purified from calf liver an enzyme, ribokinase, which is capable of phosphorylating ribose to ribose 5-phosphate, thus permitting the sugar to enter the pentose phosphate pathway. The entrance of ribose into the pathway, and the sequence of its conversion to glucose are shown in Figure 1.

Since ribose appears to be of importance in mammalian metabolism, we have undertaken a study of certain phases of ribose metabolism in man. This paper presents data concerning the fate of ribose after its intravenous administration in both unlabeled and C^{14} labeled form to both normal and diabetic subjects. Preliminary results ese studies have been published (13), as well a report of the hypoglycemia induced by ribose infusion (14). Previous publications (15, 16) from this laboratory have described the physiologic disposition in man of the related pentoses D-xylose, D- and L-arabinose, and D-lyxose, as well as the effects of insulin on the blood levels of these sugars.

MATERIALS AND METHODS

Part of the D-ribose used in these experiments was a generous gift of Dr. Hewitt G. Fletcher, Jr., of the Section on Carbohydrates, Laboratory of Chemistry of this Institute, and part was purchased from the Pfanstiehl Co., Waukegan, Ill. The sugar was pure, by the criteria of both optical rotation and paper chromatography. Seven and a half per cent sterile pyrogen-free solution of the sugar was used for infusion. Ribose 1-C^{14}, specific activity 2.01 μc. per mg. and 0.89 μc. per mg., was obtained from the National Bureau of Standards and was found to be solely ribose by chromatography and radioautography. Purified phosphoglucomutase prepared according to Najjar (17) was a gift of Dr. K. Kurahashi. The glucose 1-phosphate was commercial material electrophoretically freed of glucose 1, 6-diphosphate. Ribose 5-phosphate was obtained from Schwarz Laboratories and used as the sodium salt.

Twenty-seven intravenous infusions of various amounts of D-ribose were given to six fasting normal male and one female volunteers, 19 and 24 years of age, and to three subjects with diabetes mellitus. All of the normal subjects were maintained on a 250 Gm. carbohydrate diet prior to study. Diabetics received their last dose of crystalline insulin 24 hours before each experiment. Administration of the ribose solution was carried out either over a 15 minute period or, after a small priming dose, by constant infusions, using a Bowman pump. By the technique already described (15), blood specimens were obtained, those for glucose and ribose determination at 3 to 10 minute intervals, and those for phosphate and pyruvate analysis at 20 minute intervals. In eleven experiments 0.1 U per Kg. body weight of crystalline insulin was given intravenously in order to ascertain the effects of insulin on blood levels and urinary excretion of the pentose. Fractional urines were obtained for 24 hours and preserved by freezing.

Analytical procedures. Blood and urine glucose were estimated by employing the glucose oxidase method devised in this laboratory (15). Ribose was analyzed by the orcinol method (18), using blood filtrates and urine in which glu e had been destroyed by glucose oxidase. Serum inorganic phosphate was determined by the method

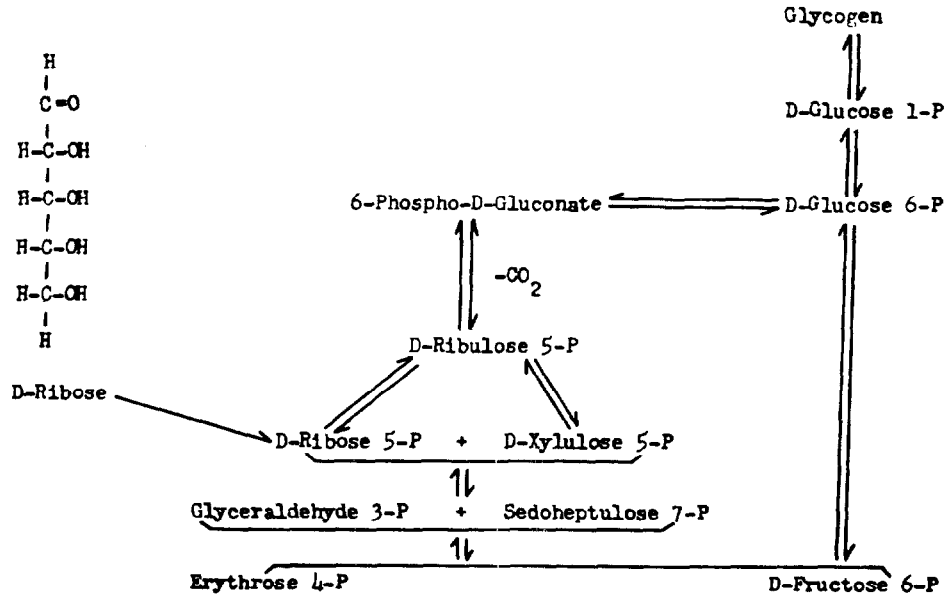

FIG. 1. THE SEQUENCE OF REACTIONS FOR THE ENTRANCE OF RIBOSE INTO THE PENTOSE PHOSPHATE PATHWAY AND ITS CONVERSION TO GLUCOSE

of Fiske and Subbarow (19) and blood pyruvate by the method of Segal, Blair, and Wyngaarden (20).

Expired CO_2 was collected and isolated as barium carbonate, as previously described (15). The CO_2 content of expired air was measured in a Cambridge CO_2 analyzer. Radioactivity in the CO_2 was determined by liberating the gas from barium carbonate and diffusing it by the method of Eisenberg (21) into hyamine base, which was counted by the technique of Passmann, Radin, and Cooper (22) in a liquid scintillation counter (Packard Co., La Grange, Ill.). The overall efficiency of CO_2 counting was 39 per cent. Cumulative excretion of $C^{14}O_2$ was estimated according to Berlin, Tolbert, and Lawrence (23).

Blood radioactivity was assayed in the following manner: Ten ml. of blood was added to an equal volume of 7 per cent perchloric acid for protein precipitation. An aliquot of resultant filtrate was neutralized with 5 N potassium hydroxide, and the precipitated $KClO_4$ centrifuged. A portion of the neutralized filtrate was then concentrated to 1.5 ml. under a nitrogen stream, and 0.5 ml. of this was added to 10 ml. of dioxane phosphor containing 5 per cent naphthalene and 0.8 per cent diphenyloxazole (24) for scintillation counting.

Urinary C^{14} counting was performed as in our previous communication (15).

Urine was chromatographed on Whatman No. 1 paper in a descending system for eight hours, employing methyl ethyl ketone, acetic acid, water, 6:1:1 as the solvent (R_F glucose, 0.07; ribose, 0.23). When tracer amounts of C^{14} pentose had been used, unlabeled ribose was added as a marker, the markers being visualized with analine hydrogen phthalate spray (25). Sugars were eluted by shaking paper strips with water in a small flask. The eluates were concentrated and assayed for activity in the same manner as whole urine. Recovery of sugars chromatographed in this was ranged from 93 to 103 per cent.

Glucose was isolated as crystalline potassium gluconate from the urine of the diabetic subject, who received C^{14} ribose by treating the urine according to the procedure of Moore and Link (26). The potassium gluconate was recrystallized twice from methyl alcohol. One portion of the gluconate was oxidized to CO_2 and counted as above, and another was degraded by the periodate method of Eisenberg (27), whereby carbon atoms one and six, individually, and two to five, collectively, could be isolated as CO_2 and counted.

The assay for phosphoglucomutase activity was that of Klenow (28).

RESULTS

The disappearance from blood of ribose administered intravenously

Semilogarithmic plots of decreasing blood ribose levels determined after the 15 minute infusion of various amounts of the sugar are shown in Figure 2. It is apparent that an entirely linear curve is obtained only when 3 Gm. amounts were injected, and that doses larger than 3 Gm. produced a lag phase before the onset of a linear disappearance. The deviation from a straight line of the blood ribose values in the lag phase does not represent a distribution gradient between blood and the total ribose compartment, for deviations due to this phenomenon lead to initial values higher than the linear curve. Likewise, the deviation is not due to a distribution gradient of ribose between

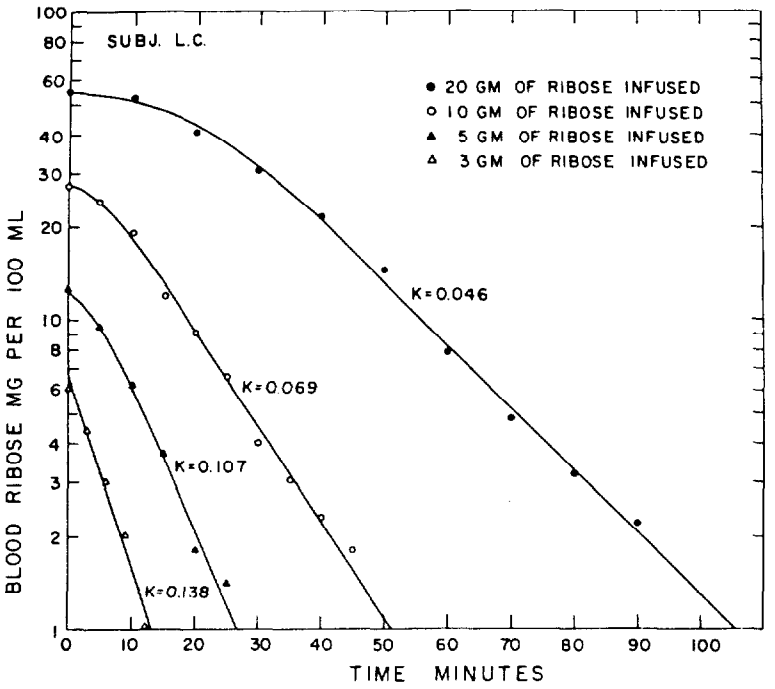

FIG. 2. SEMILOGARITHMIC DISAPPEARANCE CURVES OF RIBOSE FROM BLOOD AFTER 15 MINUTE INFUSION OF VARIOUS AMOUNTS OF PENTOSE (SUBJECT L. C.)

plasma and red blood cells, for experimentally determined ribose concentrations were essentially the same in whole blood and plasma during this early phase. These results indicate that the removal of ribose from blood is a first order kinetic process only when the ribose blood level is below some critical value.

Although developed for the analysis of substrate enzyme relationships, the mathematical treatment of Lineweaver and Burk (29) has been applied to the more complex problem of sugar transport across cell membranes (30). This type of analysis has been applied here to the clearance of ribose from blood. Using the data from the studies in Subject L. C. (Figure 2) and plotting the reciprocal of the initial disappearance rate in mg. per 100 ml. per minute versus the reciprocal of the blood ribose concentration in mg. per 100 ml., one obtains the straight line curve shown in Figure 3. From the curve, the K_S, or ribose concentration, at the point of half saturation of the arance system, can be derived. The K_S is 10 g. per 100 ml. Thus the system is completely saturated at initial blood levels of 20 mg. per 100 ml., and linear curves should be obtained when the blood level is below this value.

In Table I are given the proportional rate constants for the disappearance of ribose from blood, as derived from a knowledge of biological halftime, and expressed in terms of per cent of ribose disappearing per minute. K^1 equals K times 100. In

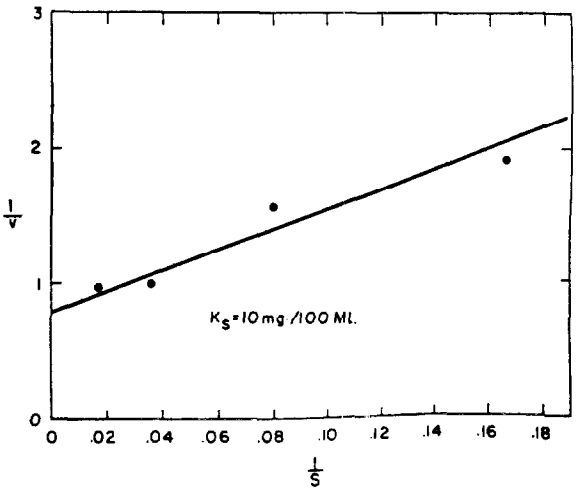

FIG. 3. GRAPH OF THE RECIPROCAL OF THE INITIAL VELOCITY IN MILLIGRAMS PER 100 MILLILITERS PER MINUTE VERSUS THE RECIPROCAL OF THE INITIAL CONCENTRATION IN MILLIGRAMS PER 100 MILLILITERS OBSERVED AFTER THE INFUSION OF VARIOUS DOSES OF RIBOSE IN SUBJECT L. C.

the normal subject, the rate constant ranges from 3.8 to 5.8 per cent per minute after a 20 Gm. dose. As the dose decreases, the rate constant increases to as high as 13.8 for 3 Gm. It is interesting to note that as the dose is halved, in Subject L. C., Figure 1, the K value is increased by a constant factor of 1.5.

The blood glucose values in the three diabetics, C. M., M. S., and N. N., were 470, 310 and 293 mg. per 100 ml., respectively. Despite the obvious lack of adequate ability to metabolize glucose, the ribose disappearance curve was identical in pattern to the normal, with a K^1 of 4.6 to 5.1. A normal disappearance curve of the pentose D-xylose has similarly been noted in Subject C. M. (31).

The effect of insulin on blood ribose levels

The previously reported enhancement by insulin of the disappearance from blood of D-xylose L-arabinose and D-lyxose from blood (16) has been observed with D-ribose. Figure 4 reveals in Subject W. W. a control ribose disappearance

TABLE I

Ribose infusion in normal and diabetic subjects

Subject	Weight *Kg.*	Quantity infused *Gm.*	Disappearance from blood K^1 *%/min.*	Volume of distribution based on Observed C_0^1* *L.*	Volume of distribution based on Calculated C_0^1 *L.*	Urinary recovery *% admin. dose*	Time of insulin admin. relative to end of infusion *minutes*
Normals							
W. Y.	81	20	3.8	24.9	19.0	22	
W. W.	74	20	4.8	21.5	10.7	20	
W. W.	74	20	4.8	19.3	10.7	21	
J. D.	81	20	5.0	26.1	19.0	32	
J. D.	81	20	5.8	21.5	3.8	21	
L. C.	80	20	4.6	29.1	11.0	26	
L. C.	80	10	6.9	29.6	21.7	18	
W. B.	64	10	8.2	21.5	7.9		
J. D.	81	10	10.5	19.6	5.5	17	
L. C.	80	5	10.7	31.7	22.2	14	
L. C.	80	3	13.8	40.0	36.3		
J. D.	81	3	11.9	24.3	24.8		
Diabetes mellitus							
C. M.†	60	20	4.6	22.3	9.2	24	
M. S.‡	73	20	5.1	24.4	10.5	30	
N. N.§	60	20	5.1	20.8	6.7	24	
Normal plus insulin							
L. C.	80	10	9.3			17	−10
C. C.	66	10				14	+25
W. R.	66	20	4.3			18	+30
W. R.	66	20	4.5			18	+30
W. B.	64	20				23	+20
W. W.	74	20	6.0			16	+20
W. W.	74	20	6.0			18	− 7
W. W.	74	20	5.8			21	+ 2
W. W.	74	constant‖ infusion				12	+60††
W. W.	74	constant¶ infusion				8	+70††
W. W.	74	constant** infusion	7.0‡‡			5	+70††

* See text, footnote 1.
† Fifty-three year old male requiring a total of 36 U of globin and crystalline insulin daily.
‡ Twenty-three year old male requiring a total of 45 U NPH and crystalline insulin daily.
§ Thirty-one year old male requiring a total of 45 U of NPH and crystalline insulin daily.
‖ Constant infusion 137 mg. per minute after a 4 Gm. prime (Figure 5).
¶ Constant infusion 146 per minute after a 4 Gm. prime.
** Constant infusion 151 mg. per minute after a 4 Gm. prime.
†† Insulin injected at stated time after the start of the constant infusion.
‡‡ Calculated from data obtained for decreasing blood levels after cessation of the constant infusion.

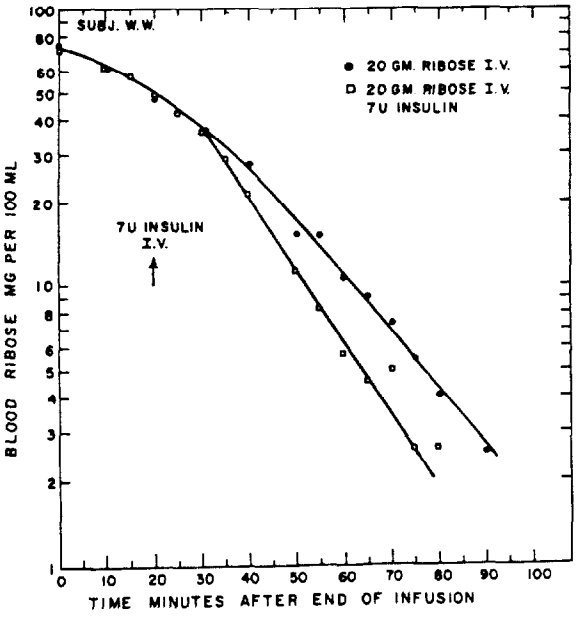

FIG. 4. THE EFFECT OF INTRAVENOUS INSULIN ON THE DISAPPEARANCE OF RIBOSE FROM BLOOD

curve, as well as one after insulin administration. As a result of the insulin injection, an earlier onset of the linear phase and an increase in the rate constant of disappearance were seen. In another experiment in Subject W. W. (Table I), insulin was injected during the ribose infusion so that its effect might be observed at high blood ribose values.

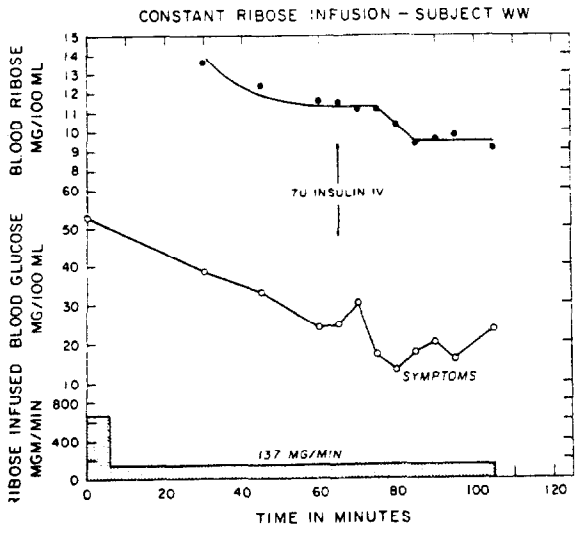

FIG. 5. BLOOD LEVELS OF RIBOSE AND GLUCOSE OBSERVED DURING THE CONSTANT INFUSION OF RIBOSE

Effects of intravenous insulin administration.

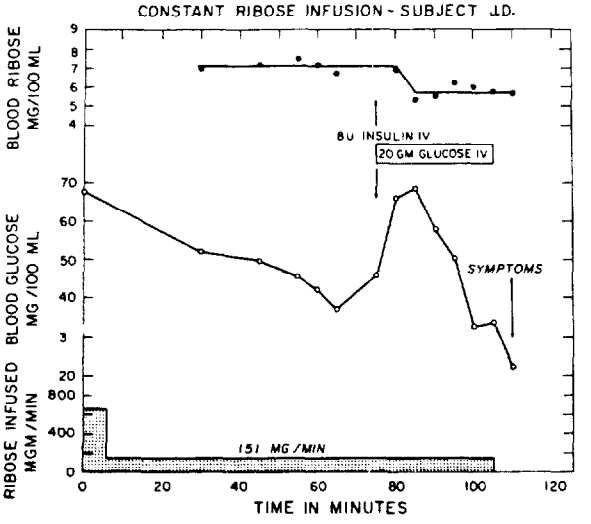

FIG. 6. BLOOD LEVELS OF RIBOSE AND GLUCOSE OBSERVED DURING THE CONSTANT INFUSION OF RIBOSE

Effects of intravenous insulin and intravenous glucose administration.

No clear effect was seen, however, until the level had fallen to about 30 mg. per cent, at which point the curve deviated from the control and became superimposable on the insulin curve shown.

Because of the unusual kinetics of ribose disappearance and the rapid decline in blood levels, the effect of insulin was tested on ribose blood levels maintained at a low constant value by continuous ribose infusion, a technique used previously (16). Figures 5 and 6 demonstrate these studies. After insulin, the ribose level falls, with a subsequent attainment of a new constant blood level. The intravenous administration of a sufficient quantity of glucose to raise the blood sugar to the control level (Figure 6) did not prevent the insulin effect, thus indicating that the response of ribose to insulin is independent of hypoglycemia *per se*. The symptoms of hypoglycemia occurring after insulin injection were not alleviated by the presence of ribose in the body.

The distribution of ribose in body fluids

The volume of distribution of ribose calculated from C_0' [1] values obtained by graphic extrapolation

[1] The concentration of ribose in plasma water (C′) has been calculated from the analytical value on whole blood (C) divided by 0.8, the approximate water content of blood. Volume of distribution equals Q/C_0' in which C_0' equals $C_0/0.8$, the concentration of ribose in

of the linear phase of the ribose disappearance curve to t_0 averaged 16.1 L. in 12 studies on five normal subjects (Table I). There is a wide range, however, in the volume of distribution calculated by this method, depending on the dose of ribose due to the kinetics of disappearance from blood. The fact that the C_0' obtained by extrapolation of the decay curve after a 3 Gm. dose was essentially equal to the observed C_0' may indicate that complete mixing has occurred. Assuming that equilibrium of distribution had been achieved during the 15 minute infusion in the other experiments, the observed C_0' appears suitable for use in the calculation. Employing the latter value, the average value of distribution is 25.8 L. with very little variation with the dose. The diabetics appear to have a normal ribose "space," as determined by both of these methods of estimation.

Calculations of the above type present the inherent difficulty of estimating the amount present in the body at time zero, the theoretical moment of mixing, as well as the concentration at that time, mainly due to the fact that ribose has a metabolic fate other than simple distribution and excretion. Under these circumstances, a preferable type of calculation is that employed by us for determining the volume of distribution of xylose (16) and based on the theoretical development of Dominguez, Goldblatt, and Pomerene (32, 33) and others (34). This treatment requires the constant infusion type of experiment shown in Figures 5 and 6. From knowledge of the concentration of ribose in blood at equilibrium, the composite rate of loss of ribose from the compartment, which is equal to the rate of infusion, and the rate constant of ribose disappearance from blood as determined following the completion of the infusion, the volume of distribution for Subject W. W. was calculated to be 14 L. before insulin administration. Since this individual weighed 74 Kg., the ribose compartment was equal to 19 per cent of body weight, a distribution essentially in extracellular fluid. This finding parellels that for D-xylose (15).

The volume of the ribose compartment after insulin was calculated to be 16.6 L. or 22.4 per cent of body weight. This increase in ribose "space" due to insulin is similar in magnitude to that seen with D-lyxose, but much smaller than that observed with D-xylose or L-arabinose.

plasma water at the theoretical moment of mixing, and Q is the quantity infused (15).

The urinary excretion of ribose

The urinary excretion of ribose as orcinol reactive material is shown in Table I. Appropriate correction has been made for the small amount of glucuronic acid which is orcinol reactive in urine. An average total of 21 per cent of the dose was excreted by the normal subject after a 15 minute ribose infusion, nearly all of this appearing in a 90 minute postinfusion period. The diabetic and the normal subject who received insulin excreted renally amounts of ribose within the control range. Data on Subject L. C. who received various ribose doses suggest that the urinary excretion decreases as the dose is diminished. When 20 Gm. of ribose was given at a constant slow rate, only 5 to 12 per cent was found in the urine. This appears to be a consequence of the slow infusion rate, and is not related to the insulin given during these experiments. In one such experiment, urine collected prior to insulin injection revealed a urinary loss of 8 mg. per minute, while 146 mg. per minute was being infused. This excretion rate of 5.5 per cent per minute approximates very well the total excretion of 5 per cent of the dose. These results are in contrast to those obtained with D-xylose. The total urinary loss of this pentose was approximately the same following the rapid injection of various doses, as well as after slow constant infusion (16).

The effect of ribose on blood levels of glucose, pyruvate and inorganic phosphate

It has been reported that the infusion of ribose into the normal and some diabetic individuals causes a lowering of blood glucose of about 30 mg. per cent (14). A typical response of blood glucose to ribose is demonstrated in Figure 7 in Subject W.W. Immediately after ribose injection, only slight fluctuation in glucose levels occurred. However, about 30 minutes after the end of the infusion, the blood glucose began to decline, reached a nadir in about 30 minutes, and then returned to approximately the control level. The effect was observed when blood ribose values had decreased to low levels. This hypoglycemic effect

TABLE II

Change in blood glucose and phosphate after infusion of various doses of ribose

Subject	Amount infused *Gm.*	Control blood glucose *mg. per 100 ml.*	Minimal blood glucose *mg. per 100 ml.*	Decrease in inorganic phosphate *mg. per 100 ml.*
L. C.	20	71	30	
L. C.	10	84	50	0.6
L. C.	5	67	61	0.9
L. C.	0 (saline)*			
J. D.	20	63	37	0.3
J. D.	20	91	59	
J. D.	10	53	45	1.2
J. D.	13.7†	68	37	
J. D.	0 (saline)*			0.3

* Two hundred sixty-seven ml. of normal saline was infused for 15 minutes and blood for phosphate determination was obtained at 20 minute intervals.

† Four Gm. prime followed by constant infusion of 151 mg. per minute.

of ribose is shown again in the same subject (Figure 5) after a 4 Gm. priming dose and the ant infusion of ribose at a slow rate.

able II reveals the effect of different doses of ribose on blood glucose values in two subjects. A marked reduction in blood glucose occurred after 20 and 10 Gm. doses, with but a questionable change after 5 Gm. in Subject L. C. Subject J. D.'s blood glucose fell reproducibly after 20 Gm. infusions but decreased only slightly after 10 Gm. was given. In contrast to the latter result, when Subject J. D. was given ribose by constant infusion (Figure 6), his blood glucose levels had fallen from 68 to 52 mg. per cent after receiving 8 Gm. and to a low value of 37 mg. per cent after receiving only 13.7 Gm.

Though blood glucose values as low as 15 mg. per cent were observed, no subject complained of hypoglycemic symptoms, and only in Subject W. W. could some slight signs (blanching of lips and a peculiarity of gaze) be detected. When 0.1 U per Kg. of insulin was given during the ribose-induced hypoglycemia (Figure 5), greater hypoglycemia occurred, and frank symptoms were then observed.

A fall in serum inorganic phosphate was consistently seen after ribose infusion (seven studies).

our normals receiving 20 Gm. the decrease ged from 0.3 to 0.7 mg. per cent. One diabetic showed a decline of 0.6 mg. per cent. The significance of these results appears to be open to question when one observes the blood phosphate changes in a given subject who received various ribose doses (Table II). Subject L. C. shows a greater phosphate fall after a 5 Gm. than after a 10 Gm. dose, and J. D., a greater fall after a 10 Gm. than after a 20 Gm. dose. Both individuals respond to saline administration with a 0.3 mg. per cent fall in phosphate, this being equal to the response of J. D. to a 20 Gm. infusion of ribose.

The blood pyruvate changes observed after 20 Gm. infusions in four normals and one diabetic were variable and insignificant, ranging from − 0.28 to + 0.10 mg. per cent. The relationship of inorganic phosphate and pyruvate to glucose and ribose levels is shown in Figure 7. Worthy of note is a comparison of the effects of ribose and insulin on the blood phosphate and pyruvate in a given individual. In the experiment shown in Figure 5, the phosphate had fallen 0.5 mg. per cent and the pyruvate was unchanged from the control value at the time when the blood glucose had decreased to 23 mg. per cent (60 minutes). The

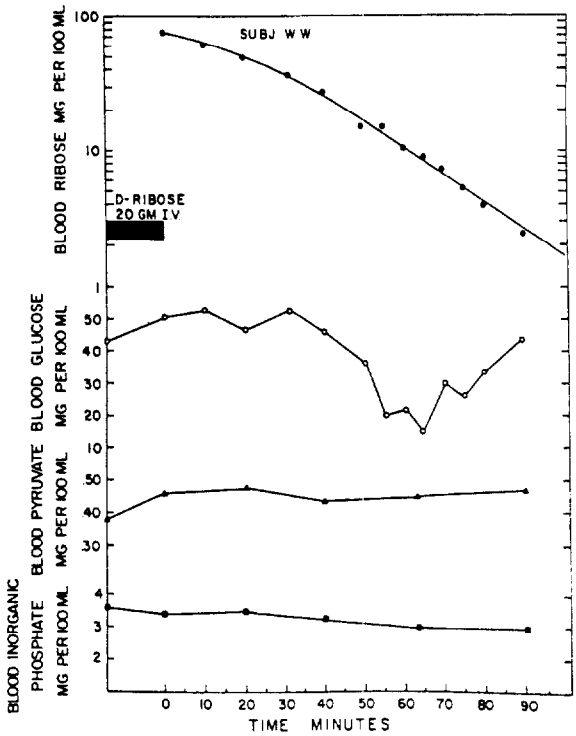

FIG. 7. THE EFFECT OF THE ADMINISTRATION OF RIBOSE ON BLOOD GLUCOSE, PYRUVATE AND SERUM INORGANIC PHOSPHATE LEVELS

A significant decrease in blood glucose level is noted.

injection of insulin and concomitant fall in glucose was followed by a further fall in phosphate of 1 mg. per cent and a rise of blood pyruvate from 0.51 mg. per cent to 1.40 mg. per cent within 28 minutes. The lowest blood glucose value after insulin in this study is about equal to the lowest value seen after ribose in the same subject (Figure 7) where little change occurred in phosphate or pyruvate values.

Studies with phosphoglucomutase

The lack of a significant increase in blood pyruvate and questionable decrease in serum inorganic phosphate, accepted parameters for indicating increased peripheral glucose utilization (35–37), suggested that the ribose-induced hypoglycemia was not due to an insulin-like effect. Quantitative determination of urinary glucose after ribose infusion (14) eliminated glucosuria as a cause and indicated that the hypoglycemia might be the result of a defect in the hepatic homeostatic mechanism for maintenance of blood glucose.

One of the important reactions in the formation of glucose from glycogen is the conversion of glucose 1-phosphate to glucose 6-phosphate by the enzyme phosphoglucomutase. Recently, Sidbury (38) and Ginsburg and Neufeld (39) have observed an inhibition of this enzyme by galactose 1-phosphate, a fact which could explain the hypoglycemia seen in galactosemics after galactose infusion (40). Because of these observations, studies were performed to demonstrate the effect of ribose 5-phosphate on phosphoglucomutase activity.

The assay system of glucose 6-phosphate dehydrogenase and triphosophopyridine nucleotide detects the formation of glucose 6-phosphate from glucose 1-phosphate by an increase in reduced triphosophopyridine nucleotide recorded as an increase in optical density at 340 mμ as in Figure 8. Two types of inhibition of phosphoglucomutase have been observed. The first can be shown by adding increasing amounts of ribose 5-phosphate to the reaction mixture. The uppermost curve in Figure 8 shows the enzymatic activity when 0.5 μM of ribose 5-phosphate is added simultaneously with the substate glucose 1-phosphate. This curve represents a 5 per cent inhibition of the enzyme. When 2.5 μM and 5 μM of ribose ester are added, one obtains a 59 and 72 per cent inhibition, respectively. We believe this to be an ion effect of ribose 5-phosphate, for Cori, Colowick, and Cori (41) and Klenow (42) have shown the enzyme to be sensitive to changes in salt concentration of both inorganic ions and organic phosphates.

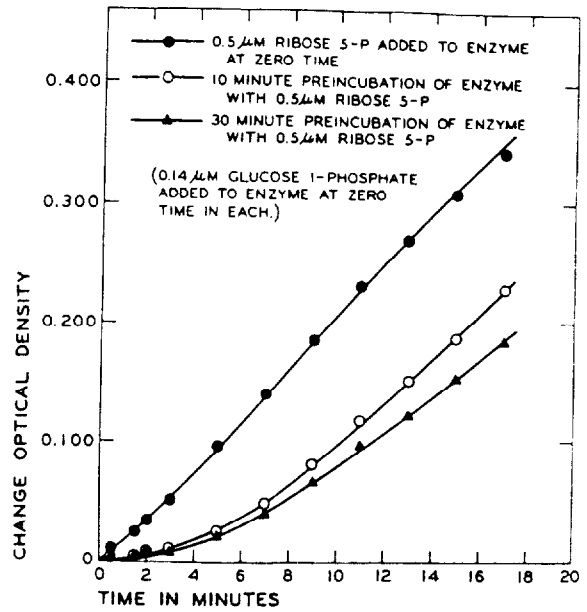

FIG. 8. THE INHIBITION OF PHOSPHOGLUCOMUTASE BY RIBOSE 5-PHOSPHATE

The ordinate represents the optical density change at 340 mμ due to the formation of reduced triphosphopyridine nucleotide by the action of glucose 6-phosphate dehydrogenase on the glucose 6-phosphate formed by the mutase reaction.

The second type of inhibition can be shown by preincubation of the enzyme with the ribose ester. The results of this preincubation experiment are shown in Figure 8. Here is seen a profound initial inhibition of the mutase reaction which is eventually overcome. Klenow and Emberland (43) have demonstrated that phosphoglucomutase will convert ribose 5-phosphate to ribose diphosphate. Since, to be active, the enzyme must be in a phosphorylated state, the inhibition seen in this type of experiment may be interpreted as a dephosphorylation of the enzyme during the preincubation period due to ribose diphosphate formation.

The fate of C^{14} after injection of ribose-1-C^{14}

In an effort to obtain information concerning the metabolic disposition of ribose, two studies

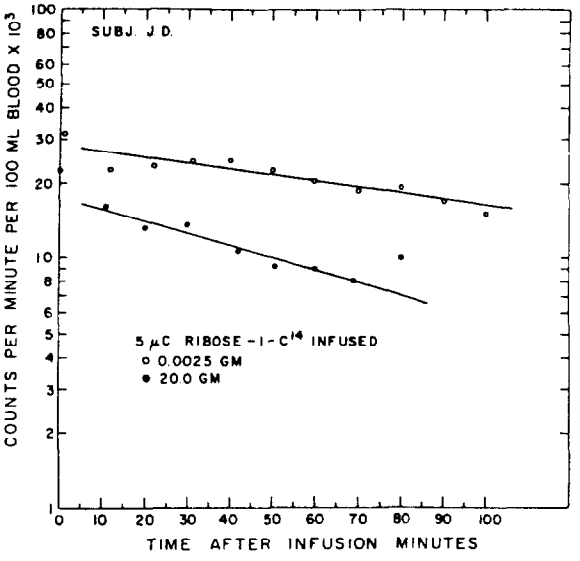

FIG. 9. A SEMILOGARITHMIC PLOT OF THE C^{14} DISAPPEARANCE FROM BLOOD AFTER RIBOSE-1-C^{14} INFUSION BOTH AS TRACER AND LOAD DOSES

w[illegible] carried out in one normal individual in which [illegible]ate of the label was determined following intravenous administration of ribose-1-C^{14}. In one experiment, 5 μc. of ribose-1-C^{14} was infused as a tracer dose (2.5 mg.). In the other, 5 μc. of the labeled material plus 20 Gm. of unlabeled ribose were infused from the same flask. The disappearance of C^{14} from blood, and the appearance of the label as expired $C^{14}O_2$, as well as the excretion of the label in urine were determined.

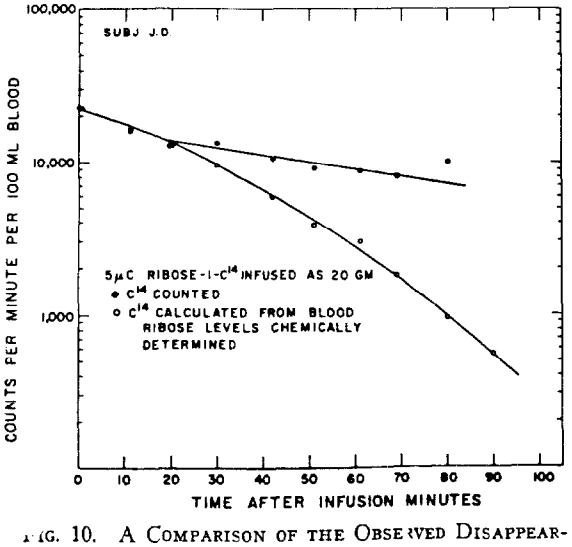

FIG. 10. A COMPARISON OF THE OBSERVED DISAPPEARANCE OF C^{14} FROM BLOOD AND THAT CALCULATED FROM CHEMICALLY DETERMINED RIBOSE LEVELS

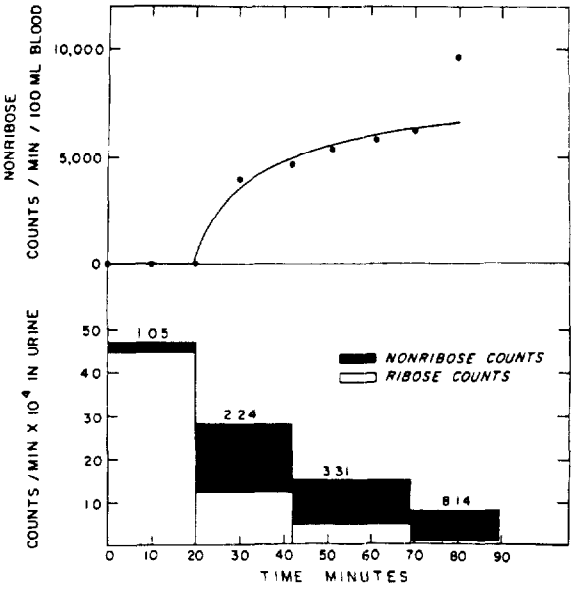

FIG. 11. THE SIMULTANEOUS APPEARANCE OF NONRIBOSE C^{14} IN BLOOD AND URINE

Numbers above bars indicate the ratio of total C^{14} counts to ribose counts in urine.

The disappearance of C^{14} from blood

Figure 9 demonstrates the disappearance curve of C^{14} from blood plotted semilogarithmically. The linear curves denote that C^{14} disappears according to first order kinetics. From the biologic halftime of disappearance, the rate constant of disappearance for the tracer dose and the 20 Gm. dose have been calculated to be 0.61 and 1.16 per cent per minute, respectively. These small rate constants are in marked contrast to the rather large ones observed for the linear disappearance of ribose chemically determined. Also worthy of note is the fact that the C^{14} decay constant for the larger dose is about three times as great as that for the tracer, an observation which differs from that noted for orcinol-determined ribose, namely that the rate constant is inversely proportional to the dose.

The constrast between the C^{14} and chemical disappearance curves for the load (20 Gm.) study is shown in Figure 10.[2] All of the data indicate

[2] The total C^{14} activity values of the curve based on chemically determined ribose were calculated by multiplying the analytical value in mg. per cent by the specific activity of ribose at zero time $\frac{(C^{14}\text{ counted})}{(\text{mg. per cent})}$ assuming the specific activity of ribose remained constant during the period of study.

that after ribose-1-C^{14} injection, as the ribose disappears from the blood, nonribose C^{14} appears. The rate of appearance of this nonribose C^{14} in blood is shown in Figure 11.

The urinary C^{14} excretion after labeled ribose injection

A total of 10 per cent of the C^{14} administered in tracer dosage appeared in the urine, whereas 40 per cent of the 5 μc. dose given as a load was excreted via this route. During the first hour after injection, about four times as much label appeared in urine in the latter study as in the former. This difference probably explains the greater rate constant for C^{14} disappearance from blood after the 20 Gm. dose.

The concomitant administration of carrier ribose with C^{14} ribose permitted comparison of urinary excretion of C^{14} and orcinol reactive material. Whereas the cumulative urinary excretion of C^{14} was 40 per cent, only 20 per cent of administered ribose as measured by orcinol was excreted by this route. The appearance of orcinol reactive material and total C^{14} in urine specimens obtained during the 90 minute postinfusion period is shown by the bar graph in Figure 11. The numbers above each bar represent the ratio of the total C^{14} activity to that due to orcinol-determined ribose. It is quite clear that with time, an increase in the proportion of total counts to ribose counts takes place, a phenomenon which parallels the increasing nonribose counts in blood.

Urine from these studies was chromatographed on paper, and the ribose area eluted and counted. Urine collected during the first hour after the tracer dose was chosen for study, since it contained nearly all of the C^{14} excreted. It was found that 92 per cent of the C^{14} eliminated after the tracer dose was unaltered ribose. A similar study of urine from the load experiment (20 to 42 minute period, Figure 11) revealed that only 42 per cent of the C^{14} applied to the paper could be located in the ribose band. This gives a ratio of total counts to ribose counts of 2.4 corroborating the ratio calculated (2.24) from urine counting and chemical data. The nature of the metabolite or metabolites in this urine remains to be identified.

Excretion of ribose as $C^{14}O_2$

The specific activity of expired $C^{14}O_2$ is shown in Figure 12. The CO_2 is appreciably labeled 5 minutes after the end of the 15 minute infusion period in both tracer and load studies on Subject J. D. However, there are marked differences between the curves in these two experiments. In the tracer experiment, the specific activity of the $C^{14}O_2$ increased at a faster rate, reached a peak about 40 minutes earlier, and maintained a much higher activity throughout the interval of observation. Both of the curves appear to show a period of constant specific activity after the peak labeling has been reached, a finding not encountered in studying the $C^{14}O_2$ excretion after the administration of other labeled pentoses (44).

The determination of the cumulative C^{14} in the expired air for six hours revealed 48 per cent of the tracer C^{14} but only 16 per cent of the C^{14} given in 20 Gm. Since a large quantity of C^{14} was found in the urine when the 20 Gm. was injected, however, comparison of the amount of metabolism to $C^{14}O_2$ may better be based not on the dose, but on the dose corrected for the six hour urinary excretion. When this is done, it is seen that 50 per cent of the retained activity is converted to CO_2 six hours after the tracer dose and 24 per cent after the larger dose. It seems apparent when comparing the $C^{14}O_2$ and urinary C^{14} excretion data above that when a load of ribose is given, a large quantity of metabolite is excreted in urine with reduction in the amount appearing as expired $C^{14}O_2$.

The metabolism of ribose-1-C^{14} in diabetes mellitus and evidence for the existence of the pentose phosphate pathway in man

Five μc. of ribose-1-C^{14} was given intravenously to a 53 year old, 60 Kg., unstable diabetic (Subject C. M., Table I) whose last dose of crystalline insulin was given 24 hours prior to the study. Blood glucose during the period of observation was 475 mg. per cent and acetone was present in the urine.

The specific activity of the expired $C^{14}O_2$ can be seen in Figure 12. Compared to results obtained in the tracer study in the normal (J. D.), the specific activity of the $C^{14}O_2$ is much lower, and the shape of the curve greatly altered. In the

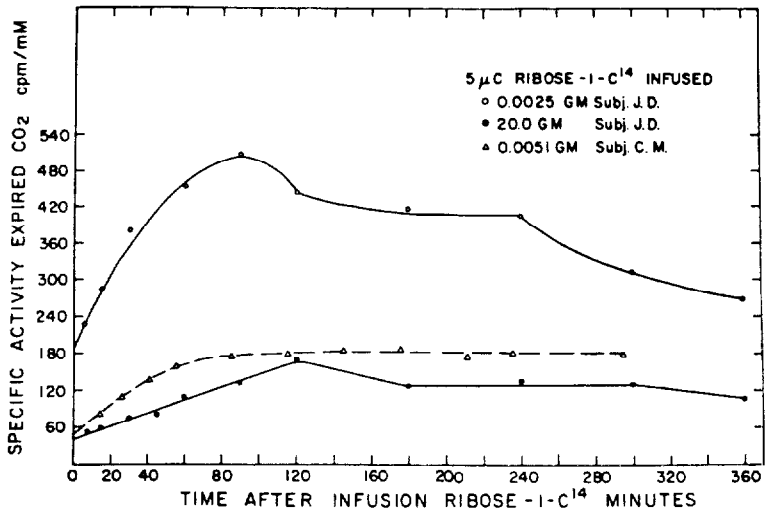

FIG. 12. THE SPECIFIC ACTIVITY OF EXPIRED $C^{14}O_2$ AFTER THE INFUSION OF RIBOSE-1-C^{14}

diabetic, the labeling of CO_2 reaches a low maximum and then remains constant throughout the ·od of observation. In five hours, the cumu- e C^{14} excretion was only 13 per cent of the retained activity (administered dose of C^{14} corrected for urinary excretion). The difference from the normal in the specific activity of $C^{14}O_2$ and the low cumulative excretion of C^{14} as CO_2 from ribose-1-C^{14} in the diabetic are not reflected in the disappearance curve of ribose from blood. The latter is unaltered from the normal in this subject and in the other diabetics tested (Table I), and suggests that the defect in the conversion of the C^{14} of ribose to $C^{14}O_2$ in the diabetic occurs at a stage later than the clearance of the sugar from blood.

Urine was collected for 24 hours, hourly specimens being · btained for the first 5 hours. Twenty-eight per cent of the C^{14} administered was excreted via this rou·e in 5 hours, and 35 per cent in 24 hours. Ribose and glucose in the hourly urine specimens were separated by paper chromatography, eluted from the paper and then counted. All of the radioactivity applied to the paper was found to reside in the ribose and glucose areas after development of the chromatogram. The cumulative excretion of C^{14} in ribose and glucose : he diabetic urine is shown in Figure 13. Five s after ribose-1-C^{14} injection, ribose C^{14} excretion in the urine had almost ceased, the urinary pentose accounting for only 10 per cent of the C^{14} dose. Eighteen per cent of the administered label resided in glucose at this time. The 10 per cent excretion of C^{14} as ribose is essentially the same as that observed in the normal. It thus appears that the large amount of glucosuria occurring in this subject did not cause an increase in ribose excretion.

The specific activity of the urinary glucose was calculated and found to be essentially constant at 25 counts per minute per mg. throughout the first five hours. Since the urinary glucose is derived from the body pool of glucose, one may assume that all of the glucose in the body has this specific activity. Assuming also a glucose space of 17 per cent of body weight (45), and knowing the glucose concentration in plasma water,[1] a glucose pool of approximately 59 Gm. can be calculated for this diabetic. If this glucose is labeled to the extent of 25 counts per minute per mg., then approximately 30 per cent of the ribose C^{14} injected (5 million counts) is present in the body as glucose at any time during the first five hours. Almost 70 per cent of the administered label has been accounted for (28 per cent in urine, 30 per cent in body glucose, and 10 per cent in expired CO_2) five hours after injection.

The fact that the specific activity of body glucose is essentially constant for five hours may explain the constant specific activity obtained for expired CO_2 (Figure 12). It would appear that ribose is converted rapidly to glucose

and that the oxidation of glucose is the means by which C^{14} appears in CO_2. The low specific activity of expired $C^{14}O_2$ and decreased excretion of C^{14} in CO_2 seen in the diabetic may be interpreted as being due not only to the low specific activity in an expanded glucose pool, but also to the defect in glucose metabolism known to exist in the diabetic.

According to the reactions of the pentose phosphate pathway (1), radioactive glucose derived from ribose-1-C^{14} should be labeled predominantly in carbons one and three. Indeed, in the intact mouse, Hiatt (11) observed that ribose-1-C^{14} gave rise to C^{14} glucose with 34 per cent of the activity in carbon one, 56 per cent in carbon three and a small quantity of activity in the other carbons. In order to determine whether ribose-1-C^{14} was converted to glucose by this pathway in man, glucose, from urine obtained during the fourth hour after ribose injection, was isolated as crystalline potassium gluconate. An aliquot was converted to CO_2 to obtain the total radioactivity in carbon one to carbon six collectively. Another portion was degraded so as to obtain carbon one and carbon six, individually, and carbon two to carbon five, collectively, as CO_2. Thirty-one per cent of the activity in carbon one to carbon six resided in carbon one, 6 per cent in carbon six and the remainder in the carbon two to carbon five fraction, presumably the activity being in carbon three. These figures correspond with Hiatt's results in the mouse, and give evidence that ribose was metabolized in man by conversion to glucose via the pentose phosphate pathway.

DISCUSSION

The kinetics of the disappearance of D-ribose from blood differ from those of D-glucose (46) or D-xylose (15) by being dose dependent. Our kinetic data indicate that the system for clearing ribose from blood is saturated after the infusion of small amounts of the sugar. That the assumption has some validity may be derived from the expired $C^{14}O_2$ data after tracer and 20 Gm. dose of labeled ribose. With the larger dose, the maximum specific activity of the $C^{14}O_2$ appeared at a later time and reached a much lower value (Figure 12).

The rate constants of disappearance of ribose from blood ranged from 3.8 to 13.8 per cent per minute, depending on the dose. Rate constan observed after the infusion of small amounts ribose are larger than those observed after the i fusion of various hexoses and other pentoses (15 Only D-galactose disappears from blood at a ra similar to that of ribose (47). At comparable do levels, the rate constant of ribose removal fro blood is four to five times as large as that of oth pentoses (15). Helmreich and Cori (48) ha similarly reported that injected ribose disappea from the body of the nephrectomized rat at rat much greater than those of other pentoses.

The average total urinary excretion of ribos after a 15 minute infusion was 21 per cent of th dose. A much smaller amount was excreted afte constant infusion of the sugar at a slow rate None of our studies was designed to determin accurately the renal mechanism for excretin ribose, and we are not aware of any careful stud ies of the renal handling of this pentose comparabl to those reported for xylose (49). One differenc between the renal handling of ribose and xylose i suggested from the present studies. Plasma glucose levels of about 300 mg. per cent cause a saturation of the xylose tubular reabsorbing mechanism and increase the renal clearance o xylose (50). No such effect was observed in the ribose studies in diabetics, for with blood glucose levels of about 300 mg. per cent, these subjects excreted no greater amounts of ribose in urine than the subjects with normal blood glucose levels.

Normally, small amounts of ribose can be detected in urine (51). There have been several reports of a ribosuria associated with muscular dystrophy (52) and neoplastic disease (53). Whether this ribosuria is a primary defect in these diseases, or is merely a consequence of tissue breakdown has not been established definitely.

The present studies with ribose-1-C^{14} in the normal subject have clearly demonstrated that the ribose escaping urinary excretion is rapidly metabolized, with prompt excretion of a portion of the first labeled carbon as CO_2. Since $C^{14}O_2$ appeared so soon after the injection of the labeled sugar, it is not likely that bacterial degradation of ribose that may have entered the gastrointestinal tract can be held responsible. Furthermore, the C^{14} ribose experiment in the diabetic has shown that the ribose not excreted in urine is largely converted to glucose by the sequence of reactions

of the pentose phosphate pathway as seen in Figure 1. Since ribose is converted to glucose, it appears probable that the $C^{14}O_2$ in expired air is derived from the oxidation of labeled glucose.

The phosphorylation of ribose to form ribose 5-phosphate appears to be a prerequisite step for ribose metabolism, and a D-ribokinase has been isolated from mammalian tissue (12). The question arises as to whether the serum phosphate drop after ribose infusion in man reflects this metabolic step. Since there seems to be no correlation between the dose of ribose and the phosphate fall, nor a difference in the phosphate decline after saline or ribose infusion (Table II, Subject J. D.), it appears unlikely that the observed phosphate changes have real significance. A fall in serum phosphate has been observed after the infusion of other pentoses (15).

Strangely enough, though ribose has been shown to be converted to glucose, hypoglycemia results after ribose infusion. This finding is apparently species specific response, for Naito (7) reports that after the injection of ribose into the mouse and rabbit, there is an increase in the level of fermentable reducing substance, presumably glucose, in blood. A hypoglycemic response in man has been reported after the infusion of galactose into subjects with congenital galactosemia (40) and some normals (54), and although several laboratories report increases in blood glucose after fructose infusion (55, 56) one group of investigators has observed a hypoglycemic response to fructose (57). The production of hypoglycemia by ribose infusion is not an effect shared by xylose or other pentoses (15).

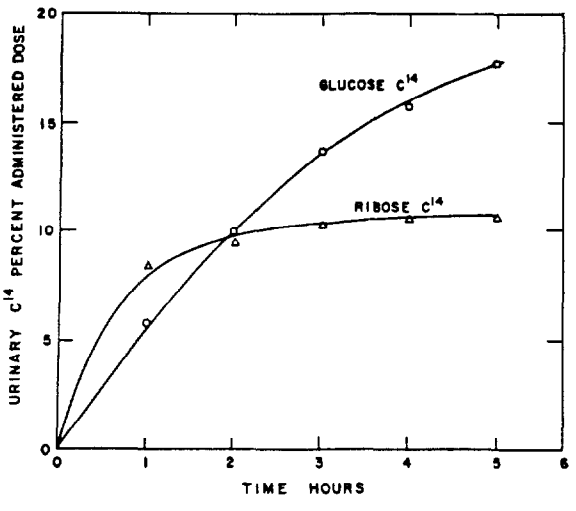

FIG. 13. URINARY EXCRETION OF C^{14} AS RIBOSE AND GLUCOSE AFTER INFUSION OF LABELED RIBOSE TO A DIABETIC SUBJECT

The lack of symptomatic response to ribose-induced hypoglycemia is of interest. Manifestations of hypoglycemia may be dependent on several factors, the rate of glucose fall, epinephrine release, and the low level of glucose actually encountered. It seems probable that the rate of fall in these experiments was sufficiently rapid and the glucose level low enough (15 to 30 mg. per cent) to cause symptoms. The lack of symptoms implies perhaps that neural tissue was able to utilize ribose or that sufficiently high glucose levels existed intracellularly. Indeed, Sable (58) has shown that ribose 5-phosphate is metabolized to hexose phosphate by brain homogenates. However, Park and co-workers (59) have demonstrated that ribose is unable to cross the blood-brain barrier in the rat, a fact which makes it doubtful that ribose itself could obviate a hypoglycemic response. In support of this is the fact that when insulin-induced hypoglycemia occurred, ribose did not appear to alleviate the symptoms.

Since the level of glucose in blood is dependent on the relative rates of entry and loss of glucose from blood, one has to consider the possibilities that ribose has either inhibited the hepatic mechanism determining the rate of entry of glucose into blood or increased the peripheral utilization of glucose via an insulin-like effect. It is conceivable that ribose causes a liberation of insulin from the pancreas. Hiatt (60) has obtained evidence that the hypoglycemic response to ribose in the dog is mediated by the pancreas. However, the observations we have made in man of a lack of blood phosphate and pyruvate change, associated with ribose-induced hypoglycemia, have led us to the belief that this effect of ribose may be primarily an hepatic one.

Under the conditions of our *in vitro* studies, the results indicate that an inhibition of phosphoglucomutase activity, causing a decrease in the rate of glycogen breakdown in the liver, could be responsible for the hypoglycemia reported here. Since we have only studied the ribose phosphate effect on one enzyme, we cannot state that this

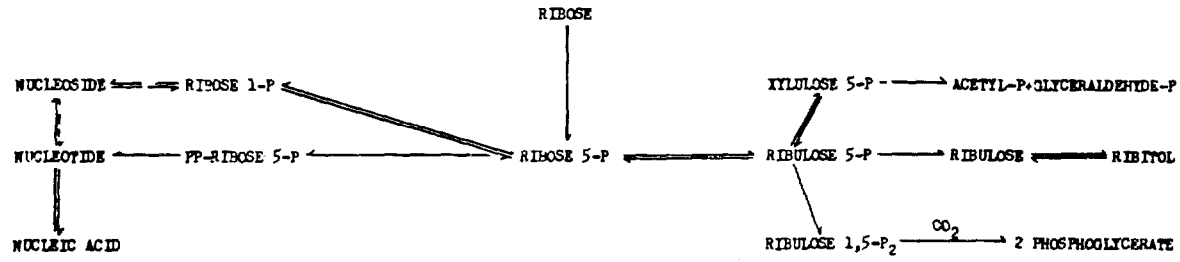

FIG. 14. FATES OF RIBOSE OTHER THAN ENTRANCE INTO THE PENTOSE PHOSPHATE PATHWAY OF METABOLISM

may be the sole locus of a possible defect in hepatic glucose output. In addition, it is only by conjecture and inference that we can extend observations made about an isolated enzyme to the intact cell or organism.

In the experiments with phosphoglucomutase *in vitro*, the direct addition of ribose ester caused an inhibition which could be ascribed to the presence of nonspecific organic phosphate (41). Therefore, it was necessary to demonstrate a more specific inhibition, and this was done with the preincubation type of experiment. In these studies, ribose 5-phosphate was used in excess of glucose 1-phosphate. However, since gram quantities of ribose are being metabolized in the *in vivo* experiments, it does not seem unreasonable that ribose 5-phosphate would be in excess of normally occurring amounts of glucose 1-phosphate. It is conceivable that the presence of significant amounts of intracellular ribose phosphate might well change the ionic environment of the mutase to produce the first type of inhibition. Possibly the ribose phosphate interacts with the enzyme and glucose 1,6-diphosphate according to Klenow and Emberland (43) to cause depletion of the latter cofactor and a state of dephosphorylation of the enzyme. This sequence would correspond to but not be identical to our preincubation period. It is interesting to point out that our *in vitro* observations parallel those observed of the effects of galactose 1-phosphate on the mutase reaction (38), that both sugars cause hypoglycemia, and that there has been shown an accumulation of intracellular galactose 1-phosphate in galactosemics (61), subjects who are known to have a hypoglycemic response to galactose infusion (40).

In both our *in vitro* studies and those reported with galactose 1-phosphate (38), isolated rabbit enzyme has been used. Though rabbits respond to ribose injection with a rise in blood glucose (7), this could be due to kinetic or other factors operating to rid the cell more quickly of ribose phosphate by conversion to glucose.

The observation of Levine, Goldstein, Huddlestun, and Klein (62) that insulin increases the volume of distribution of various sugars in the eviscerate dog has been corroborated in other animal species (59, 63). We have reported that in man, insulin causes an enhancement of the disappearance of the infused pentoses D-xylose, L-arabinose, and D-lyxose,, whereas D-arabinose is unaffected (16). Since the blood level of D-ribose is affected by insulin, this pentose may be grouped with the others similarly affected in the category of insulin responsive sugars. Although Goldstein, Henry, Huddlestun, and Levine (64) postulated a specificity of configuration for insulin responsive sugars, namely that carbon atoms one to three resembled the structure of these carbons in D-glucose, this has not been found to be the case by other investigators (54, 63). In man, D-lyxose, which differs from the glucose structure in carbon two, and D-ribose, which differs in carbon three, are insulin responsive. The response of these two sugars to insulin, however, is minimal compared to the response of D-xylose and L-arabinose, sugars which have the glucose configuration in carbon one to carbon three. At this time though, the relationship of the structure of a sugar to its insulin responsiveness is unsettled; the theory that insulin acts by facilitating the transport of sugars across the cell membrane is quite tenable.

The fates of ribose other than entrance into the pentose phosphate pathway are shown in Figure 14. Once isomerized to ribulose 5-phosphate, ribose 5-phosphate has several fates. An epimerization to xylulose 5-phosphate may ensue (65), whereupon a cleavage of the latter sugar

ccurs (in bacteria) to form an acetyl phosphate nd glyceraldehyde phosphate (66). Free ribuose has been reported to be present in normal uman urine (67), suggesting that ribulose phosphate is dephosphorylated. Recently Hollmann nd Touster (68) have demonstrated the presence of an enzyme in mammalian tissue, which is capable of reducing free ribulose to the sugar alcohol ribitol. Ribulose phosphate is also capable of being phosphorylated to ribulose 1,5-diphosphate (69) which acts as an important intermediate in the fixation of CO_2 in plants by forming wo molecules of phosphoglycerate (70).

Ribose 5-phosphate may be changed to ribose 1-phosphate by the enzyme phosphoribomutase (71). Ribose 1-phosphate, interacting with purine bases under the influence of a nucleoside phosphorylase described by Kalckar (72), forms nucleosides which theoretically may be phosphorylated to yield a nucleotide, the structural component of nucleic acids. The main pathway for the formulation of nucleotides, however, involves the pyrophosphorylation of ribose 5-phosphate to form phosphoribosylpyrophosphate (73, 74). A summary of the reaction sequence of this pathway has recently been published by Wyngaarden (75).

According to Soskin and Levine (76), ribose forms a negligibly small part of the daily carbohydrate intake in man. It is ingested chiefly as a component of the nucleic acids of meat. Whether any part of this ingested ribose, or the ribose we infused, is incorporated intact into body nucleic acid is not known. Studies have suggested that ribose isolated from animals is synthesized from glucose or carbon fragments by enzymes involved in the pentose phosphate pathway of metabolism (77, 78). Recently, however, it has been shown that ribose may be incorporated directly into nucleic acid (79) in the HeLa cell in tissue culture.

SUMMARY

The metabolism of D-ribose was studied in man by the infusion of both labeled and unlabeled sugar. This pentose was found to be rapidly and extensively metabolized, a principal fate being conversion to body glucose via the pentose phosphate pathway. Although converted to glucose, the ribose infused caused a lowering of blood glucose, presumably by inhibiting the enzyme phosphoglucomutase, thus preventing glycogen breakdown in the liver. Ribose was found to be insulin responsive, although its response was smaller than that observed with other pentoses.

REFERENCES

1. Horecker, B. L., and Mehler, A. H. Carbohydrate metabolism. Ann. Rev. Biochem. 1955, **24**, 207.
2. Wood, H. G. Significance of alternate pathways in the metabolism of glucose. Physiol. Rev. 1955, **35**, 841.
3. Bloom, B., Eisenberg, F., Jr., and Stetten, D., Jr. Glucose catabolism in liver slices via the phosphogluconate oxidate pathway. J. biol. Chem. 1955, **215**, 461.
4. Bloom, B. Fraction of glucose catabolized via the Embden-Meyerhof pathway; alloxan-diabetic and fasted rats. J. biol. Chem. 1955, **215**, 467.
5. Bloom, B. Catabolism of glucose by mammalian tissues. Proc. Soc. exp. Biol. (N. Y.) 1955, **88**, 317.
6. Marks, P. A., and Feigelson, P. Pathways of glycogen formation in liver and skeletal muscle in fed and fasted rats. J. clin. Invest. 1957, **36**, 1279.
7. Naito, Y. Biochemical studies on D-ribose with special reference to the mechanism of absorption of sugars from the intestinal tract. J. Biochem. (Tokyo) 1944, **36**, 131.
8. Herrmann, H., and Hickman, F. H. The utilization of ribose and other pentoses by the cornea. Bull. Johns Hopk. Hosp. 1948, **82**, 287.
9. Horecker, B. L., Gibbs, M., Klenow, H., and Smyrniotis, P. Z. The mechanism of pentose phosphate conversion to hexose monophosphate. I. With a liver enzyme preparation. J. biol. Chem. 1954, **207**, 393.
10. Katz, J., Abraham, S., Hill, R., and Chaikoff, I. L. The occurrence and mechanism of the hexose monophosphate shunt in rat liver slices. J. biol. Chem. 1955, **214**, 853.
11. Hiatt, H. H. Glycogen formation via the pentose phosphate pathway in mice *in vivo*. J. biol. Chem. 1957, **224**, 851.
12. Agranoff, B. W., and Brady, R. O. Purification and properties of calf liver ribokinase. J. biol. Chem. 1956, **219**, 221.
13. Segal, S., Foley, J., and Wyngaarden, J. B. The metabolism of D-ribose in man. Fed. Proc. 1957, **16**, 245.
14. Segal, S., Foley, J., and Wyngaarden, J. B. Hypoglycemic effect of D-ribose in man. Proc. Soc. exp. Biol. (N. Y.) 1957, **95**, 551.
15. Wyngaarden, J. B., Segal, S., and Foley, J. Physiological disposition and metabolic fate of infused pentoses in man. J. clin. Invest. 1957, **36**, 1395.
16. Segal, S., Wyngaarden, J. B., and Foley, J. The effect of insulin on the blood levels of infused pentoses in man. J. clin. Invest. 1957, **36**, 1383.

17. Najjar, V. A. The isolation and properties of phosphoglucomutase. J. biol. Chem. 1948, **175**, 281.
18. Hurlbert, R. B., Schmitz, H., Brumm, A. F., and Potter, V. R. Nucleotide metabolism. II. Chromatographic separation of acid-solube nucleotides. J. biol. Chem. 1954, **209**, 23.
19. Fiske, C. H., and Subbarow, Y. The colorimetric determination of phosphorus. J. biol. Chem. 1925, **66**, 375.
20. Segal, S., Blair, A. E., and Wyngaarden, J. B. An enzymatic spectrophotometric method for the determination of pyruvic acid in blood. J. Lab. clin. Med. 1956, **48**, 137.
21. Eisenberg, F. Conference on Liquid Scintillation Counting. Northwestern University, Aug., 1957, In press.
22. Passmann, J. M., Radin, N. S., and Cooper, J. A. D. Liquid scintillation technique for measuring carbon-14-dioxide activity. Analyt. Chem. 1956, **28**, 484.
23. Berlin, N. I., Tolbert, B. M., and Lawrence, J. H. Studies on glycine-2-C^{14} metabolism in man. I. The pulmonary excretion of $C^{14}O_2$. J. clin. Invest. 1951, **30**, 73.
24. Furst, M., Kallman, H., and Brown, F. H. Increasing fluorescence efficiency of liquid-scintillation solutions. Nucleonics 1955, 13(4), 58.
25. Partridge, S. M. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. Nature (Lond.) 1949, **164**, 443.
26. Moore, S., and Link, K. P. Carbohydrate characterization. I. The oxidation of aldoses by hypoiodite in methanol. II. The identification of seven aldo-monosaccharides as benzimidazole derivatives. J. biol. Chem. 1940, **133**, 293.
27. Eisenberg, F., Jr. Degradation of isotopically-labeled glucose via periodate oxidation of gluconate. J. Amer. chem. Soc. 1954, **76**, 5152.
28. Klenow, H. Some properties of the phosphoribomutase reaction. Arch. Biochem. 1953, **46**, 186.
29. Lineweaver, H., and Burk, D. The determination of enzyme dissociation constants. J. Amer. chem. Soc. 1934, **56**, 658.
30. Crane, R. K., Field, R. A., and Cori, C. F. Studies of tissue permeability. I. The penetration of sugars into the Ehrlich ascites tumor cells. J. biol. Chem. 1957, **224**, 649.
31. Segal, S., Wyngaarden, J. B., and Foley, J. Unpublished data.
32. Dominguez, R., Goldblatt, H., and Pomerene, E. Kinetics of the excretion and utilization of xylose. Amer. J. Physiol. 1937, **119**, 429.
33. Dominguez, R., and Pomerene, E. Kinetics of the disappearance of galactose from the plasma after a rapid intravenous injection. Amer. J. Physiol. 1944, **141**, 368.
34. Jokipii, S. G., and Turpeinen, O. Kinetics of elimination of glucose from the blood during and after a continuous intravenous injection. J. clin. In 1954, **33**, 452.
35. Amatuzio, D. S., Shrifter, N., Stutzman, F. L., Nesbitt, S. Blood pyruvic acid response to ir venous glucose or insulin in the normal and in tients with liver disease and with diabetes mell J. clin. Invest. 1952, **31**, 751.
36. Soskin, S., Levine, R., and Hechter, O. The r tion between the phosphate changes in blood muscle, following dextrose, insulin and epineph administration. Amer. J. Physiol. 1941, **134**, 4
37. Nichols, N. The effect of glycogen deposition liver phosphorus. J. clin. Invest. 1955, **34**, 171
38. Sidbury, J. B., Jr. Inhibition of phosphogluco tase with galactose phosphate. Abstracts of pers presented at the National Meeting of Amer. Chem. Soc., N. Y. 1957, p. 27c.
39. Ginsburg, V., and Neufeld, E. F. Inhibition of pl phoglucomutase by galactose 1-phosphate, a r sible factor in galactose toxicity. Abstracts papers presented at the National Meeting of Amer. Chem. Soc., N. Y. 1957, p. 27c.
40. Bruck, E., and Rapoport, S. Galactosemia in an fant with cataracts. Amer. J. Dis. Child. 1945, 267.
41. Cori, G. T., Colowick, S. P., and Cori, C. F. T enzymatic conversion of glucose 1-phosphoric es to 6-ester in tissue extracts. J. biol. Chem. 19 **124**, 543.
42. Klenow, H. The nature of the salt inhibition some reactions catalyzed by phosphoglucomut preparations. Arch. Biochem. 1955, **58**, 288.
43. Klenow, H., and Emberland, R. The enzymatic re tion between ribose 1-phosphate and glucose 6-diphosphate. Arch. Biochem. 1955, **58**, 276.
44. Segal, S., and Foley, J. The metabolic fate of labeled pentoses in man. In preparation.
45. Shreeve, W. W., Baker, N., Miller, M., Shipl R. A., Incefy, G. E., and Craig, J. W. C^{14} stud in carbohydrate metabolism. II. The oxidation glucose in diabetic human subjects. Metaboli 1956, **5**, 22.
46. Amatuzio, D. S., Stutzman, F. L., Vanderbilt, M. and Nesbitt, S. Interpretation of the rapid int venous glucose tolerance test in normal individu and in mild diabetes mellitus. J. clin. Invest. 19 **32**, 428.
47. Stenstam, T. Peroral and intravenous galact tests. Acta med. scand. Suppl. **177**, 1946.
48. Helmreich, E., and Cori, C. F. Studies of tiss permeability. II. The distribution of pentoses b tween plasma and muscle. J. biol. Chem. 19 **224**, 663.
49. Smith, H. W. The Kidney, Structure and Functi in Health and Disease. New York, Oxford Un Press, 1951.
50. Shannon, J. A. The tubular reabsorption of xyl in the normal dog. Amer. J. Physiol. 1938, 1 775.

51. White, A. A., and Hess, W. C. Paper chromatographic detection of sugars in normal and dystrophic human urines. Arch. Biochem. 1956, **64**, 57.
52. Tower, D. B., Peters, E. L., and Pogorelskin, M. A. Nature and significance of pentosuria in neuromuscular diseases. Neurology 1956, **6**, 37.
53. Leonardi, G., and Amalfi, M. La ribosuria in soggetti normali e in soggetti leucemici e neoplastici. Boll. Soc. ital. Biol. Sper. 1950, **26**, 1051.
54. Papper, S., Saxon, L., and Alpert, H. C. Effects of cortisone on the galactose tolerance tests of normal men. J. Lab. clin. Med. 1957, **50**, 384.
55. Miller, M., Drucker, W. R., Owens, J. E., Craig, J. W., and Woodward, H., Jr. Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J. clin. Invest. 1952, **31**, 115.
56. Papper, S., Saxon, L., Prout, T. E., and Alpert, H. C. The effect of cortisone on the fructose and glucose tolerance tests of normal men. J. Lab. clin. Med. 1956, **48**, 13.
57. Renold, A. E., Winegrad, A. I., Froesch, E. R., and Thorn, G. W. Studies on the site of action of arylsulfonylureas in man. Metabolism 1956, **5**, 757.
58. Sable, H. Z. Pentose metabolism in extracts of yeast and mammalian tissues. Biochim. Biophys. Acta 1952, **8**, 687.
59. Park, C. R., Post, R. L., Kalman, C. F., Wright, J. H., Jr., Johnson, L. H., and Morgan, H. E. The transport of glucose and other sugars across cell membranes and the effect of insulin *in* Ciba Foundation Colloquia on Endocrinology. London, J. and A. Churchill, 1956, vol. 9, p. 240.
60. Hiatt, H. H. Personal communication.
61. Schwartz, V., Goldberg, L., Komrower, G. M., and Holzel, A. Some disturbances of erythrocyte metabolism in galactosaemia. Biochem. J. 1956, **62**, 34.
62. Levine, R., Goldstein, M. S., Huddlestun, B., and Klein, S. P. Action of insulin on the permeability of cells to free hexoses as studied by its effect on the distribution of galactose. Amer. J. Physiol. 1950, **163**, 70.
63. Drury, D. [illegible] and Wick, A. N. Mechanism of insulin action. Diabetes 1955, **4**, 203.
64. Goldstein, M. S., Henry, W. L., Jr., Huddlestun, B., and Levine, R. Action of insulin on the transfer of sugars across cell barriers. Common chemical configuration of substances responsive to action of the hormone. Amer. J. Physiol. 1953, **173**, 207.
65. Stumpf, P. K., and Horecker, B. L. The role of xylulose 5-phosphate in xylose metabolism of *Lactobacillus pentosus*. J. biol. Chem. 1956, **218**, 753.
66. Horecker, B. L., Heath, E. C., Hurwitz, J., and Ginsburg, A. Pentose fermentation in *Lactobacillus pentosus*. Fed. Proc. 1957, **16**, 198.
67. Futterman, S., and Roe, J. H. The identification of ribulose and L-xylulose in human and rat urine. J. biol. Chem. 1955, **215**, 257.
68. Hollmann, S., and Touster, O. The L-xylulose-xylitol enzyme and other polyol dehydrogenases of guinea pig liver mitochondria. J. biol. Chem. 1957, **225**, 87.
69. Horecker, B. L., Hurwitz, J., and Weissbach, A. The enzymatic synthesis and properties of ribulose 1, 5-diphosphate. J. biol. Chem. 1956, **218**, 785.
70. Weissbach, A., Horecker, B. L., and Hurwitz, J. The enzymatic formation of phosphoglyceric acid from ribulose diphosphate and carbon dioxide. J. biol. Chem. 1956, **218**, 795.
71. Guarino, A. J., and Sable, H. Z. Studies on phosphomutases. II. Phosphoribomutase and phosphoglucomutase. J. biol. Chem. 1955, **215**, 515.
72. Kalckar, H. M. Differential spectrophotometry of purine compounds by means of specific enzymes. III. Studies of the enzymes of purine metabolism. J. biol. Chem. 1947, **167**, 461.
73. Kornberg, A., Lieberman, I., and Simms, E. S. Enzymatic synthesis of pyrimidine and purine nucleotides. I. Formation of 5-phosphoribosylpyrophosphate. J. Amer. chem. Soc. 1954, **76**, 2027.
74. Remy, C. N., Remy, W. T., and Buchanan, J. M. Biosynthesis of the purines. VIII. Enzymatic synthesis and utilization of α-5-phosphoribosylpyrophosphate. J. biol. Chem. 1955, **217**, 885.
75. Wyngaarden, J. B. Intermediary purine metabolism and the metabolic defects of gout. Metabolism 1957, **6**, 244.
76. Soskin, S., and Levine, R. Carbohydrate metabolism *in* Diseases of Metabolism, G. G. Duncan, Ed. Philadelphia, W. B. Saunders Co., 1952, pp. 15-88.
77. Hiatt, H. H. Biosynthesis of ribose of HeLa cell grown in tissue culture. Fed. Proc. 1957, **16**, 58.
78. Marks, P. A., and Feigelson, P. The biosynthesis of nucleic acid ribose and glycogen glucose in the rat. J. biol. Chem. 1957, **226**, 1001.
79. Hiatt, H. H. Studies of ribose metabolism. I. The pathway of nucleic acid ribose synthesis in a human carcinoma cell in tissue culture. J. clin. Invest. 1957, **36**, 1408.

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Metabolism of D-Ribose in Diabetes Mellitus

Edwin L. Bierman, M.D., Eugene M. Baker, Ph.D., Irvin C. Plough, M.D., and William H. Hall, M.D., Denver

D-ribose, administered intravenously to normal subjects, disappears rapidly from blood, is sparingly excreted in urine, and produces a significant decrease in blood glucose levels.[1-4] C^{14}-labeled ribose has been traced to glucose[3,4] and CO_2[3] in man, presumably via metabolism over pentose phosphate pathways.[3,5] In addition, this sugar is an efficient glycogen precursor in several mammals.[6-8]

Ribose metabolism may be insulin dependent, since administration of this hormone enhances the removal of ribose from the blood stream.[3] On the other hand, insulin appears to have no effect on transport of this pentose into muscle cells.[9]

e hypoglycemic effect of ribose in normal subjects is a property shared by galactose,[10] but not by other pentoses[11] or fructose.[12] It is noteworthy in this regard that, of several monosaccharides tested, only glucose, galactose, and ribose stimulated the release of insulin from dog pancreas.[13] Ribose may possibly produce a diminution of hepatic glucose outflow. It has been suggested that ribose impairs hepatic glycogenolysis, since this pentose can inhibit phosphoglucomutase in vitro.[3]

Segal and his co-workers observed decreased blood glucose levels following the intravenous administration of 20 gm. of ribose to three diabetic subjects.[1] Results in the present study, using larger doses of ribose given to patients with both mild and severe diabetes, confirm this finding and show in addition that several aspects of the metabolism of this pentose remain unaltered in the diabetic state.

METHODS

Five per cent solutions of D-ribose, obtained from Pfanstiehl Laboratories, Inc., Waukegan, Illinois, were prepared using sterile pyrogen-free water and were checked bacteriologically for sterility. Four mild adult diabetic and four severe juvenile-type diabetic patients were each given an intravenous infusion of ribose (40 or 50 gm.) administered at a constant rate over a one-hour period following an overnight fast. Insulin was withheld for at least twenty-four hours before the test. Heparinized venous blood samples and timed urine collections were obtained prior to the infusion and at hourly intervals thereafter.

Blood glucose was measured directly in blood filtrates prepared by the Somogyi method[14] using the prepared enzymatic reagent, Glucostat, Worthington Biochemical Corporation (glucose oxidase, horse-radish peroxidases, phosphate buffer, and O-dianisidine) as described by Saifer and Gerstenfeld.[15] Ribose added to glucose solutions had no influence on color development by this method.

Ribose in blood was determined by the orcinol method following treatment of the filtrates with glucose oxidase (Pfanstiehl).[12] Nonesterified fatty acids (NEFA) in plasma were measured by the method of Dole.[16]

Glucose and ribose were determined on diluted urine by the methods described for blood. However, prior to incubation with Glucostat, diluted urine samples were treated with ion exchange resins to remove interfering substances. A method described by Salomon and Johnson[17] was modified as follows: Urine was diluted to contain glucose concentrations from .015 to .075 mg./ml., a range in which the color response with the glucose oxidase reagent is linear. For this purpose, preliminary estimations of urine glucose content with Tes-Tape (urine sugar analysis paper, Lilly) were found to be satisfactory. To approximately 1 gm. of a mixture of equal parts of freshly regenerated Amberlite IR 120 and Amberlite IR 45 ion exchange resins in a 13 × 100 mm. test tube, 2 to 3 ml. of the dilute urine sample were added following washing of the resin with several aliquots of the sample. The tubes were then mechanically shaken for ten minutes and the resin allowed to settle. When low urine dilutions (less than 1:25) were used, an increase in resin treatment time was found to be necessary to remove effectively all interfering sub-

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stances. Duplicate 1 ml. aliquots of resin treated dilute urine samples were then removed for incubation with the glucose oxidase reagent. By this procedure, recovery of glucose added to varying dilutions of urine was found to be essentially complete.

Urine ketopentoses were measured by a method previously described.[15] Ketones in urine were estimated with Acetone Test Reagent, Denco (sodium nitroprusside, sodium bicarbonate, and ammonium sulfate).

RESULTS

The disappearance of ribose from blood (half time = 27 ± 3 minutes)* in eight diabetic subjects was not significantly different ($p > .100$) from that observed previously in normals (half time = 23 ± 4 minutes)[4] (figure 1). Further, mild and severe diabetics removed ribose at similar rates ($p > .050$).

PENTOSES IN BLOOD AND URINE FOLLOWING 50gm RIBOSE INFUSIONS IN 8 NORMALS AND 7 DIABETICS (MEAN VALUES)

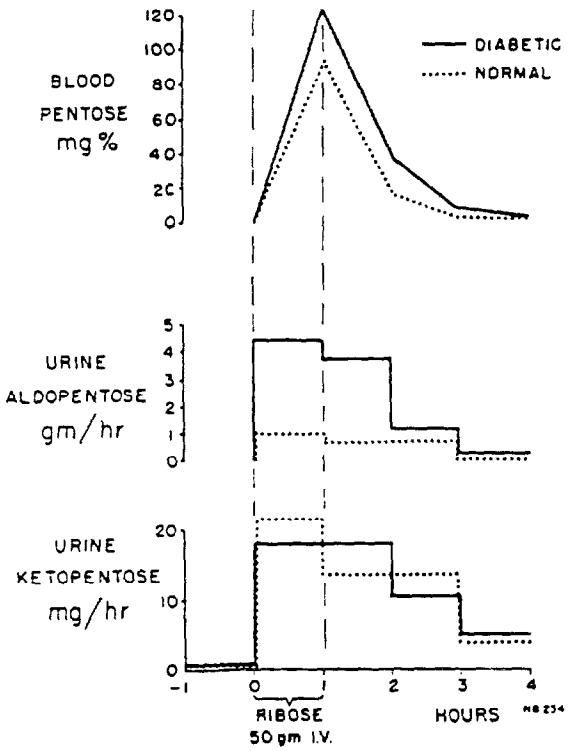

FIGURE 1

The rate of excretion of ribose in urine was greater ($p < .005$) in diabetics in the first three hours following infusion than in normals[4] (figure 1) and resulted in the elimination of 20 ± 10 per cent of the dose in four hours. Ketopentose excretion rates, however, were similar.

Blood glucose concentration decreased 21 ± 11 per cent from fasting values in seven of the eight subjects (table 1). No hypoglycemic response was observed in one diabetic (No. 8) who had been difficult to regulate. Minimum glucose values were reached one to three hours following the start of infusion. This effect was of similar magnitude ($p > .200$) in mild and severe diabetics and was not associated with symptoms. In addition, a prolonged fall in the renal excretion rate of glucose was observed following infusion (table 2).

A decrease of 33 ± 18 per cent in plasma nonesterified fatty acid concentration occurred in those subjects who responded with a fall in blood glucose (table 1). Four of these exhibited varying degrees of ketonuria prior to infusion. In all cases, urine ketones were considerably reduced in two hours (figure 2).

In one mild diabetic examined, no ribose was present in spinal fluid obtained immediately after infusion.

MILD DIABETIC ACIDOSIS (16 yr ♀, 39 kg)
COURSE FOLLOWING RIBOSE INFUSION

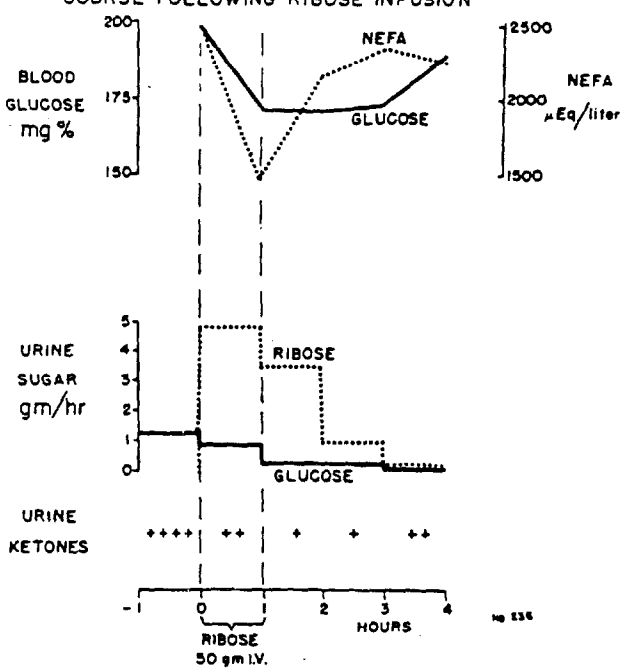

FIGURE 2

DISCUSSION

The rate of disappearance of ribose from blood was not impaired in diabetics, confirming similar observations by Segal and Foley.[3] However, results in the present study differ in that diabetic subjects excreted more than twice as much of the administered dose as has

*Data expressed as: mean for each series of observations ± the standard deviation of the individual observations.

TABLE 1

Effect of ribose* on blood glucose and nonesterified fatty acids

Subject	Sex	Age	Weight (kg.)	Insulin dose (units/day)	GLUCOSE Initial value (mg. per cent)	GLUCOSE Per cent change Hours 1	2	3	4	NEFA Initial value (μEq./L.)	NEFA Per cent change Hours 1	2	3	4
(A) Mild diabetic														
1	M	42	79	0	87	—1	—45	—46	—33	723	—16	—20	—26	—11
2	M	46	92	35	140	13	—11	—16	—1	896	—5	+29	+56	+77
3	M	47	96	15	149	0	—6	—13	—13	1,528	—18	—25	+2	+9
4	M	48	74	0	162	—9	—19	—17	—19	742	—31	—47	—33	—9
(B) Severe diabetic														
5	F	16	39	60	200	—15	—15	—14	—6	2,481	—40	—13	—6	—9
6	F	36	50	80	188	—20	—14	—5	—13	896	—4	—25	—11	+16
7	M	17	78	100	226	—9	—17	—12	—	1,312	—44	—60	+14	—
8	M	30	80	60	129	+5	0	+9	+9	824	+4	+4	—13	—17

*Dose = 50 gm. in all cases except Subject No. 5, 40 gm.

TABLE 2

Effect of ribose on urine glucose excretion (gm./hr.)

Subject	Period following infusions (hours) Control	0 to 1	1 to 2	2 to 3	3 to 4
(A) Mild diabetic					
1	.06	.03	.01	.01	.01
2	.20	.12	.04	.03	.04
3	.06	.01	.02	—	—
4	.01	.02	.01	.01	.01
(B) Severe diabetic					
5	1.22	.83	.20	.22	.06
6	.18	.22	.03	.17	.16
7	1.14	1.24	.17	.06	.06

been reported for normals.[2-4] This suggests some impairment of ribose utilization in the diabetic, but it is possible that renal tubular reabsorption of ribose was partially blocked by glucose, a phenomenon well defined for another pentose, xylose.[19]

Although conversion of ribose to glucose occurs in both diabetic[3] and normal subjects,[4] ribose produces a decrease in blood glucose levels. This differs from the effect produced by the administration of insulin independent hexoses such as fructose and galactose, since they contribute further to diabetic hyperglycemia.[20,21] However, these experiments do not rule out enhanced conversion of ribose to glucose in diabetes. Possibly, stimulation of endogenous insulin release could account for some effect on blood glucose, since no hypoglycemia results from ribose infusions given to pancreatectomized dogs.[22,23]

As has also been noted in normal subjects,[3,4] no symptoms were observed that could be associated with the lowering of blood sugar. It is unlikely that D-ribose can be metabolized by the brain in vivo, since evidence obtained in the rat[9] and in this study indicates that the sugar does not pass the blood brain barrier.

The utilization of ribose appears to alleviate the excessive fat mobilization characteristic of the uncontrolled diabetic. The decrease in plasma nonesterified fatty acid concentration is similar to that observed when ribose is administered to normal subjects.[4] In accord with this observation, all diabetics exhibited clearing of ketonuria when present prior to infusion. This is in harmony with the demonstration that stimulation of metabolism via the pentose phosphate pathway leads to a very marked acceleration of lipid synthesis, both in normal[24] and diabetic[25] rat liver homogenates.

Several ketopentoses, intermediates in pathways of pentose metabolism, are normally excreted in urine at an average rate of 4 mg./day, predominantly as L-xylulose.[18] Dietary supplementation with carbohydrate or protein, or administration of triiodothyronine, increases urinary L-xylulose excretion, possibly reflecting accelerated metabolism via the glucuronic acid pathway.[18] A marked increase in ketopentose excretion, predominantly D-xylulose, results from the administration of ribose to normal subjects.[4] The similar high rates of excretion noted in diabetics following ribose administration suggests that insulin is not required for metabolism of ribose over pentose pathways.

Thus, a comparison of ribose disappearance rate, blood glucose levels, plasma nonesterified fatty acid concentration, and ketopentose excretion, in normal and diabetic subjects suggests that the utilization of ribose is not altered in diabetes. The increased urinary excretion of ribose in the diabetic appears contradictory.

Further study of the renal tubular reabsorptive mechanism for ribose is needed, however, before it can be concluded that this effect reflects impaired metabolism. It has been reported that, in one diabetic examined, the elimination of a tracer dose of C^{14}-labeled ribose as $C^{14}O_2$ was diminished;[3] this finding, however, has been ascribed to defective metabolism following conversion of ribose to glucose.

Since ribose can be utilized by the diabetic, does not contribute to hyperglycemia, and limits fat mobilization, further investigation of the possible beneficial effect of administration of this sugar in uncontrolled diabetes is warranted.

SUMMARY

D-ribose, given intravenously to mild and severe diabetic patients, produced a decrease in blood glucose concentration and urine glucose excretion.

Ribose disappeared from blood at a normal rate; however, increased amounts were excreted in urine. Ketopentose excretion was not altered in diabetics.

A drop in plasma nonesterified fatty acid levels paralleled the fall in blood glucose and was associated with a reduction in urine ketones.

SUMMARIO IN INTERLINGUA

Le Metabolismo de d-Ribosa in Diabete Mellite

Le administration intravenose de d-ribosa a patientes con grados leve e sever de diabete produceva un reduction del concentration de glucosa in le sanguine e del excretion de glucosa in le urina.

Le ribosa dispareva ab le sanguine con un rapiditate normal. Tamen, augmentate quantitates de illo esseva excernite in le urina. Le excretion de cetopentosa non esseva alterate in le patientes diabetic.

Un reduction del nivellos de nonesterificate acidos grasse in le plasma occurreva parallel al reduction del glucosa in le sanguine e esseva associate con un reduction del cetones in le urina.

REFERENCES

[1] Segal, S., Foley, J., and Wyngaarden, J. B.: Hypoglycemic effect of d-ribose in man. Proc. Soc. Exp. Biol. & Med. 95:551-55, 1957.

[2] Hiatt, H. H.: The pentose phosphate pathway in human metabolism. J. Clin. Invest. 36:899, 1957.

[3] Segal, S., and Foley, J.: The metabolism of d-ribose in man. J. Clin. Invest. 37:719-35, 1958.

[4] Bierman, E. L., Baker, E. M., and Plough, I. C.: The effect of intravenous administration of d-ribose and d-xylose on urinary excretion of ketopentoses in man. Report No. 232, U.S. Army Medical Research and Nutrition Laboratory, 1959.

[5] Hiatt, H. H.: Studies of ribose metabolism. III. The pathway of ribose carbon conversion to glucose in man. J. Clin. Invest. 37:651-54, 1958.

[6] Hiatt, H. H.: Glycogen formation via the pentose phosphate pathway in mice in vivo. J. Biol. Chem. 224:851-69.

[7] McCormick, D. B., and Touster, O.: The conversion in vivo of xylitol to glycogen via the pentose phosphate pathway. J. Biol. Chem. 229:451-61, 1957.

[8] Naito, Y.: Biochemical studies on d-ribose, with special reference to the mechanism of absorption of sugars from the intestinal tract. J. Biochem. (Tokyo) 36:131-61, 1944.

[9] Park, C. R., Johnson, L. H., Wright, J. H., Jr., and Batsel, H.: Effect of insulin on transport of several hexoses and pentoses into cells of muscle and brain. Am. J. Physiol. 191:13-18.

[10] Papper, S., Saxon, L., Alpert, H. C.: The effects of cortisone on the galactose tolerance tests of normal men. J. Lab. Clin. Med. 50:384-90, 1957.

[11] Wyngaarden, J. B., Segal, S., and Foley, J. B.: Physiological disposition and metabolic fate of infused pentoses in man. J. Clin. Invest. 36:1395-1407, 1957.

[12] Miller, M., Craig, J. W., Drucker, W. R., and Woodward, H., Jr.: The metabolism of fructose in man. Yale J. Biol. Med. 29:335-60, 1956.

[13] Pozza, G., Galansino, G., Hoffield, H., and Foá, P. P.: Stimulation of insulin output by monosaccharides and monosaccharide derivatives. Am. J. Physiol. 192:497-500, 1958.

[14] Somogyi, M.: A method for the preparation of blood filtrates for the determination of sugar. J. Biol. Chem. 86:655-63, 1930.

[15] Saifer, A., and Gerstenfeld, S.: The photometric microdetermination of blood glucose with glucose oxidase. J. Lab. Clin. Med. 51:448-60, 1958.

[16] Dole, V. P.: A relation between nonesterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35:150-54, 1956.

[17] Salomon, L. L., and Johnson, J. E.: Enzymatic microdetermination of glucose in blood and urine. Anal. Chem. 31:453-56, 1959.

[18] Baker, E. M., Bierman, E. L., and Plough, I. C.: Factors influencing the urinary excretion of ketopentoses by normal men. Metabolism. In press.

[19] Smith, H. W.: The Kidney. Structure and Function in Health and Disease. New York, Oxford Univ. Press, 1951, pp. 101-102.

[20] Greenman, L.: Alterations in blood glucose following intravenous galactose. J. Biol. Chem. 183:577-85, 1950.

[21] Miller, M., Drucker, W. R., Owens, J. E., Craig, J. W., and Woodward, H., Jr.: Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J. Clin. Invest. 31:115-25, 1952.

[22] Hiatt, H. H., quoted by Segal, S.: Pentose metabolism in man (editorial). Diabetes 7:147-49, 1958.

[23] Bierman, E. L.: Unpublished observations.

[24] Siperstein, M. D., and Fagan, V. M.: Studies on the relationship between glucose oxidation and intermediary metabolism. I. The influence of glycolysis on the synthesis of cholesterol and fatty acid in normal liver. J. Clin. Invest. 37:1185-95, 1958.

[25] Siperstein, M. D., and Fagan, V. M.: Studies on the relationship between glucose oxidation and intermediary metabolism. II. The role of glucose oxidation in lipogenesis in diabetic rat liver. J. Clin. Invest. 37:1196-201, 1958.

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Oral Administration of D-Ribose in Diabetes Mellitus

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SUMMARY

Thirty-four subjects were categorized on the basis of their glucose tolerance as normal (thirteen subjects), probable diabetics (six subjects), mild diabetics (five subjects), tolbutamide-responsive diabetics (five subjects) and insulin-dependent diabetics (five subjects). Fifteen grams of d-ribose dissolved in six ounces of water were administered orally to each of the subjects and the effect on serum glucose concentration was measured. The hypoglycemic effect of d-ribose varied in an orderly and progressive manner through the different categories of glucose intolerance. As their glucose intolerance increased in severity, the subjects were significantly less responsive to the blood glucose lowering effect of d-ribose.

The serum immunoreactive insulin concentration in response to the oral administration of d-ribose was measured in the normal and mild diabetic subjects. The mild diabetics had a mean peak increase in immunoreactive insulin which was 2.6-fold greater than the normal subjects; however, the insulin concentrations attained were not significant in either group. Thus the insulinogenic response as determined in peripheral venous blood does not account for the differential hypoglycemic effect of d-ribose. DIABETES *19*: 11-16, 1970.

It has been shown that the intravenous infusion of d-ribose will consistently reduce the serum glucose concentration in normal human subjects.[1-3] Few studies have been constructed in diabetic patients, however. In 1957 Segal et al. reported decreased blood glucose levels following the intravenous infusion of d-ribose in three insulin-dependent diabetic subjects.[1] They suggested that the mechanism of the hypoglycemic effect of ribose did not depend on insulin release. Bierman et al.[4] reported that four "mild adult" diabetic and four "severe" diabetic patients responded to an infusion of d-ribose with a mean decrease in blood glucose of similar magnitudes. They noted, however, that one of the "severe" diabetics responded with an increase in blood glucose concentration.

Preliminary studies in our laboratory suggested that diabetic patients responded to d-ribose in a different manner than nondiabetics. The purpose of the present study was to determine if the glycemic response to a standard oral dose of d-ribose was related to varying degrees of glucose intolerance and insulin secretion.

MATERIAL AND METHODS

Thirty-four subjects (thirteen male, twenty-one female) were classified according to glucose tolerance. Each of twenty-four subjects who had repeatedly normal fasting blood glucose concentrations was segregated on the basis of two oral glucose tolerance tests (performed one week apart) into one of three categories: normal (thirteen subjects: seven male, six female), probable diabetics (six subjects: two male, four female), or mild diabetics (five subjects: one male, four female). Each subject consumed a diet containing 300 gm. of carbohydrate for at least three days prior to the tests. After a fasting baseline blood specimen was obtained, Glucola,* seven ounces, was given orally over a five-minute period. Subsequent blood specimens were obtained by venipuncture at 30, 60, 90 and 120 minutes. Assignment to one of the three categories was made using the criteria of Fajans and Conn[5] adjusted to our glucose oxidase method which averages approximately 12 mg. per 100 ml. lower than the Somogyi-Nelson method within the 100 to 160 mg./per 100 ml. range. Only the subjects who fell into the same category both times the test was done were chosen for further studies.

From the University of California, Irvine, Orange, California 92668.

*Ames Company, Division of Miles Laboratory, Inc., Elkhart, Indiana.

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id early diabetic subjects. Diabe
hemical aspects of insulin. *In*: B.
all, (Eds.). Excerpta Medica Foun
p. 227, 1965.
is, D. M.: Plasma insulin respon
le of normal weight and obese d
jects. Diabetes *15*:867-74, 1966.
E. W., Herron, A. L., and Brenn
in response to glycemic stimul
release to carbohydrate intolera
J. Clin. Invest. *46*:323-35, 1967.
erman, E. L., and Porte, D., Jr
insulin levels in the evaluation
glucose in diabetic and nondiabe
46:1549-57, 1967.
R.: Plasma insulin response to
uced by glucose infusion in hum
1, 1963.
S. A.: Comparison of the mechanis
intolerance in subclinical diabe
and during postpartum oral con
J. Clin. Endocr. *29*:807-18, 196
n, H., and Stoddard, F. J.: Acqui
tus in women receiving oral con
:7:307, 1968 (Abstract).
chardson, B. L., and Stoddard, F.
response during prednisolone gluc
etic mothers of heavy infa
3.
, J. W., Traisman, H. S., and M
rtisone-modified glucose tolerance
:1460-68, 1966.

The fourth category consisted of five subjects (one male, four female) with known diabetes who had been symptomatic with elevated fasting blood sugars and glycosuria when control by diet alone was attempted but were being satisfactorily maintained on tolbutamide therapy. The mean fasting blood glucose of the tolbutamide-responsive diabetics was 139 mg./100 ml. on the morning of the ribose test, while the newly discovered mild diabetics had a mean fasting blood glucose of 67 mg./100 ml. The fifth category consisted of five subjects (two male, three female) with insulin-dependent diabetes of known duration greater than five years. The mean age and weight of the individual categories were not significantly different.

All subjects (except two normals) who were segregated on the basis of glucose tolerance were given two oral d-ribose tests within two weeks following their assignment to a specific category. Diabetic patients receiving tolbutamide or insulin and two of the normal subjects were given a single ribose test. Tolbutamide was discontinued seventy-two hours before, or insulin was withheld at least twenty-four hours before the d-ribose test. The insulin-dependent diabetics had been using either NPH or Lente insulin and had received their last injection no later than forty-eight hours prior to the morning of testing. All tests were performed in the morning with the subjects in the post-absorptive state. After obtaining a fasting blood specimen, fifteen grams of d-ribose dissolved in six ounces of tap water were given orally over a five-minute period. Blood specimens were obtained by venipuncture at 15, 30, 45, 60, 75, 90 and 120 minutes. Serum glucose was determined by the glucose oxidase method of Ware and Marbach.[6] In the normal and mild diabetic subject categories, the serum was also assayed for immunoreactive insulin concentration by the method of Morgan and Lazarow.[7]

RESULTS

The mean serum glucose responses of each group to oral d-ribose are depicted graphically in figure 1. Normal subjects had a mean maximum decrease of 27 ± 2 (S.E.M.) mg. per 100 ml. in serum glucose concentration with the nadir of the response at sixty minutes. Subjects in the probable diabetic category responded in a similar way with a mean maximum decrease of 22±3.1 mg. per 100 ml. at sixty minutes. Subjects in the mild diabetic category evidenced a mean maximum decrease of 20 ± 3.1 mg. per 100 ml. but the nadir of the response was delayed to seventy-five minutes. Diabetic patients who had been treated with tolbutamide

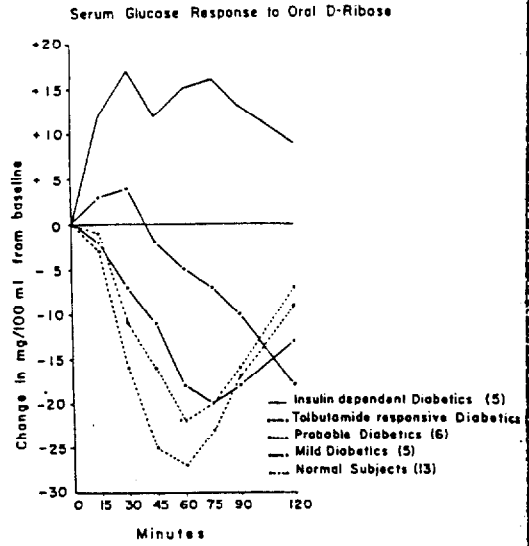


FIG. 1. Mean serum glucose responses following the oral administration of d-ribose. The numbers in parentheses indicate the number of subjects in each category.

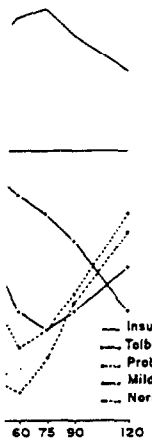
showed an initial increase in blood glucose concentration followed by a delayed mean maximum decrease of 18 ± 25.3 mg. per 100 ml. which may not have reached its nadir at 120 minutes when the test was terminated. Insulin-dependent diabetics showed a hyperglycemic response with a mean maximum increase of 17 ± 46.6 mg. per 100 ml. The elevated glucose concentration had not yet returned to the baseline at 120 minutes.

If blood glucose concentration at a single time after d-ribose administration is used to characterize each of the five categories of subjects, the best separation is demonstrated at forty-five minutes. The forty-five-minute glucose concentrations of all subjects tested are plotted in figure 2 in which the gradually decreasing degree of hypoglycemic response with increasing severity of glucose intolerance can be appreciated. The significance of the differences of the responses at forty-five minutes of the abnormal groups in relation to the normal is depicted in table 1.

The reproducibility of the serum glucose responses in the twenty-two subjects in whom the test was performed twice is shown in table 2.

Because it was felt that the insulinemic response to d-ribose administration might prove to be an important consideration in attempting to explain the differences in the glycemic response, serum immunoreactive insulin

Response to Oral D-Ribose



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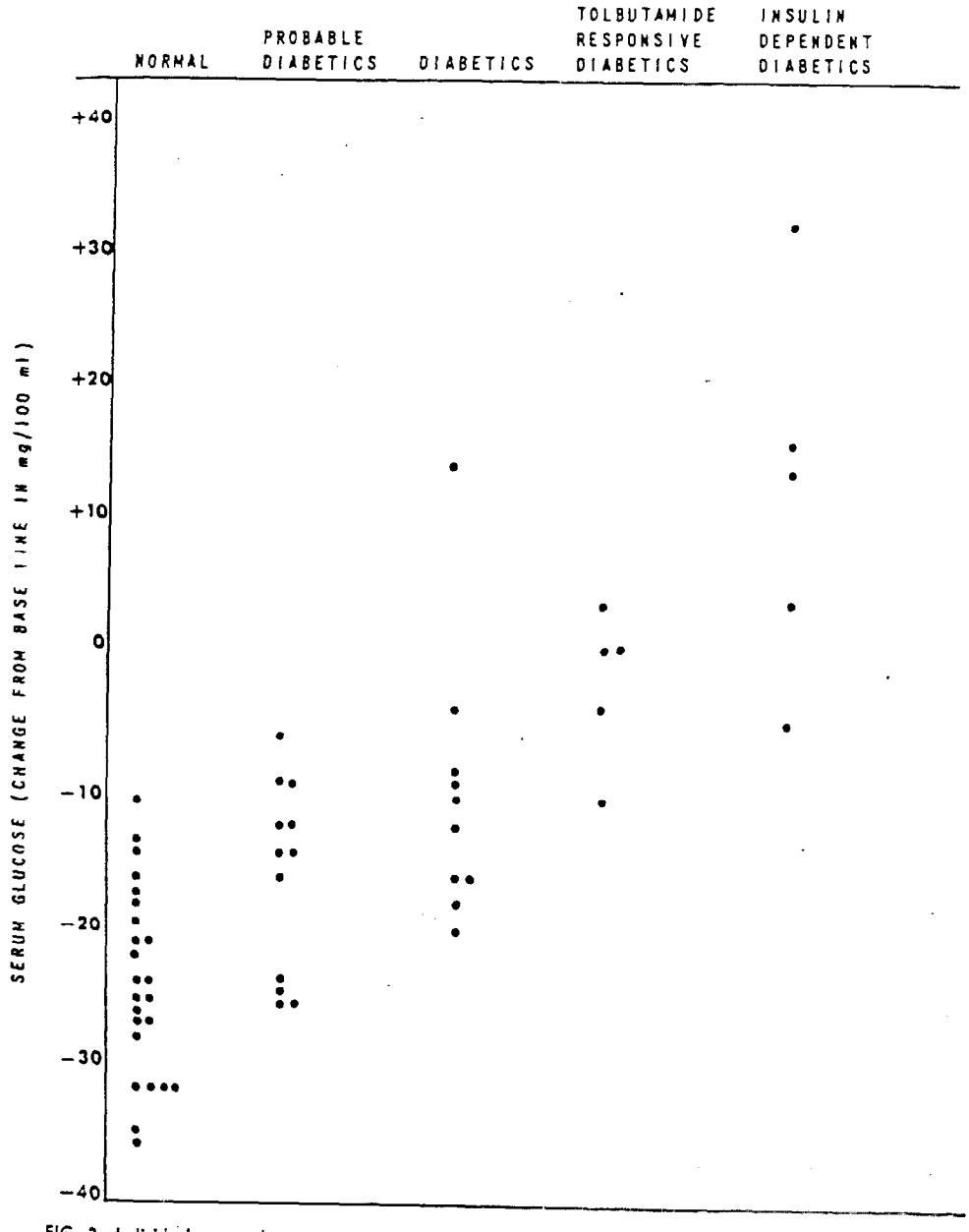

FIG. 2. Individual serum glucose responses at forty-five minutes following the oral administration of d-ribose.

TABLE 1

Serum glucose response to oral d-ribose at forty-five minutes

	Decrease at 45 min. in mg./100 ml.		Per cent decrease at 45 min.		
	Mean	S.E.M.	Mean	S.E.M.	p value
Normals	24	1.5	37	2.4	
Probable diabetics	16	2.1	25	3.2	0.005
Mild diabetics	11	2.5	18	3.2	0.001
Tolbutamide-responsive	2	2.1	2	2.0	0.001
Insulin-dependent	(+)12	6.3	(+) 7	3.4	0.0001

TABLE 2

Reproducibility of serum glucose responses to d-ribose. Difference in mg./100 ml. of the maximum decrease from the baseline serum glucose of results obtained from testing the same subjects on two separate occasions.

Subjects	Mean	Range
Normals	3.5	0- 9
Probable diabetics	8.6	6-17
Mild diabetics	7.8	0-19

concentration was determined in the normal and mild diabetic subject categories (figure 3). The normal subjects evidenced a slight mean increase of 6 μU. per ml. at fifteen minutes which fell rapidly to baseline concentration. The mild diabetics started from a higher mean baseline of 23 μU. per ml. as compared to 15 μU. per ml. for the normal subjects and evidenced an apparently exaggerated but delayed mean increase of 16 μU. per ml. at thirty minutes. Neither the response of the normal nor the mild diabetic was significant; both had a p value > 0.5. There was great disparity in the serum insulin responses in those subjects in whom the test was performed twice.

DISCUSSION

It is apparent in figures 1 and 2 that the decrease in serum glucose concentrations subsequent to the ingestion of d-ribose varies in a rather orderly and progressive manner through the different categories of glucose intolerance. Subjects with the greatest glucose intolerance are least responsive to the blood glucose lowering effect of d-ribose. This is contradictory to the findings of Bierman et al.[4] who reported an effect of similar magnitude in the "mild" and "severe" diabetic patients. The discrepancy between our findings and those of the above investigators may be due to the lack of a clear definition of "mild" and "severe" diabetics, since two of the four "mild" diabetics used insulin. Even the grouping of diabetics into insulin and non-insulin-dependent categories may not provide a sufficient indication of the severity of the diabetic state. De Belle et al.[8] studied the effect of tolbutamide in juvenile diabetes mellitus and found that patients who were diabetic less than two-and-a-half years had a significant hypoglycemic effect from tolbutamide, in contrast to those subjects who were diabetic three years or more and had no response to tolbutamide. The insulin-dependent subjects in the present study were diabetic for five years or longer and none had a significant decrease in their serum glucose concentration following the ingestion of d-ribose. Thus the careful categorization of our subjects may account for the distinct difference obtained in the responses of the various groups to d-ribose which was not apparent in previous reports.[1,4]

It appears (figure 3) that a greater insulinemic response in normal subjects is not an acceptable explanation for the hypoglycemic effect of ribose. This cannot be stated with certainty, unfortunately, because the fifteen-minute specimen which was the first blood sample obtained after the ingestion of the d-ribose showed the greatest concentration of immunoreactive insulin in the normal subjects and it is conceivable, but unlikely, that an earlier and greater response might have occurred.

Recently, we have characterized the response of serum glucose and immunoreactive insulin concentrations to the intravenous administration of 15 gm. of d-ribose in normal subjects.[3] We concluded that although the concentration of serum insulin was transiently elevated during the infusion of d-ribose, it was insufficient in itself to account for the markedly delayed hypoglycemia. Although we have not studied the insulinemic response to the infusion of d-ribose in diabetics, it appears that the above conclusion may apply to the present study wherein the ribose was administered orally. The apparently exaggerated insulin levels in the mild diabetics in response to d-ribose may be analogous to the response of some diabetics to the administration of glucose and tolbutamide.[9] D-ribose has been given by mouth to dogs[10] and human subjects[11] with resultant decreases in serum glucose concentrations similar to those in the present study. This is the first report of the oral administration of d-ribose to human subjects with the

inutes

ease at 45 min.

S.E.M.	p value
2.4	
3.2	0.005
3.2	0.001
2.0	0.001
3.4	0.0001

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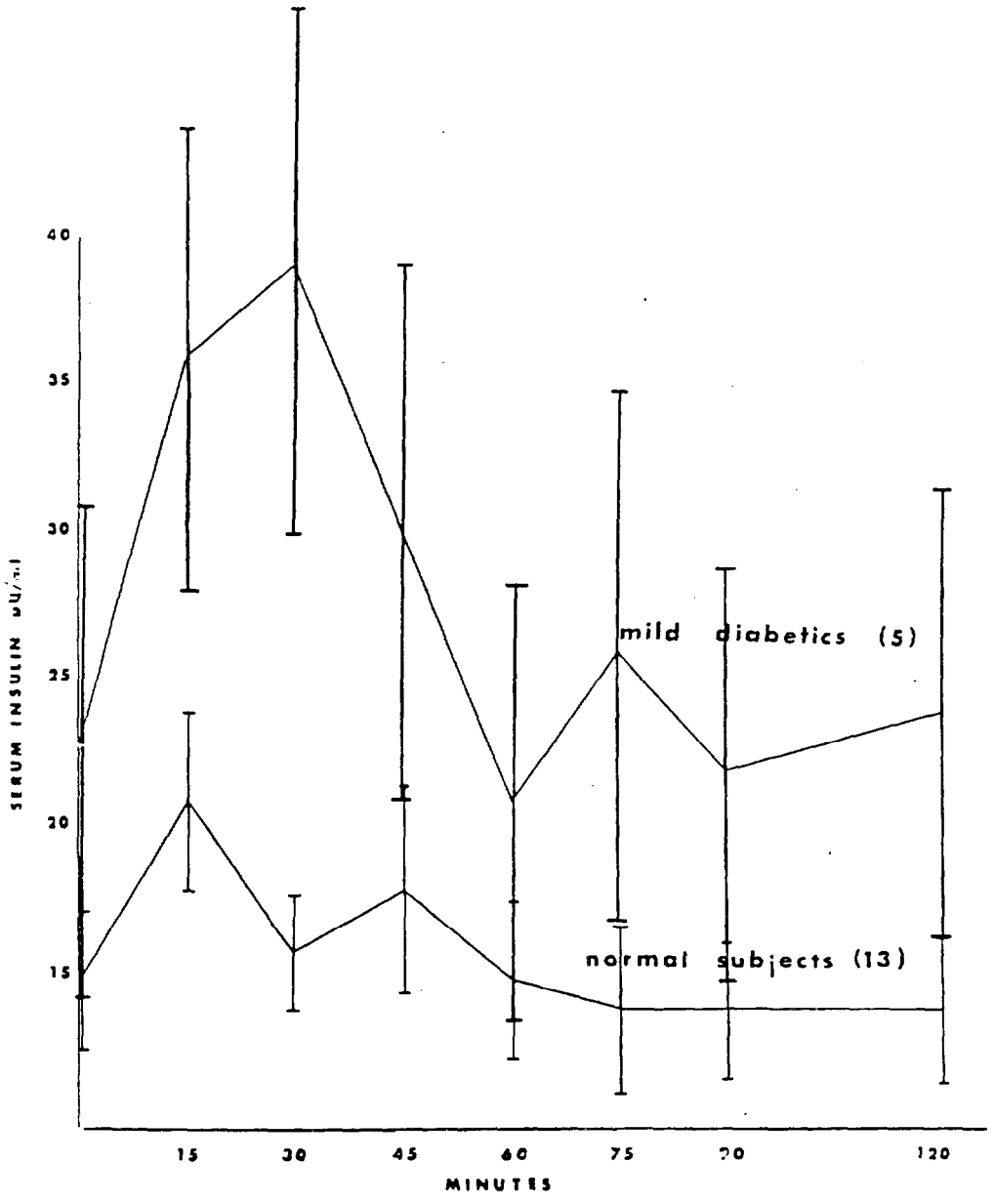

FIG. 3. Mean serum insulin response following the oral administrationof d-ribose. The numbers in parentheses indicate the number of subjects tested. The vertical bars represent the S.E.M.

measurement of the effect on serum immunoreactive insulin concentrations.

We have observed that in normal human subjects the oral administration of 15 gm. of d-ribose resulted in a mean maximum decrease of 47 per cent from the baseline serum glucose concentration, as compared to a mean maximum decrease of 22 per cent when the same quantity of d-ribose was infused intravenously over a twenty-minute period. Although this would appear to correspond with studies which have shown that oral glucose is a greater insulinogenic stimulus than the same quantity of glucose administered intravenously,[12-14] in fact, the contrary has become evident. When d-ribose was administered orally to normal subjects, the mean peak increase in serum immunoreactive insulin was 6 μU./ml. whereas the intravenous administration of the same quantity of d-ribose produced a mean peak increase of 14 μU./ml. A more potent insulinogenic effect of intravenously, as compared to orally, administered d-ribose has also been shown in dogs.[10] Thus, there is a dissociation in the magnitude of the insulin response and the degree of hypoglycemia.

Of particular potential interest is the response to the oral administration of d-ribose in tolbutamide-responsive diabetics. These subjects had a mean maximum decrease of 16 per cent from their baseline serum glucose level and this was observed at 120 minutes after ingestion of d-ribose which happened to be the termination of the study period. Additional and more prolonged studies in this type of diabetic appear to be warranted and might reveal d-ribose to be of value as an adjunctive oral hypoglycemic agent.

ACKNOWLEDGMENT

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REFERENCES

[1] Segal, S., Foley, J., and Wyngaarden, J. B.: Hypoglycemic effect of d-ribose in man. Proc. Soc. Exp. Biol. Med. 95:551-55, July 1957.

[2] Segal, S., and Foley, J.: The metabolism of d-ribose in man. J. Clin. Invest. 37:719-35, May 1958.

[3] Steinberg, T., Ortman, P., Poucher, R., Cochran, B., Jr., and Gwinup, G.: The effect of d-ribose infusion on serum immuno-reactive insulin and glucose concentration. Metabolism 16:40-46, Jan. 1967.

[4] Bierman, E. L., Baker, E. M., Plough, I. C., and Hall, W. H.: Metabolism of d-ribose in diabetes mellitus. Diabetes 8:455-58, Nov.-Dec. 1959.

[5] Fajans, S. S., and Conn, J. W.: An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. Diabetes 3:296-304, Sept. 1954.

[6] Ware, A. G., and Marbach, E. P.: Application of a glucose oxidase method to serum. Clin. Chem. 11:792, Aug. 1965.

[7] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Diabetes 12:115-26, Mar.-Apr. 1963.

[8] de Belle, R., Belmonte, M. M., and Colle, E.: Effect of intravenous tolbutamide in juvenile diabetes mellitus. Diabetes 16:215-18, Apr. 1967.

[9] Perley, M., and Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes 15:867-74, Dec. 1966.

[10] Halter, J. B., Schwartz, M. Z., and Goetz, F. C.: The effect of oral and intravenous d-ribose on plasma insulin levels in unanesthetized dogs. Proc. Soc. Exp. Biol. Med. 127:1147-51, April 1968.

[11] Beaconsfield, P., and Ginsburg, J.: Hypoglycemia after oral ribose. Lancet 2:153, 1967.

[12] Elrick, H., Stemmler, L., Hlad, C. J., Jr., and Arai, Y.: Plasma insulin response to oral and intravenous glucose administration. J. Clin. Endocr. 24:1076-82, Oct. 1964.

[13] Perley, M. J., and Kipnis, D. M.: Plasma insulin responses to oral and intravenous glucose: Studies in normal and diabetic subjects. J. Clin. Invest. 46:1954-62, Dec. 1967.

[14] McIntyre, N., Holdsworth, C. D., and Turner, D. S.: Intestinal factors in the control of insulin secretion. J. Clin. Endocr. 25:1317-24, Oct. 1965.

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HYPOGLYCÆMIA AFTER ORAL RIBOSE

SIR,—The pentose, D-ribose, causes hypoglycæmia after intravenous injection in animals[1] and man.[2,3] The mechanism is obscure, since the pentose is rapidly cleared from the blood[4] and converted into glucose after intravenous injection.[5] A transient rise in serum-insulin level has been recorded after intravenous injection of ribose,[6] but the investigators questioned whether this was sufficient to account for the hypoglycæmia. In view of the therapeutic possibilities of this hypoglycæmic effect, especially since the pentose is a constituent of many animal and plant foods, the effect on blood-sugar level of the administration of D-ribose by mouth was investigated in 3 normal people and 3 diabetics.

D-ribose (1 g. per kg. body-weight) was dissolved in water and taken fasting by 3 healthy young adults, 1 patient in whom diabetes had arisen late in life and whose blood-sugar levels are controlled by diet and chlorpropamide, and 2 young women with severe diabetes since childhood controlled by insulin. The diabetics had had no medication for 36 hours before the test. Blood-glucose levels were measured at half-hourly intervals by a glucose-oxidase method.[7]

The results in the accompanying table show a fall in blood-glucose levels in the healthy adults 30–60 minutes after the pentose solution was taken; and 2 hours later they were still well below normal. The blood-glucose level also fell in the elderly diabetic, though to a lesser degree than in the healthy young adults. In the 2 subjects with severe diabetes, no response was observed with doses of 1 g. per kg.

It is important in studies of this sort to measure blood-sugar levels by a method specific for glucose. In 1 of the healthy adults (no. 1) this was done simultaneously by the Technicon 'AutoAnalyzer' ferricyanide method (which measures total reducing substances) and the glucose-oxidase method. Blood-sugar analysis, in mg. per 100 ml., by the ferricyanide method gave 76 and 78 ("control values"), and 110, 112, 80, 60, and 50 (after ingestion of ribose), respectively, the apparent rise in blood-sugar level after ribose administration presumably being due to its absorption from the gut. Chromatographic estimation of ribose confirmed its presence in the plasma after oral ingestion—60 mg. per 100 ml. was found after 60 minutes—but there was none detectable in the control period.

Hypoglycæmia after oral ribose ingestion may be related to the release of insulin, and studies are in progress of pancreatic, pituitary, and adrenocortical response to the administration of D-ribose. Experiments in animals have shown that when labelled pentose is injected intravenously, radioactivity is incorporated in the insulin molecule.

These results suggest that present ideas about changes in blood-glucose and hormone levels after ingestion of a sugar or a protein-and-fat meal[8] require re-evaluation. Thus far, the hypoglycæmic effect of pentoses normally present in the diet has not been taken into consideration.

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1. Slovter, H. A., Ikeda, M., Sakata, K. *Am. J. Physiol.* 1964, 207, 407.
2. Sezai, S., Foley, J., Wyngaarden, J. B. *Proc. Soc. exp. Biol. Med.* 1957, 95, 551.
3. Bierman, E. L., Baker, E. M., Plough, I. C., Hall, W. H. *Diabetes*, 1964, 9, 455.
4. Wyngaarden, J. B., Segal, S., Foley, J. *J. clin. Invest.* 1957, 36, 1395.
5. Hiatt, H. H. *ibid.* 1958, 37, 651.
6. Steinberg, T., Ortman, P., Poucher, R., Cochran, B., Gwinup, G. *Metabolism*, 1967, 16, 40.
7. Middleton, J., Griffiths, H. *Br. med. J.* 1957, ii, 1525.
8. Ginsburg, J., Boucher, B., Beaconsfield, P. Unpublished.
9. Rabinowitz, D., Merimee, T. J., Marfezzoli, R., Burgess, J. A. *Lancet*, 1966, ii, 454.
10. Western Interstate Commission on Higher Education Joint Data Collection Project, Spring, 1966, N.I.C.H.H.D. contract no. PH 43-65-989.
11. McQuarrie, I. *Am. J. Dis. Child.* 1954, 87, 399. Haworth, J. C., Coodin, F. J. *Pediatrics, Springfield*, 1960, 25, 748.
12. Cornblath, M., Joassin, G., Weisskopf, B., Swaiman, K. *Pediat. Clins of N. Am.* 1966, 13, 905.
13. Nelson N. *J. biol. Chem.* 1944, 153, 375.
14. Rock, J. A., Gerende, L. J. *J. Am. med. Ass.* 1966, 198, 231.

BLOOD-GLUCOSE LEVELS BEFORE AND AFTER INGESTION OF D-RIBOSE IN 3 HEALTHY PEOPLE AND 3 DIABETICS

Subject no.	Age (yr.)	Sex	Condition	Blood-glucose in mg. per 100 ml. (minutes after ingestion of D-ribose, 1 g. per kg. body-weight)									
				−40	−10	30	60	90	120	150	180	210	240
1	29	M	Healthy	100	102	85	82	88	58	52	48	..	..
2	40	F	,,	70	74	68	47	70	58	47	..	..	..
3	27	M	,,	64	62	51	45	51	51	48	46	45	49
4	70	M	Diabetic on chlorpropamide	118	93	96	88	78	65	68	73	83	..
5	20	F	Diabetic on insulin	207	198	207	229	242	233	210	228	..	..
6	33	F	,, ,, ,,	256	260	242	260	230	238	234	206	..	..

HYPOGLYCÆMIA AND MENTAL RETARDATION

SIR,—A recent survey of 23,443 patients of 19 Western United States institutions for the mentally retarded revealed only 5 cases with the diagnosis of "encephalopathy associated with other disorders of carbohydrate metabolism".[10] This diagnosis includes glycogenosis and hypoglycæmia of various causation. Mental retardation has been established as a sequela of untreated hypoglycæmia in childhood,[11] particularly in the newborn period.[12] The preceding facts led us to investigate the incidence of hypoglycæmia in 1279 inmates of this institution for the mentally retarded by the following method.

RELATION BETWEEN BLOOD-SUGAR LEVELS (mg. per 100 ml.) DETERMINED BY DEXTROSTIX AND BY SOMOGYI-NELSON METHODS IN 136 MENTALLY RETARDED SUBJECTS AFTER 24-HOUR FAST

Initial screening with dextrostix	Somogyi-Nelson method			
	≤40 mg.	40–50 mg.	>50 mg.	Total
≤40 mg. ..	6	4	6	16
>40 <65 mg.	6	8	106	120
Total	12	12	112	136

After a 24-hour fast, peripheral blood was obtained by finger-prick. The blood-glucose level was estimated by the reagent-strip glucose-oxidase-colour-indicator method ('Dextrostix'), in accordance with the manufacturer's recommendations. Subjects with estimated blood-glucose levels below 40 mg. per 100 ml., or above 40 but below 65 mg. per 100 ml., had a second specimen of blood drawn by venepuncture. The blood-sugar level was then determined by the Somogyi-Nelson method.[13] Despite the reported inadequacy of 'Dextrostix' as a mass screening tool,[14] our yield of cases for further study was 10·63%,—that is, 136 subjects of the 1279 screened had an estimated blood-glucose level below 65 mg. per 100 ml. by the method. As depicted in the table, 24 (17·6%) of the 136 cases (i.e., 1·9% of the 1279 screened subjects) were further identified as hypoglycæmic by the Somogyi-Nelson method. Preliminary studies of the 24 cases under controlled hospital conditions seem to confirm these findings.

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Serum Levels of Glucose, Insulin, and C-Peptide during Long-Term D-Ribose Administration in Man*

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Summary. D-ribose was given orally and/or intravenously to nine healthy subjects at doses ranging from 83.3 to 222.2 mg/kg per hour for at least four hours. The serum ribose level increased in a dose-dependent manner to maximum concentrations of 75 to 85 mg/dl. The serum glucose level decreased after the beginning of continuous ribose administration and was reduced as long as ribose was being administered. The oral or intravenous administration of 166.7 mg/kg per hour of ribose resulted in a 25% decrease in serum glucose. Higher intravenous doses of ribose did not provoke a further decrease in serum glucose concentration. Oral administration of 166.7 mg/kg per hour led to an increase in serum insulin concentrations from a mean of 8.4 (range 6.4–11.5) to 10.4 (range 6.3–15.4) μU/ml ($p < 0.05$). In contrast, intravenous administration did not change serum insulin concentrations significantly. The serum c-peptide concentration remained unchanged regardless of treatment. We conclude that the variations in plasma insulin concentrations do not account for the observed decrease in mean serum glucose concentrations accompanying D-ribose administration.

Key words: (D-)ribose – Glucose – Insulin – C-peptide

For more than forty years it has been known that the administration of ribose to normal humans or experimental mammals results in a transient decrease in serum glucose concentration (1943 intraperitoneal injection in rats [10], 1946 oral administration in man [20]). The mechanisms that mediate this hypoglycemic effect are poorly understood. A transient increase in peripheral venous insulin levels following bolus administration of ribose has been variably reported. These studies utilizing bolus administration could not answer the question of whether the observed, transient insulin peak can completely explain the observed decrease in serum glucose concentration. No studies have been done using long-term administration of ribose. It is not known if continuous ribose administration will provoke sustained changes in serum concentrations of glucose and insulin.

The investigation of endocrinological mechanisms involved in the glucose-lowering effect of ribose might be of clinical importance. The administration of ribose can prevent or temper the exercise-induced symptoms occurring in some cases of myoadenylate deaminase deficiency [21]. A release of insulin might contribute to the beneficial effect of ribose by activating muscle glycogen synthetase [9]. Insulin also stimulates the uptake of ribose [6] and glucose [12] by muscle cells.

The present study was carried out in nine healthy volunteers to answer two questions: whether the glucose-lowering effect would persist and whether changes in serum insulin level would occur during continuous ribose administration[1].

Materials and methods

Subjects and materials: Twelve tests were performed in nine healthy subjects (8 men, 1 woman: initials JM), ages 23 through 37 years. D-ribose was obtained from Fluka or Sigma. A sterile and

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[1] The results of this study concerning the basic metabolic data of ribose, including steady state ribose concentration, intestinal absorption, and urinary excretion, have been published previously [5].

Table 1. Serum levels of glucose, ribose, insulin, and c-peptide during oral and intravenous ribose administration: values prior to intake and average values obtained during the third hour (i.v. experiments) or third and fourth hour (oral experiments) of ribose administration at each dose

Subject	Body weight [kg]	Body length [cm]	Dose [mg/kg per hour]	Serum Glucose [mg/dl] (change compared with initial value)		Serum ribose [mg/dl]	Serum insulin [μU/ml]	Serum c-peptide [p-mol/ml]
Oral administration 83.3 and 166.7 mg/kg per hour								
AH	104.0	204	initial	76.8		1.8	7.5	0.50
			83.3	71.1	(−7.4%)	5.8	7.3	0.40
			166.7	56.7	(−26.2%)	29.1	11.8	0.61
MW	84.0	177	initial	75.5		1.9	10.7	0.46
			83.3	62.9	(−16.7%)	6.4	9.6	0.58
			166.7	53.7	(−28.9%)	37.0	15.4	0.54
MG	65.5	178	initial	83.5		3.4	7.5	0.48
			83.3	73.0	(−12.6%)	3.7	5.4	0.30
			166.7	56.3	(−32.5%)	27.4	6.3	0.32
WB	71.8	190	initial	59.6		0.7	7.5	0.84
			83.3	66.4	(+11.5%)	2.6	5.6	0.46
			166.7	53.1	(−10.9%)	23.1	7.0	0.63
Oral administration only 166.7 mg/kg per hour								
BL	68.5	178	initial	89.2		1.6	11.5	n.d.
			166.7	59.0	(−33.9%)	35.2	15.1	n.d.
JS	74.0	188	initial	71.8		1.3	6.4	0.41
			166.7	61.7	(−14.0%)	42.4	6.9	0.54
JM	66.3	173	initial	63.5		3.8	9.1	0.66
			166.7	53.6	(−15.7%)	39.5	12.5	0.80
WE	72.5	176	initial	68.0		2.7	6.6	0.41
			166.7	51.9	(−23.6%)	24.5	8.0	0.45
Average values oral administration (8 observations except 83.3 mg/kg per hour: only 4)								
			initial	73.5		2.2	8.4	0.54
			83.3	68.4		4.6	7.0	0.44
			166.7	55.8		32.3	10.4	0.56
Intravenous administration								
AH	106.0	204	initial	73.9		1.5	8.7	0.46
			83.3	64.0	(−13.3%)	9.3	8.4	0.56
			166.7	53.3	(−27.8%)	38.7	6.6	0.52
			222.2	56.8	(−23.1%)	77.7	5.0	0.35
JS	74.0	188	initial	72.8		1.8	7.7	0.39
			83.3	59.1	(−18.8%)	6.4	10.0	0.52
			166.7	57.2	(−21.5%)	45.4	7.2	0.45
			222.2	60.9	(−16.3%)	85.6	7.7	0.36
MW	65.5	178	initial	79.5		2.3	5.7	n.d.
			166.7	64.8	(−18.5%)	59.9	6.1	n.d.
SV	78.5	182	initial	88.5		1.1	14.0	0.70
			166.7	59.3	(−33.0%)	38.3	14.4	0.83
Average values intravenous administration								
			initial	78.7		1.7	9.0	0.52
			166.7	58.7		45.6	8.5	0.62

pyrogen-free 8.33 g/100 ml solution was prepared for the intravenous administration.

Analytical methods: The serum ribose concentration was measured according to the method of Roe and Rice [15] as modified by Miller [11]. Glucose was measured in the serum by the glucose-oxidase method. Urine samples were screened with a test strip for glucosuria. Insulin in serum was measured using the radioimmunoassay (RIA) of Cambridge

Medical Diagnostics, Billerica, Mass., USA (lowest standard 5 μU/ml, normal values for fasting persons below 20 μU/ml). C-peptide in serum was measured using the RIA of Ire-Medgenix, Fleurus, Belgium (sensitivity 0.05 pmol/ml, normal values 0.36 to 1.12 pmol/ml).

Procedure: All experiments were started in the morning after an overnight fast. Two hours prior to and during ribose administration the subjects rested on a couch. Two hours before the beginning of ribose administration they drank 500 ml of table water per hour to establish a steady state of diuresis and to correct for any changes in blood chemistry due to the intake of the water during ribose administration. Every 30 min the subjects emptied the bladder completely, and each urine sample was checked for glucosuria.

Blood samples were taken from an indwelling antecubital catheter every 30 min. In the experiments with intravenous administration, the contralateral antecubital vein was cannulated for ribose infusion. Catheter patency was ensured by maintenance infusion of saline at a rate of 100 ml/h. Blood samples were centrifuged immediately and kept at 4° C for determinations on the same day or frozen at −20° C for determinations within a few days.

For oral administration, ribose was dissolved in table water. The concentration was calculated to provide an intake of 400 ml of fluid per hour during administration. Every 5 min the subject drank 1/12 of the total amount. The intravenous administration was performed using a 8.33 g/100 ml ribose solution which is equivalent to 10 g/100 ml of hexose. The difference between the 400 ml orally administered fluid and the infused volume was balanced by oral intake of table water.

Doses used ranged from 83.3 to 222.2 mg/kg body weight per hour. A dose of 222.2 mg/kg per hour was administered only intravenously to avoid the diarrhea caused by oral administration at doses above 200 mg/kg per hour [5]. Ribose was administered for five hours per dose in the oral experiments and for four hours per dose in the intravenous experiments. Continuous oral ribose administration at a dose of 166.7 mg/kg per hour was given to 8 subjects (Table 1). In four experiments, this administration was preceded by the continuous intake of half this dose. Infusions of ribose at a dose of 166.7 mg/kg per hour were performed in four experiments. In two of these experiments, this infusion was preceded by infusion of half the dose for four hours and followed by infusion of 222.2 mg/kg per hour for another four hours.

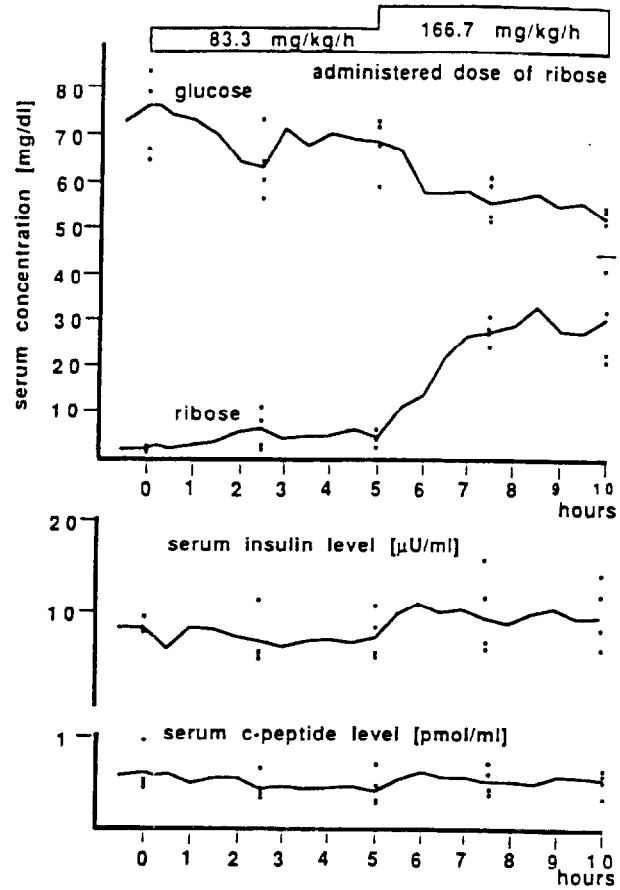

Fig. 1. Serum levels of ribose, glucose, insulin, and c-peptide in four subjects (AH, MG, MW, WB) during oral ribose administration at two doses. The points indicate single values measured at the beginning, after $2^1/_2$ h, and after 5 h of ribose administration at each dose. The line represents the average value of the data obtained every 30 min

Results

To study the dose dependency and long-term effects of continuous ribose administration on blood glucose concentration, four subjects (AH, MW, MG, WB) first received ribose orally at a dose of 83.3 mg/kg per hour fractionated for five hours, and then double that dose (166.7 mg/kg per hour) for the next five hours (Fig. 1). Average serum ribose concentrations of 4.6 mg/dl (range 2.6 to 6.4) and 29.2 mg/dl (range 23.1 to 37.0), respectively, were achieved. Glucose serum concentration decreased from an average initial value of 73.9 mg/dl (range 59.6 to 83.5) to 68.4 mg/dl (62.9 to 73.0) and 55.0 mg/dl (53.1 to 56.7), respectively. The serum insulin concentration was 8.3 μU/ml (range 7.5 to 10.7) at the beginning, and 7.0 μU/ml (5.4 to 9.6) and 10.1 μU/ml (6.3 to 15.4) following the respective dosages. The c-peptide serum level was unchanged (average concentrations 0.57, 0.44, and 0.53 pmol/ml, respectively).

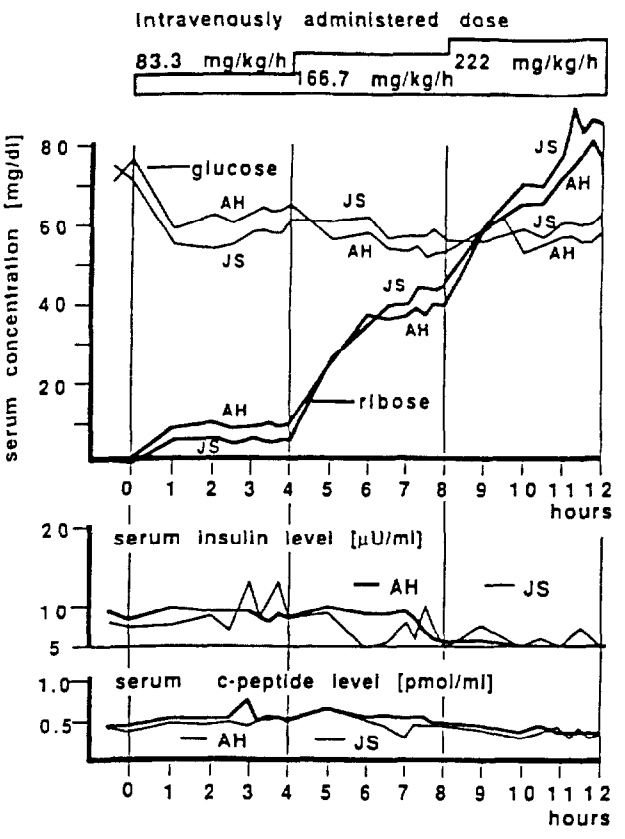

Fig. 2. Serum levels of ribose, glucose, insulin, and c-peptide in two subjects (AH, JS) during intravenous ribose administration at three doses. Blood samples were taken every 30 min

In the eight subjects who received ribose orally at a dose of 166.7 mg/kg per hour, the serum glucose level decreased from an average initial level of 73.5 mg/dl (range 59.6 to 89.2) to 55.8 mg/dl (range 51.9 to 61.7) during ribose intake (−24.1%). The average initial serum insulin level was 8.4 µU/ml (range 6.4 to 11.5), and the average during the fourth and fifth hours of administration was 10.4 µU/ml (range 6.3 to 15.4), significance $p < 0.05$ (Wilcoxon signed rank test paired observations). The average values for c-peptide were 0.54 pmol/ml (range 0.41 to 0.84) and 0.56 pmol/ml (range 0.32 to 0.80), respectively (difference not significant).

Two subjects (AH and JS) received three different doses of ribose intravenously, each for four hours (Fig. 2). The serum glucose levels dropped by about 20 mg/dl independently of serum ribose concentrations (maximum concentrations >80 mg/dl). The serum insulin level showed a slight decrease, while the c-peptide level was unchanged. Two more experiments (MW, SV) with intravenous administration also resulted in a marked decrease of serum glucose levels without changes in serum levels of insulin and c-peptide (Table 1).

In the four experiments with intravenous administration at a dosage of 166.7 mg/kg per hour, the serum glucose level decreased from an average initial value of 78.7 mg/dl (range 72.8 to 88.5) to 58.7 mg/dl (range 53.3 to 64.8) during the last hour of infusion (−25.4%). The corresponding data for insulin were 9.0 µU/ml (range 5.7 to 14.0) and 8.5 µU/ml (range 6.1 to 14.4), and for c-peptide, 0.52 pmol/ml (range 0.39 to 0.70) and 0.62 pmol/ml (range 0.45 to 0.83).

No glucose could be detected in any urine sample.

Discussion

Our data document a sustained decrease in serum glucose concentrations during ribose administration. This effect is astonishing, since ribose can be converted to glucose via the pentose phosphate pathway [7, 8], and for that reason an increase would have been expected, rather than the decrease observed. This glucose-lowering effect is not shared with other pentoses such as D-xylose and D-arabinose [14, 17] and it is not species-specific (also seen in rats [10] and in dogs [2, 4]).

The decrease in serum glucose concentration was asymptomatic in all cases. Glucose concentrations of 15 mg/dl (enzymatic determination) without any clinical symptoms during ribose administrations have been reported [16]. Thus it has been speculated that the nervous system can utilize ribose instead of glucose [18]. However, there are data to suggest that ribose cannot penetrate the blood-brain barrier to any measurable extent [12].

It has been postulated that the fall in serum glucose with ribose administration is due to enhanced insulin secretion, and previous studies of ribose-induced hypoglycemia have generally shown a transient increase in insulin concentration. However, no long-term experiments have been done. Therefore, it could not be determined whether the decrease in serum glucose concentration is an effect of a transient insulin peak, or whether other mechanisms must be considered.

In our experiments, we have shown that long-term administration of ribose was associated with a sustained reduction in serum glucose concentration over the entire period of ribose administration. In the four cases using oral administration of ribose at two different doses, this hypoglycemic effect seemed to correlate with the serum ribose level, which increased in a dose-dependent fashion. However, in the intravenous experiments leading

to higher serum ribose concentrations, the glucose concentration could not be further lowered by increasing either the dose or the serum ribose concentration. This might be due to saturation of the glucose-lowering mechanism at serum ribose concentrations higher than about 30–40 mg/dl.

Both oral and intravenous experiments showed a marked increase in the serum ribose concentration with higher doses. This effect can be attributed to saturation of the ribose utilization (intake minus renal excretion) and a decrease in renal ribose clearance at higher serum ribose concentrations [5].

Changes in serum insulin concentrations were either minimal or nonexistent, and no changes in c-peptide concentrations were recorded during long-term ribose administration. In contrast, the serum glucose level showed a marked and sustained decrease in all experiments as long as ribose was administered. Therefore, it seems unlikely that insulin is responsible for the decreased glucose levels during long-term ribose administration.

There is also evidence that the insulin peak observed during short-term ribose administration cannot completely explain the decrease in serum glucose levels. After ribose infusions over 5 min (3.75 gm) and 20 min (15 gm) in nine normal subjects, a two- to threefold transient increase of serum insulin was reported [19]. By 10 min the insulin level had returned to baseline values. The intravenous injection of an amount of insulin that provoked an equal elevation in serum insulin levels produced no decrease in serum glucose concentrations.

These results contrast with a report on the insulin output of dog pancreas. During a one-hour period of ribose infusion (1 or 2 mg/kg per min) into the portal vein of dogs, a twofold increase of plasma insulin concentration in the pancreatic veins was reported [2]. This difference between the brief insulin increase in peripheral venous blood and the sustained effect when pancreatic output is measured led to speculations about an increased rate of deposition of insulin in the liver by increased metabolism of carbohydrates such as ribose [3]. However, our findings of unchanged c-peptide concentrations argue against this possibility.

There is no difference in the mean glucose decrease during intravenous versus oral administration at a dosage of 166.7 mg/kg per hour, making the involvement of gastrointestinal hormones unlikely. Changes in human growth hormone and in cortisol were excluded in a previous study [1].

Since a renal loss of glucose has been excluded, some investigators have looked for indications of increased peripheral glucose utilization. A decrease in serum inorganic phosphate [7, 16, 18], but insignificant changes in blood pyruvate [13, 16, 18] and unchanged lactic acid levels [13] have been reported. Increased peripheral utilization of glucose, however, is accompanied by a large decrease in blood inorganic phosphate and an increase in blood pyruvate. Therefore, increased glucose utilization is not likely to cause the decrease in serum glucose levels, although we did not specifically measure this factor.

Since the data on the investigated hormones cannot completely explain the effect of ribose on the serum glucose level, and increased glucose utilization is unlikely, further mechanisms must be identified. It is known that phosphoglucomutase, the rate-limiting enzyme in the formation of glucose from glycogen in the liver, is inhibited by ribose [18]. This and other, still-unknown effects on enzyme activities might cause or contribute to the glucose-lowering effect of ribose.

References

1. Ginsburg J, Boucher B, Beaconsfield P (1970) Hormonal changes during ribose-induced hypoglycemia. Diabetes 19:23–27
2. Goetz FC, Maney JW, Greenberg BZ (1967) The regulation of insulin secretion: effects of the infusion of glucose, ribose, and other sugars into the portal veins of dogs. J Lab Clin Med 69:537–557
3. Goodman C, Goetz FC (1970) Oral and intravenous D-ribose and plasma insulin in healthy humans: effects of route of administration and of epinephrine and propranolol. Metabolism 19:1094–1103
4. Halter JB, Schwarty MZ, Goetz FC (1968) The effect of oral and intravenous D-ribose on plasma insulin levels in unanesthetized dogs. Proc Soc Exp Biol Med 127:1147–1151
5. Gross M, Reiter S, Zöllner N (1989) Metabolism of D-ribose administered continuously to healthy persons and to patients with myoadenylate deaminase deficiency. Klin Wochenschr 67:1205–1213
6. Helmreich E, Cori CF (1957) Studies on tissue permeability. II. The distribution of pentoses between plasma and muscle. J Biol Chem 224:663–679
7. Hiatt HH (1957) The pentose phosphate pathway in human metabolism. J Clin Invest 36:899
8. Hiatt HH (1958) Studies of ribose metabolism. III. The pathway of ribose carbon conversion to glucose in man. J Clin Invest 37:651–654
9. Kida Y, Esposito-Del Puente A, Bogardus C, Mott DM (1990) Insulin resistance is associated with reduced fasting and insulin-stimulated glycogen synthase phosphatase activity in human skeletal muscle. J Clin Invest 85:476–481
10. Larson HW, Bradshaw PJ, Ewing ME, Sawyer SD, Blatherwick NR (1943) The metabolism of d-ribose. Fed Proc 2:66
11. Miller B (1974) Der D-Xylose-Test. In: Englhardt A, Lommel H (eds) Malabsorption. Maldigestion, Bd 2. Verlag Chemie, Weinheim, pp 155–163
12. Park CR, Johnson LH, Wright JH, Batsel H (1957) Effect of insulin on transport of several hexoses and pentoses into cells of muscle and brain. Am J Physiol 191:13–18

13. Pizzichini MR, Marcolongo R, Marinello E (1979) Ribose tolerance in gouty patients. J Clin Chem Clin Biochem 17:436
14. Pozza G, Galansino G, Hoffeld H, Foa PP (1958) Stimulation of insulin output by monosaccharides and monosaccharide derivates. Am J Physiol 192:497–500
15. Roe JH, Rice EW (1948) A photometric method for the determination of free pentoses in animal tissues. J Biol Chem 173:507–512
16. Segal S, Foley J, Wyngaarden JB (1957) Hypoglycemic effect of D-ribose in man. Proc Soc Exp Biol Med 95:551–555
17. Segal S, Wyngaarden JB, Foley J (1957) The effect of insulin on blood levels of infused pentoses in man. J Clin Invest 36:1383–1394
18. Segal S, Foley J (1958) The metabolism of D-ribose in man. J Clin Invest 37:719–735
19. Steinberg T, Ortman P, Poucher R, Cochran B, Gwinup G (1967) The effect of D-ribose infusion on serum immunoreactive insulin and glucose concentration. Metabolism 16:40–46
20. Wuest HM, Solmssen UV (1946) The urinary excretion of d-ribose in man. Arch Biochemistry 11:199–201
21. Zöllner N, Reiter S, Gross M, Pongratz D, Reimers CD, Gerbitz K, Paetzke I, Deufel T, Hübner G (1986) Myoadenylate deaminase deficiency: successful symptomatic therapy by high dose oral administration of ribose. Klin Wochenschr 64:1281–1290

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Buchbesprechungen

W. Steinbrich, G.P. Krestin (Hrsg.) **Kernspintomographie der Abdominal- und Beckenorgane. Einbindung in klinisch-radiologische Untersuchungskonzepte.** Springer, Berlin Heidelberg New York London Paris Tokyo Hong Kong 1990. XVI, 264 S., 204 Abb., 23 Tab., geb. DM 180,–

Das vorliegende Buch enthält, basierend auf den Erfahrungen großer Zentren, die Zusammenstellung sämtlicher Referate eines Symposiums im März 1990 mit der Zielsetzung, zum einen den aktuellen Stand der diagnostischen Wertigkeit der Kernspintomographie im Abdominal- und Beckenbereich zu erörtern, zum anderen den derzeitigen Schwerpunkt der wissenschaftlichen Arbeit aufzuzeigen.

Da sowohl die Technik als auch die Entwicklung von neuen Kontrastmitteln vor allem in jüngster Zeit sehr schnell fortschreiten, ist die aktuelle Neubewertung des diagnostischen Procedere von großem Interesse. Vor allem die Referate, die die Leberdiagnostik zum Thema haben, zeigen, daß erhebliche Verbesserungen erzielbar sind mit Einführung von schnellen Gradienten-Echosequenzen, Flußkompensationsverfahren und neuen Kontrastmitteln wie dem superparamagnetischen Eisenoxid in Ergänzung zu dem bisher verwendeten Gadolinium-DTPA. Abgehandelt wird auch die lokalisierte In-vivo-Spektroskopie der Leber, die neue Einblicke in biochemische Zusammensetzung und den Stoffwechsel bietet, die mit konventionellen nichtinvasiven Methoden bisher nicht möglich sind. Die Gefäßdiagnostik mittels MRT, eine faszinierende Methode, die erst am Beginn ihrer Entwicklung steht, ist ebenfalls Thema eines Referats. Auch in der Diagnostik von Erkrankungen von Milz, Nieren, Nebennieren und gynäkologischen Tumoren sehen die Referenten Vorteile für die MRT, jedoch werden auch die Grenzen und die Überlegenheit der Konkurrenzmethoden aufgezeigt, z.B. im Bereich des Pankreas oder in der Diagnostik des männlichen Beckens.

Das Buch ist für alle Radiologen wertvoll, die die Kernspintomographie auch des Abdomens und Beckens durchführen, um bei dem schnellen Fortschreiten der Methoden den aktuellen Stand der diagnostischen Verfahren zu kennen und anzuwenden. L. Ziegler, D. Hahn (München)

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W. Wiegand: **Kernspintomographie von Auge, Orbita und Sehnerv.** (Bücherei des Augenarztes, Band 122) Ferdinand Enke, Stuttgart 1990. XII, 131 S., 352 Abb., 7 Tab., kart. DM 72,–

In der sehr klar gegliederten Monographie gibt der Autor eine äußerst informative Übersicht über Kernspintomographie (KST) in der Ophthalmologie.

In gut verständlicher Weise werden zunächst die physikalischen Grundlagen der KST besprochen. Es schließt sich ein Abschnitt über die Patientenauswahl und die Durchführung der KST an, dann ausführliche Darstellungen der normalen Anatomie, der Befunde bei intraokularen und intraorbitalen Erkrankungen (140 KST-Untersuchungen bei 120 Patienten) sowie von Artefakten. Abschließend wird der Stellenwert der KST im Vergleich zu anderen bildgebenden Verfahren besprochen, die ophthalmologischen Indikationen werden zusammengefaßt sowie ein Ausblick in weitere Entwicklungen gegeben.

In allen Abschnitten gelingt es dem Autor, sowohl seine Kompetenz für diese Untersuchungstechnik als auch seine kritische Einschätzung der KST im Vergleich zu den anderen bildgebenden Methoden darzustellen. Bei den intraokularen und orbitalen Erkrankungen stellt der Autor ausführlich das – für die Differentialdiagnostik wichtige – Signalverhalten dar und führt, teilweise durch Literaturzitate belegte, Erklärungen für unterschiedliches Signalverhalten an bzw. weist auf die Korrelationen zwischen KST und pathologischen Gewebeveränderungen hin. Wünschenswert wäre – wie für die intraokularen Raumforderungen – eine tabellarische Zusammenfassung der Kriterien für orbitale Erkrankungen. Die diskutierten Befunde werden durch ausgezeichnetes und sehr informatives Bildmaterial ergänzt. Das Buch kann allen Ophthalmologen sowie interessierten HNO-Ärzten, (Neuro-)Radiologen und Neurologen sehr empfohlen werden. G. Hasenfratz (München)

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Indirect mechanism of insulin release from dog pancreas during infusions of D-ribose

FREDERICK C. GOETZ, BERYL Z. GREENBERG, JOHN W. MANEY, RUTH WITTMERS, AND DEMETRE NICOLOFF
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GOETZ, FREDERICK C., BERYL Z. GREENBERG, JOHN W. MANEY, RUTH WITTMERS, AND DEMETRE NICOLOFF. *Indirect mechanism of insulin release from dog pancreas during infusions of D-ribose.* Am. J. Physiol. 227(5): 1158–1165. 1974.—D-Ribose causes insulin release, but evidence for direct action on pancreatic beta cells is contradictory. This suggests an indirect mechanism, possibly involving liver or portal vein. Further acute experiments were carried out: *1*) ribose infusions into hepatic artery and right atrium were as effective as those into portal vein in stimulating directly measured pancreatic insulin output; pancreatic artery infusion was ineffective. *2*) In 11 cross-circulation experiments, arterial blood from donor dogs receiving a dilute intra-atrial infusion of ribose was led into pancreatic arteries of test dogs. Insulin output of test dogs regularly increased within 1 min. *3*) In 14 experiments, isolated dog pancreas-plus-duodenum responded to perfusion of glucose but not ribose. *4*) After acute hepatectomy a slight rise in peripheral vein insulin was seen during infusion of ribose. It is concluded that ribose-induced insulin release in dogs depends on an indirect humoral mechanism. It is suggested that metabolism of ribose in various tissues, including red blood cells, yields substances such as reduced pyridine nucleotides with direct insulin-releasing properties. Glucose may share such a mechanism.

pentose; isolated perfused dog pancreas; hepatectomy; indirect insulin-release mechanism

IT WAS PREVIOUSLY SHOWN that small amounts of D-ribose introduced into the portal vein in dogs triggered the release of insulin from the pancreas (8). This occurred in spite of a fall in arterial glucose levels and in the absence of measurable levels of arterial ribose. Investigations of ribose effects on peripheral blood insulin levels in dogs, rabbits, and humans have shown that both oral and intravenous administrations of D-ribose are effective in causing insulin release (6, 9, 10, 24, 27, 28). The present paper describes further investigations of the mechanism of insulin release from the dog pancreas after ribose administration.

MATERIAL AND METHODS

Cannulation techniques. Healthy mongrel dogs weighing about 20 kg were used. They were fed unmeasured amounts of standard dog chow. Sodium pentobarbital was used for anesthesia, and care was taken to insure adequate breathing; artificial respiration was not required. Plastic cannulas (Teflon) were placed in a femoral artery for sampling of systemic arterial blood and in other vessels as mentioned in specific experiments.

Pancreatic venous blood was obtained from a cannula placed in the splenic vein, with ligatures placed so that virtually pure pancreatic blood from the distal end of the pancreas could be collected. Blood flow was estimated by direct measurement in a graduated vessel. The estimate of partial pancreatic insulin output thus obtained was found previously to be well correlated with total pancreatic insulin output (8). This technique was more convenient and sensitive than sampling of the more dilute pancreaticoduodenal vein blood. The term "insulin output" is used in this paper to indicate partial pancreatic insulin output into the splenic vein, as described here.

All infusions were carried out at a rate of 0.76 ml/min with a Harvard constant-infusion apparatus.

Assay methods. Insulin content of plasma was measured by a double-antibody radioimmunoassay method previously described (7); as a reference standard a sample of dog insulin with an assumed potency of 25 U/mg was used.[1]

All assays were performed in duplicate after coding of the samples so that the technician performing the assay was ignorant of their sequence in the experiment. As a further precaution to insure objectivity, the assay results were calculated by computer rather than by graph, with use of a program devised by Meinert and McHugh (21).

Ribose and glucose concentrations in blood were measured by estimation of the reducing activity of the sample (ferricyanide method, AutoAnalyzer), before and after treatment of the sample with glucose oxidase-peroxidase. Concentrations of glucose and ribose were then calculated from standard curves for each sugar. Recoveries of both ribose and glucose were considered satisfactory (90–110%) in the range 5–100 mg/100 ml, and concentrations of ribose as low as 2 mg/100 ml were regularly detected in experiments with standard solutions of ribose. Furthermore, comparisons of ribose recoveries with a method using orcinol (29) showed good agreement up to concentrations of 100 mg/100 ml of ribose. Above this concentration of ribose, however, recoveries of both ribose and glucose were erratic, with recoveries of glucose as low as 60% of the

[1] Dog insulin was kindly furnished by Dr. I. A. Mirsky of the University of Pittsburgh.

theoretical value. The explanation for this effect of high concentrations of ribose on the glucose oxidase method was not apparent. Because of such discrepancies, concentrations of sugar in samples of high ribose content are approximate values only.

A single lot of D-ribose (Mann Research Laboratories) was used for all experiments. The purity of the D-ribose was confirmed by thin-layer chromatography. It was also used as a reference standard for the assays of ribose in plasma. Reagent grade D-glucose (Merck) was used throughout.

Infusion of ribose at various sites. To investigate the relative effectiveness of several infusion sites, three experiments were performed:

1) Ribose, 3.75 %, was infused directly into the hepatic artery in three dogs, after a control period of isotonic saline infusion.

2) Portal vein (A) and right atrium (B) ribose infusions were compared in eight dogs. Four dogs in the set received the infusions in the order A-B, and four dogs received the infusions in the order B-A. This design allowed the elimination of the effect, if any, of the order of infusion on the pancreatic insulin response. A relatively low concentration of ribose was infused (1 %) to keep arterial ribose concentrations low. Since the minute output of insulin during the initial period varied somewhat from dog to dog, the results were analyzed by calculating the ratio of insulin output during ribose infusion to that during the immediately preceding three control (saline) observations; significance of differences between treatment groups was tested by analysis of variance.

3) In an exactly similar set of experiments, each dog received paired infusions of ribose into the right atrium and the pancreatic artery.

Right atrial infusions were accomplished by passing a cannula through a femoral vein into the heart. The location of the cannula tip was checked by inspection after killing the dogs at the end of each experiment.

Cross-circulation experiments. The purpose of these experiments was to test for the presence of a humoral insulin-releasing factor in the blood of dogs given an intravenous (right atrial) infusion of ribose. Accordingly, in each experiment two dogs were anesthesized and arterial blood from the "donor" dog was led through a plastic cannula into a branch of the splenic artery supplying the distal end of the pancreas of the "test" dog, as shown in the schematic drawing (Fig. 1).

Arterial pressure of the donor dog provided the flow. Blood volume of the donor dog was maintained by circulating blood back from the test dog by way of a cannula from the femoral artery of the test dog to the femoral vein of the donor dog. Insulin, glucose, and ribose concentrations were measured repeatedly in the arterial blood of the donor dog and in the arterial and pancreatic venous blood of the test dog. Observations were made before and after establishment of the cross-circulation, while the donor dog received a constant infusion of saline into the right atrium through a cannula inserted through a femoral vein. The infused solution was changed to ribose, 1 %, and further observations were made 1, 7, 15, and 30 min after the ribose entered the circulation of the donor dog. After a recovery period with

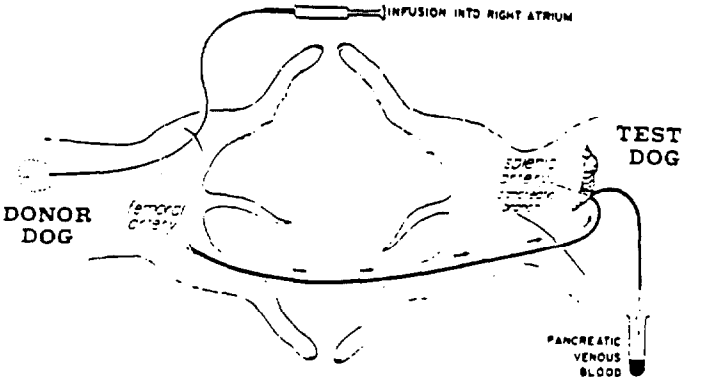


FIG. 1. Schematic drawing of method of cross-circulation of arterial blood from donor dog into pancreas of test dog.

saline infusion into the donor dog, the cross-circulation was taken down and ribose, 1 %, was infused into the right atrium of the test dog.

Control experiments were conducted according to an identical protocol, except that the donor dog received simply a continuous infusion of saline into the right atrium and no ribose. A coin was tossed to decide which were to be experimental (ribose) infusions and which were to be control experiments.

Perfusion of isolated dog pancreas and duodenum. In 14 experiments, the entire pancreas and adjacent portions of the duodenum were removed from dogs and immediately perfused through the celiac arterial axis by the method described by Idezuki and others (12). The perfusing solution was dog blood mixed with half its volume of Ringers solution, with or without added amounts of ribose or glucose calculated to raise the concentration of sugar to about 300 mg/100 ml. In three successive 15-min periods, the organs were perfused with solutions containing *1*) glucose, 40 mg/100 ml; *2*) glucose, 40 mg/100 ml, plus ribose, 300 mg/100 ml; *3*) glucose, 300 mg/100 ml, without ribose. The perfusion rate was held at 15 ± 2 ml/min. Samples of the effluent were taken at 1- to 5-min intervals. The whole effluent was analyzed for ribose and glucose. The insulin content of the cell-free supernatant was determined by immunoassay. Packed cell volume was measured on each sample.

Ribose infusion and peripheral insulin levels in hepatectomized dogs. For preliminary information on the effect of hepatectomy on the acute insulin response to ribose and glucose, a series of six experiments was carried out. In contrast to the experiments described above, insulin concentration was measured only in peripheral (femoral arterial) blood rather than in pancreatic venous blood; therefore, no measurements of minute output of insulin were possible. In healthy dogs under Brevane anesthesia, an acute hepatectomy was performed in one stage, with end-to-side anastomosis of the portal vein to the vena cava. The approximate time from induction of anesthesia to complete removal of the liver was about 2 h; during this time an intravenous infusion of glucose, 10 %, was maintained.

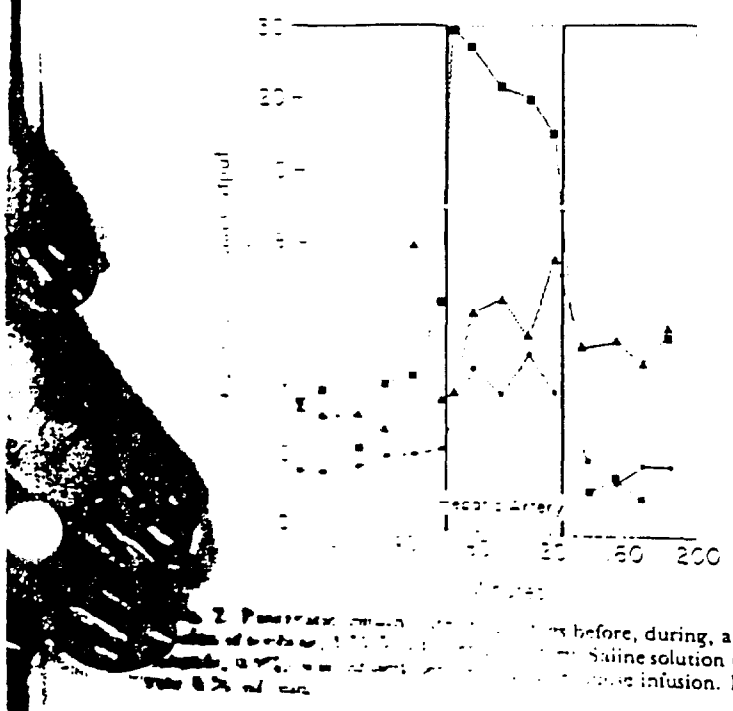
A few minutes after the removal of the liver, intravenous solutions were infused in four consecutive 15-min periods according to the following protocol: *1*) saline; *2*) ribose solution, 30 %; *3*) saline; *4*) glucose solution, 30 %.

This protocol was accomplished in three dogs. In addition, three control experiments were carried out in which the ribose infusion was replaced by a continuation of the saline infusion in the second 15-min period.

RESULTS

Comparative effects on insulin output of infusion of ribose at various sites. Since infusion of ribose in small amounts into the portal veins of dogs had been shown to be highly effective in eliciting increased insulin output from the pancreas, it was of interest to examine the effects of ribose infusion into other blood vessels. First, ribose was infused into the hepatic arteries of three dogs, after preliminary infusion with saline solution (Fig. 2). In two of the three dogs there was an immediate rise in insulin output as soon as ribose infusion was begun, although the rise was much greater in one dog than in the other. There was a prompt fall back to the baseline insulin output when the ribose infusion was stopped. In the third dog there was no clear change in insulin output associated with ribose infusion. These results suggested that the hepatic artery was just as effective a route of infusion as the portal vein.

Next, a systematic comparison was made, in the same eight dogs, of portal vein and right atrial infusions of ribose, 1% (15 min), alternating with control periods of saline infusion (30 min). The results are given in Table 1*A*. There was no rise in arterial ribose concentration in these experiments. As expected from previous experiments, infusion into the portal vein was accompanied by a highly significant increase in insulin output. However, infusions into the right atrium were also accompanied by significant increases in insulin output as compared to the appropriate control period. In fact, the increases during atrial infusion were slightly greater than those seen during portal vein infusion in the same animals. Analysis of variance also showed that dog-to-dog variation was rather large in these experiments, as is commonly seen, with very little residual unexplained variation.



... before, during, and after ... Saline solution (sodium ... infusion. Infusion

TABLE 1. *Comparison of mean insulin output (mU/min, mean ± SE) from pancreatic vein during ribose or saline infusion into right atrium and into pancreatic artery or portal vein*

Infusion Route	Infused Solution: Saline		Infused Solution: Ribose
A) Infusion into right atrium vs. infusion into portal vein (8 dogs)			
Right atrium	4.66 ± 0.84	$P^* = < .001$	8.62 ± 1.25
			$P = .047$
Portal vein	4.73 ± 0.97	$P = < .001$	7.40 ± 1.51
B) Infusion into right atrium vs. infusion into pancreatic artery (8 dogs)			
Right atrium	3.22 ± 0.43	$P = .08$	5.45 ± 1.09
			$P = .19$
Pancreatic artery	3.43 ± 0.66	$P \cong 1.0$	3.39 ± 0.50

* All *P* values are from analysis of variance.

In a second set of eight dogs, the identical plan was carried out, this time comparing right atrial with the pancreatic artery route of infusion. Systemic (femoral artery) blood ribose concentration did not rise during either ribose infusion in any of the experiments. Pancreatic venous ribose concentrations, on the other hand, rose promptly to levels of 30 to approximately 130 mg/100 ml when ribose was infused into the pancreatic artery.

Insulin output during control and ribose infusions into these two sites are shown in Table 1*B*. During ribose infusion into the right atrium, insulin output rose somewhat over control values, while during pancreatic artery infusion of ribose there was no apparent change in insulin output. Analysis of variance showed that the results were somewhat unexpected in that dog-to-dog variation was quite small, whereas residual unexplained variation was large. As the table shows, there was some overlap in the results. However, the mean value for insulin output during right atrial infusion approaches significant difference from that during the control period, whereas there was clearly no significant difference in the rate of insulin output during pancreatic artery infusion as opposed to the control period. The right atrial infusion gave a greater response on the average than the pancreatic artery infusion, although by analysis of variance the difference was not significant.

These results indicated that *1*) the portal vein per se is not essential for the ribose effect; *2*) direct infusion into the liver also is not essential, since the right atrial route is equally effective; *3*) direct infusion into the pancreatic artery, however, is not effective.

Cross-circulation experiments. The purpose of these experiments was to test the following hypothesis: the intravenous infusion of ribose is promptly followed by the appearance of a factor (distinct from ribose) in the blood which enhances insulin output from the pancreas. Since arterial blood from the donor dog receiving the ribose infusion was con-

ducted into the pancreatic circulation of the test dog through a plastic cannula, any influence on the insulin output from the test dog's pancreas must necessarily be humoral, not neural.

The changes in pancreatic insulin output in the cross-circulation experiments are presented in Table 2. The duration of the experiments was about three h, divided into seven consecutive periods indicated by letters A–G. Two groups of dogs were studied. The treatment of the dogs in both *group 1* (11 pairs) and *group 2* (10 pairs) was identical except during *period D*, when ribose, 1%, was infused at the usual rate into the right atrium of the donor dogs in *group 1*, whereas the *group 2* donor dogs continued to receive saline solution.

In *period A*, two base-line observations of pancreatic insulin output were made. After placement of all cannulas but before opening of the cross-circulation, the mean insulin output was approximately 7 mU/min in both groups of dogs, and there was no significant difference in the output between the two groups. At the beginning of *period B*, the arterial cross-circulation was opened. Thirty minutes later, at the beginning of *period C*, an infusion of saline solution was begun into the donor dogs through a cannula reaching the right atrium through a femoral vein. Insulin output at 62 and 77 min was clearly higher than in *period A* in both groups of dogs. However, the mean output in *group 1* test dogs was significantly higher ($P < .05$) than in the *group 2* test dogs at both 62 and 77 min. No explanation for this difference could be found. Since previous diet was not quantitatively controlled, one possible explanation for differences in insulin output between the two groups of dogs could be fluctuations in carbohydrate intake before the study (26).

TABLE 2. *Cross-circulation experiments: femoral artery blood of donor dogs into pancreatic artery of test dogs*

Time, min	Experimental Period	Insulin Output, mU/min, mean ± SE	
		Group 1	Group 2
	A) Base line		
0		7.0 ± 1.4	5.8 ± .9
15		8.1 ± 2.2	7.2 ± 1.0*
16	*B*) Cross-circulation on		
31		9.0 ± 1.6	5.9 ± 1.0
46		12.8 ± 2.0*	7.6 ± 1.8
47	*C*) Saline infusion into donor dog		
62		16.6 ± 2.9	8.9 ± 1.5
77		18.9 ± 3.8	10.1 ± 1.5
78	*D*) Ribose (group 1) or saline (group 2) infusion into donor dog		
79		28.5 ± 6.1*	10.6 ± 1.6
85		28.5 ± 5.6	11.1 ± 1.8
93		27.8 ± 6.3	11.6 ± 2.0
108		27.5 ± 6.5	12.9 ± 2.3
109	*E*) Saline infusion into donor dog		
124		18.6 ± 4.4*	13.0 ± 2.4
139		21.9 ± 5.1*	13.5 ± 2.2
140	*F*) Cross-circulation off; saline infusion into test dog		
155		9.5 ± 2.6	7.4 ± 1.5†
170		10.8 ± 2.5	7.8 ± 1.6
171	*G*) Ribose infusion into test dog		
178		28.2 ± 4.8*	14.3 ± 3.0*
186		27.6 ± 5.4	17.2 ± 3.6

Mean pancreatic vein insulin output in test dogs is shown during ribose or saline infusion into donor dogs. *Groups 1* and *2* were treated identically except for *period D*. *Group 1*, $n = 11$. *Group 2*, $n = 10$. * Differs from preceding sample ($P < .02$, paired t test. † Differs from preceding sample ($P < .05$, paired t test).

Period D was the crucial one, in which ribose was infused into the *group 1* donor dogs. One minute after the start of this infusion (79-min sample), there was a sharp increase in insulin output from the pancreas of the corresponding test dogs, significantly different from the output 1 min before the ribose infusion was begun (77-min sample). In contrast, the insulin output in the *group 2* test dogs showed no change. This difference was sustained through the 30 min of *period D* (85-, 93- and 108-min samples).

At the beginning of *period E*, saline infusion into the donor dog was continued or resumed. In the *group 1* dogs, the insulin output dropped back to the level before the ribose infusion; in the *group 2* dogs there was no change at this time. In *period F*, the cross-circulation was turned off and saline infusion was begun into the right atrium of the test dog. In *period G*, ribose, 1%, was substituted for saline infusion in both groups of test dogs. Both groups showed an increase in insulin output at 178 min to a level at least double that at 170 min. This change indicated that the test dog pancreases were still responsive to ribose at the end of the experiment.

Ribose, glucose, and plasma insulin were measured in arterial samples from both donor and test dogs taken at the same time as the pancreatic vein samples from the test dog. There was no detectable increase in nonglucose-reducing substances (which would include ribose) during the period of ribose infusion. Arterial insulin concentration rose somewhat in the donor dogs during the ribose infusion, the highest mean value being 82 ± 27 μU/ml (mean ± SE) of plasma as compared to the base-line mean value of 35 ± 5 ($.05 < P < .1$, paired t test). In contrast, mean arterial insulin in the donor dogs receiving saline remained steady at about 25 ± 4 μU/ml. The change in insulin concentration in the experimental dogs' arterial plasma was interpreted as reflecting the response, as expected, of the donor dogs' own pancreases. However, it was a trivial change in comparison to the change in mean insulin concentration in pancreatic venous blood in the test dogs (from 6,400 to 10,200 μU/ml) and could not account for the change in insulin output which was observed. Arterial glucose concentrations remained unchanged throughout the experiment in both sets of dogs. However, the initial glucose concentration in arterial blood of the test dogs was somewhat higher in the *group 2* (102 ± 8 mg/100 ml) than in the *group 1* dogs (83 ± 5) ($.05 < P < .1$).

These results indicated that a factor capable of inducing

immediate insulin release is present in arterial blood within 1 min after the beginning of intra-atrial infusion of ribose and that the factor is not ribose itself.

Direct perfusion of isolated dog pancreas and duodenum with ribose and glucose. Insulin and sugar concentrations in the effluent from 14 perfusion experiments are shown in Fig. 3. Packed cell volume remained constant throughout each experiment. Since flow rate was also constant, the concentrations of insulin noted were a direct reflection of the minute output of insulin from the pancreas.

Insulin output fell during the first 15 min of perfusion in most of the experiments, with a glucose concentration of about 40 mg/100 ml. During the second 15 min, when ribose concentration was rising from 0 to approximately 250 mg/100 ml, insulin output remained constant or fell further in all but two experiments. During the final 15 min, during which glucose concentration rose from about 40 to approximately 250 mg/100 ml, insulin concentration rose

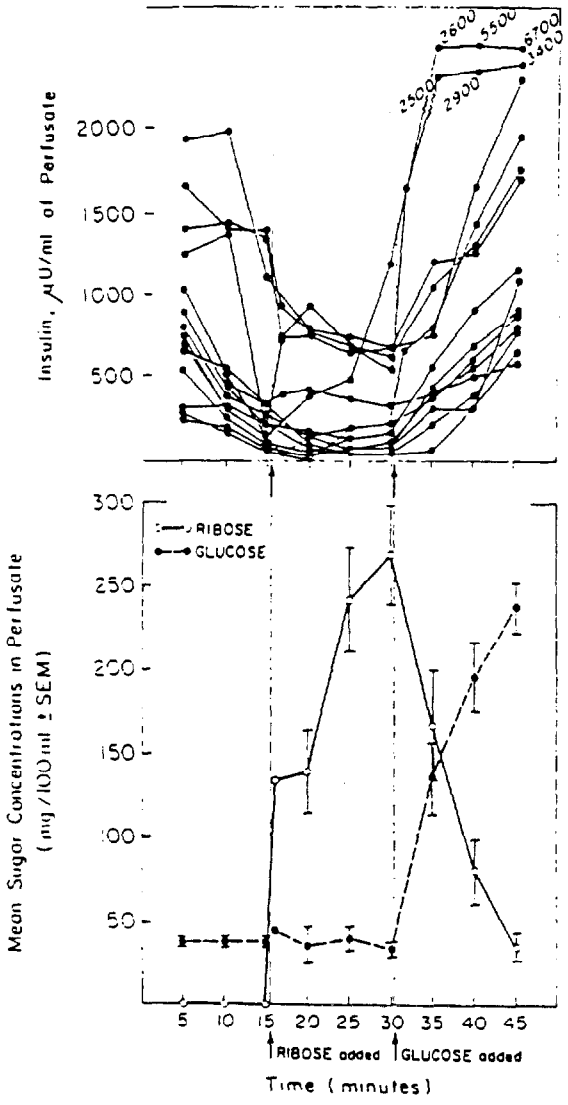


FIG. 3. Insulin and mean ribose and glucose concentrations ± SEM in effluent from 14 perfusion experiments of isolated dog pancreas and duodenum. In 3 successive 15-min periods, solutions containing *1)* glucose, 40 mg/100 ml, *2)* glucose, 40 mg/100 ml, plus ribose, 300 mg/100 ml, *3)* glucose, 300 mg/100 ml, were perfused through organs.

in all experiments, showing that the preparation was still responsive.

These results indicated no effect of ribose on insulin secretion by isolated perfused dog pancreas.

Ribose infusion and peripheral insulin levels in hepatectomized dogs. Levels of insulin and of ribose and glucose in six hepatectomy experiments are shown in Fig. 4.

In all six experiments, plasma insulin fell in the first 15 min following hepatectomy. The amount of change varied from dog to dog, but roughly it corresponded to the moderate fall in blood glucose observed in most animals. In the three dogs which received an infusion of concentrated ribose solution (30%) during the second 15-min period after hepatectomy, the fall in arterial insulin concentration was interrupted by a slight rise. The fall was resumed when ribose infusion was stopped. In the three control experiments, no change in insulin level was observed during the corresponding periods. In the final 15-min period, plasma insulin levels rose distinctly in five of the six dogs during an infusion of concentrated glucose (30%). This response confirmed the sensitivity of the islets to stimulation.

Although the number of experiments was small, it was concluded that the liver is not essential for the stimulation of insulin release by ribose or glucose.

DISCUSSION

The aim of the present study was to investigate the mechanism of the previously observed insulin release from the pancreas after administration of D-ribose.

The probability that insulin release is not the result of direct stimulation of the beta cell with D-ribose is indicated by both the in vivo and in vitro experiments reported here. Direct pancreatic arterial infusion, in vivo, was not effective in causing release of insulin. Also, on direct exposure of isolated dog pancreas to rising ribose concentrations (up to 250 mg/100 ml or more), there was no increase in insulin secretion.

The final effect on the pancreas itself was shown to be a humoral rather than a neural effect. In cross-circulation experiments where there was no neural connection between the donor dog and the responding pancreas of the test dog, there was immediate insulin release within 1 min after the beginning of the intravenous infusion of ribose. (A neurohumoral mechanism, involving rapid release of a beta-cell stimulator from the hypothalamus or other neural tissue, is a possibility which we could not test.)

It is not clear why ribose leaving the pancreas in the direct pancreatic artery infusions (Table 1) did not lead to insulin release. One theoretical possibility is that concentrations of ribose above a critical level may actually directly inhibit insulin release from the beta cells, but we have no evidence for this.

The liver is not necessary for the insulin-release mechanism to be activated, though it may still be considered as one of the sites of activation. This was demonstrated in two studies. Infusion into the right atrium of the heart proved to be as effective as portal vein infusion in producing insulin release. In preliminary experiments with acutely hepatectomized dogs, the ribose effect was still present.

It is of interest to review the work of other investigators in regard to the question of the ability of ribose to stimulate

TABLE 3. *Review of studies of insulin release from isolated pancreas preparations after ribose stimulation*

Author	Species	Pancreas Preparation	Sugar Concn mg/100 ml		Insulin Response
			Glucose	Ribose	
Ashcroft et al. (3)	Mouse	Islets	0	300	No
Grodsky	Rat	Perfused pancreas	0	300	No
Malaisse et al. (16)	Rat	Pieces	30	270	No (Suppression?)
Malaisse and Malaisse-Lagae (15)	Rat	Pieces	0	250	No
			50	250	No
			100	250	No
			150	250	Yes (additive)*
			300	250	No*
			500	250	No*
Montague et al. (22, 23)	Rat	Islets	0	300	Yes
Coore and Randle (5)	Rabbit	Pieces	0	300	No
			60	300	No
			300	300	No
Goetz et al.	Dog	Perfused pancreas	40	0–250	No

* Glucose controls increased over basal.

glucose administration, followed by a serum insulin response 10 min after glucose. On comparing this slow time course of insulin release with the immediate (within 1 min) response in the ribose cross-circulation experiments, we conclude that a gut factor is at most a minor contributor to ribose-induced insulin release.

As an alternative, it is proposed that the beta-cell trigger is a small molecule directly associated with or resulting from the metabolism of ribose in extrapancreatic tissues. Ribose is metabolized by mammals, presumably through the pentose phosphate shunt and presumably after phosphorylation by ribokinase. There is direct evidence that, along with the liver (1), the red blood cells, at least in man, pig, and rabbit (19), are capable of actively metabolizing ribose. Blood is the one substance that is common to all organs and tissues along the vascular pathway between the portal vein and the pancreas. Since the red blood cells are able to metabolize ribose, this would mean that any factor involved in the metabolic pathway of ribose metabolism could be implicated in the release mechanism of insulin. One clue is offered by studies of several investigator Work of Watkins et al. (30–32) and also of Panten et a (25) and of Hellman and Täljedal (11) indicates that a cumulation of reduced pyridine nucleotides in islets c exposure of islets or isolated secretory granules to reduce nucleotides is associated with secretion or solubilizing c insulin. These findings might imply that NADPH generate by carbohydrate being metabolized through the pentos phosphate shunt stimulates insulin release from the pancrea

There is now evidence that the red cell can act as a ca rier for metabolic substrate to tissues (2). We can speculat that it can also act as a carrier or provider of reduce pyridine nucleotides resulting from ribose metabolism i the red cells themselves or in solid organs. The reduce nucleotides could then stimulate insulin release from th beta cell. The known speed of circulation in the dog (18 is consistent with such a speculation.

The effectiveness of ribose infusions into the right atriun (Table 1) raises the possibility that the lungs could be a (the?) ribose-responsive tissue. However, in preliminary experiments ribose infusions into the left ventricle of the heart appeared to be about as effective as those into the right atrium. The possible role of the lungs in the response to ribose appears to be a matter for further investigation.

Is the indirect mode of insulin release unique to ribose? There is some evidence that at least two other carbohydrates, galactose and xylitol, may have the same property. Xylitol especially is a potent stimulus to insulin release in vivo, but the evidence on its in vitro effects is conflicting, much like that on ribose (3, 14, 15, 22, 23).

Whatever the mechanism of the ribose effect may be, it may also be part of the insulin-releasing mechanism of glucose, since glucose also can enter the pentose shunt pathway in many tissues. Our earlier concept of a "hepatoportal" signal for insulin release is not eliminated by the present results (17), but instead is broadened to include participation of other tissues.

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REFERENCES

1. AGRANOFF, B. W., AND R. O. BRADY. Purification and properties of calf liver ribokinase. *J. Biol. Chem.* 219: 221–229, 1956.
2. AOKI, T. T., M. F. BRENNAN, W. A. MULLER, F. D. MOORE, AND G. F. CAHILL, JR. Effect of insulin on muscle glutamate uptake. Whole blood versus plasma glutamate analysis. *J. Clin. Invest.* 51: 2889–2894, 1972.
3. ASHCROFT, S. J. H., L. C. C. WEERASINGHE, J. M. BASSETT, AND P. J. RANDLE. The pentose cycle and insulin release in mouse pancreatic islets. *Biochem. J.* 126: 525–532, 1972.
4. CHISHOLM, D. J., J. D. YOUNG, AND L. LAZARUS. The gastrointestinal stimulus to insulin release. I. Secretin. *J. Clin. Invest.* 48: 1453–1460, 1969.
5. COORE, H. G., AND P. J. RANDLE. Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. *Biochem. J.* 93: 66–78, 1964.
6. GINSBURG, J., B. BOUCHER, AND P. BEACONSFIELD. Hormonal changes during ribose-induced hypoglycemia. *Diabetes* 19: 23–27, 1970.
7. GOETZ, F. C., B. Z. GREENBERG, J. ELLS, AND C. MEINERT. A simple immunoassay for insulin: application to human and dog plasma. *J. Clin. Endocrinol. Metab.* 23: 1237–1246, 1963.
8. GOETZ, F. C., J. W. MANEY, AND B. Z. GREENBERG. The regulation of insulin secretion: effect of the infusion of glucose, ribose, and other sugars into the portal veins of dogs. *J. Lab. Clin. Med.* 69: 537–557, 1967.
9. GOODMAN, C., AND F. C. GOETZ. Oral and intravenous D-ribose

and plasma insulin in healthy humans: effects of route of administration and of epinephrine and propranolol. *Metabolism* 19: 1094–1103, 1970.

10. HALTER, J. B., M. Z. SCHWARTZ, AND F. C. GOETZ. The effect of oral and intravenous D-ribose on plasma insulin levels in unanesthetized dogs. *Proc. Soc. Exptl. Biol. Med.* 127: 1147–1151, 1968.
11. HELLMAN, B., AND I.-B. TÄLJEDAL. Solubilizing effect of nucleotides on isolated insulin secretory granules. *J. Cell Biol.* 47: 289–293, 1970.
12. IDEZUKI, Y., F. C. GOETZ, S. E. KAUFMAN, AND R. C. LILLEHEI. In vitro insulin productivity of preserved pancreas: a simple test to assess the viability of pancreatic allografts. *Surgery* 64: 940–947, 1968.
13. KIM, H. D., AND T. J. MCMANUS. Studies on the energy metabolism of pig red cells II. Lactate formation from free ribose and deoxyribose with maintenance of ATP. *Biochim. Biophys. Acta* 230: 12–19, 1971.
14. LIN, B. J., AND R. E. HAIST. Respiration and insulin synthesis in the islets of Langerhans. *Can. J. Physiol. Pharmacol.* 49: 559–567, 1971.
15. MALAISSE, W., AND F. MALAISSE-LAGAE. Effects of ribose and pentitols upon insulin secretion. *Arch. Intern. Physiol. Biochim.* 77: 366–367, 1969.
16. MALAISSE, W., F. MALAISSE-LAGAE, AND P. H. WRIGHT. A new method for the measurement in vitro of pancreatic insulin secretion. *Endocrinology* 80: 99–108, 1967.
17. MARKS, V., AND E. SAMOLS. Intestinal factors in the regulation of insulin secretion. *Advan. Metab. Disorders* 4: 1–38, 1970.
18. MARSHALL, R. J., Y. WANG, AND J. T. SHEPHERD. Components of the "central" blood volume in the dog. *Circulation Res.* 8: 93–99, 1960.
19. MATTHIES, H., AND L. LACHHEIN. Über eine Ribokinase in kernlosen Erythrocyten. *Naturw.* 46: 264, 1959.
20. MCINTYRE, N., C. D. HOLDSWORTH, AND D. S. TURNER. New interpretation of oral glucose tolerance. *Lancet* 2: 20–21, 1964.
21. MEINERT, C. L., AND R. MCHUGH. The biometry of an isotope displacement immunologic microassay. *Math. Biosci.* 2: 319–338, 1968.
22. MONTAGUE, W., S. L. HOWELL, AND K. W. TAYLOR. Pentitols and the mechanism of insulin release. *Nature* 215: 1088–1089, 1967.
23. MONTAGUE, W., AND K. W. TAYLOR. Pentitols and insulin release by isolated rat islets of Langerhans. *Biochem. J.* 109: 333–339, 1968.
24. NIJJAR, M. S., AND W. F. PERRY. Effects of intravenous and oral infusion of monosaccharides on serum insulin levels in rabbits. *Diabetes* 19: 155–160, 1970.
25. PANTEN, U., J. CHRISTIANS, E. v. KRIEGSTEIN, W. POSER, AND A. HASSELBLATT. Effect of carbohydrates upon fluorescence of reduced pyridine nucleotides from perifused isolated pancreatic islets. *Diabetologia* 9: 477–482, 1973.
26. SELTZER, H. S., AND Y. FUJITA. Influence of antecedent carbohydrate intake on the biphasic insulin response (abstract). *Diabetes* 22, Suppl. 1: 304, 1973.
27. STEINBERG, T., P. ORTMAN, R. POUCHER, B. COCHRAN, JR., AND G. GWINUP. The effect of D-ribose infusion on serum immunoreactive insulin and glucose concentration. *Metabolism* 16: 40–46, 1967.
28. STEINBERG, T., R. L. POUCHER, R. K. SARIN, R. B. REES, AND G. GWINUP. Oral administration of D-ribose in diabetes mellitus. *Diabetes* 19: 11–16, 1970.
29. UMBREIT, W. W., R. H. BURRIS, AND V. F. STAUFFER. Methods for the analysis of phosphorylated intermediates. In: *Manometric Techniques*. Minneapolis: Burgess, 1957, p. 274.
30. WATKINS, D. T. Pyridine nucleotide stimulation of insulin release from isolated toadfish insulin secretion granules. *Endocrinology* 90: 272–276, 1972.
31. WATKINS, D., S. J. COOPERSTEIN, P. K. DIXIT, AND A. LAZAROW. Insulin secretion from toadfish islet tissue stimulated by pyridine nucleotides. *Science* 162: 283–284, 1968.
32. WATKINS, D., S. J. COOPERSTEIN, AND A. LAZAROW. Stimulation of insulin secretion by pyridine nucleotides. *Endocrinology* 88: 1380–1384, 1971.

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Sustained Hypoglycemia in Response to Intravenous Infusion of D-Ribose in Normal Dogs

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Synopsis

The intravenous infusion of D-ribose to normal, nonanesthetized and anesthetized dogs at a rate of 3 mg/kg·min produced a sustained hypoglycemia. The mean blood glucose concentration decreased to a level about 30 mg/dl below the fasting level in 45 min after starting D-ribose infusion and remained at the same level until the infusion was discontinued. After stopping the infusion the blood glucose concentration returned to the preinfusion level in about one hour.

The plasma IRI (immunoreactive insulin) concentration in pancreatic effluent blood in anesthetized dogs increased rapidly, peaked within 5-10 min and then declined gradually, but remained above the basal level during the sustained hypoglycemia. The plasma IRI concentration in pancreatic effluent blood declined further below the basal level after stopping the infusion. In mixed blood an essentially similar pattern in time course of the plasma IRI concentration was observed in both nonanesthetized and anesthetized dogs.

No change in the plasma IRG (immunoreactive glucagon) concentration in pancreatic effluent blood and the plasma CGH (canine growth hormone) concentration in mixed venous blood was observed during or after the infusion of D-ribose.

The invariable occurrence of hyperinsulinemia during the period of progressive fall of blood glucose and sustained hypoglycemia suggests that insulin release is essential for D-ribose-induced hypoglycemia. Glucagon and growth hormone do not seem to play a role in producing a sustained hypoglycemia to prevent further progress of hypoglycemia by increasing their concentration in the blood. The pattern of insulin release during D-ribose infusion suggests the occurrence of the suppression of D-ribose-stimulated insulin release by the fall of blood glucose below the fasting level, which produces a sustained hypoglycemia at a certain level.

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It has been reported that the intravenous infusion of D-ribose at a rate of 1.9 to 3 mg/kg·min into nonanesthetized normal dogs causes hypoglycemia (Hetenyi and Ishiwata, 1968). It was concluded that this effect was due to the release of extra insulin, since D-ribose did not affect either the concentration or the turnover rate of blood glucose in pancreatectomized dogs which were

maintained on a matched intraportal infusion of insulin (Ishiwata *et al.*, 1969).

In our first report (Hetenyi and Ishiwata, 1968), an increase in the plasma insulin concentration in mixed venous blood was observed in only 2 out of 5 experiments, and only during the initial 20 minutes of the D-ribose infusion. Moreover, the course of hypoglycemia during the infusion of D-ribose suggested that blood glucose would reach a low but steady level if the infusion of D-ribose was to be continued longer. Comparing this leveling off of blood glucose with the changes in the plasma insulin concentration, it appears that there is an rapid increase in insulin release during the initial period of D-ribose infusion. This rapid increase is followed by a somewhat diminished but still higher than basal rate of insulin secretion as long as ribose is being infused. This pattern of insulin secretion leads to a steady, low level of glucose in blood.

The purpose of the experiments is to test this hypothesis and at the same time to study the possible roles of glucagon and/or growth hormone in producing this hypoglycemia.

Materials and Methods

Animals and surgical procedures

Healthy mongrel dogs fed with a standard dog chow and weighing 10 to 17 kg were used. The dogs to be used without anesthesia were trained for a week or longer to stand quietly in a Pavlov stand during the experiment. They were made to fast for about 20 hr prior to the experiment. In the experiment in the morning (8 : 30 a.m.) a plastic catheter for sampling mixed venous blood was inserted into the inferior vena cava via the saphenous vein, with the tip below the entry of the hepatic vein. Another plastic catheter for the infusion of D-ribose was inserted into the cephalic vein under local anesthesia.

The dogs to be anesthetized for the experiments were also made to fast for about 20 hr prior to the experiment. At 8 : 30 a.m., sodium pentobarbital (25 mg/kg) was injected intravenously. A catheter for sampling pancreatic venous blood was placed into the superior pancreaticoduodenal vein via the right gastroepiploic vein (Kuzuya and Kanazawa, 1969). Ligatures were placed on the main duodenal branch of the superior pancreaticoduodenal vein in order to reduce dilution by blood coming from the duodenum. The pancreatic branch of the splenic vein was also ligated in order to reduce drainage of the venous blood from the pancreas to the other vessels. The abdominal wall was closed, leaving the end of the catheter outside the abdominal wall. Catheters for sampling mixed venous blood and for infusion were inserted into the vena cava inferior via the right femoral vein, and the cephalic vein respectively. After surgery, the dog was laid on its right side.

Blood samples were taken according to a predetermined schedule. The blood loss was replaced by administering the same amount of saline, and the catheters were filled with heparinized saline. The total amount of heparin taken in by this procedure throughout the experiment was less than 1500 U. Supplementary injections of sodium pentobarbital (5 mg/kg each time) were given during the experiment when necessary.

Experimental design

All the experiments strictly followed the same time pattern, except for the period following the D-ribose infusion. During the initial 100-min period, saline was infused. This was followed by an infusion of D-ribose for 140 min. Thereafter, saline was again infused for 60 or 120 min. All infusions were given with the help of a Harvard constant-infusion pump at the rate of 0.34 or 0.48 ml/min, depending on the weight of the dogs.

Material

A single lot of D-ribose (Takeda Chemical Industry Company, Ltd., Osaka, Japan) was used for all the experiments. It was supplied as a 50 per cent solution for injection in glass ampules and was diluted with saline to a suitable amount when used.

Assay methods

The blood samples were put in glass tubes coated with heparin and stored in an ice-water bath until centrifugation. The plasma samples were kept frozen at −20°C.

Blood glucose was determined by the glucose oxidase method (Blood Sugar-Test, Boehringer, Manheim GmbH) after the deproteinization of the whole blood according to Somogyi (Somogyi, 1945). D-Ribose was determined by the method of Roe and Rice (1948) after the deproteinization of the plasma according to Somogyi. Free fatty acid (FFA) was determined as has been described by Elphick (1968), with minor modifications.

The plasma insulin concentration (IRI) was determined by the double-antibody radioimmunoassay method, using a reference standard of canine insulin (Kanazawa *et al.*, 1966). The plasma glucagon concentration (IRG) was measured by the double-antibody radioimmunoassay method (Kajinuma *et al.*, 1975), using a reference standard of porcine glucagon (Eli Lilly and Co., Indianapolis, Indiana). The rabbit anti-porcine glucagon antiserum used was the same as that previously used by Kaneto and Kosaka (1971). Despite some crossreactivity with an intestinal glucagon-like substance, the technique was shown to be able to detect an increase in the pancreatic effluent plasma IRG concentration in response to the infusion of arginine (Kaneto and Kosaka, 1971). The plasma growth hormone concentration (CGH) was determined by the double-antibody radioimmunoassay method, using a reference standard of canine growth hormone (Tsushima *et al.*, 1971).

Blood glucose, plasma FFA, plasma CGH, and hematocrit were determined in mixed venous blood. Plasma IRG was determined in pancreatic venous blood. Plasma IRI was determined in both mixed and pancreatic venous blood.

The paired *t* test was employed for the statistical analysis of the difference in the means of paired samples.

Results

Effects of intravenous infusion of D-ribose in nonanesthetized dogs

The effects of the intravenous infusion of D-ribose were observed in 6 nonanesthetized dogs. Fig. 1 summarizes the results. The blood glucose concentration began to drop within 10 min and reached a low, steady level within 45 min. Ten min after the termination of the infusion, the blood glucose concentration began to rise again and reached the basal level in about 60 min. The mean fasting blood glucose concentration ($\bar{x}\pm$S.E.M., the mean at 60 min to 100 min) was 83.0±0.11 mg/d*l* and the mean blood glucose concentration during the sustained hypoglycemia ($\bar{x}\pm$S.E.M. at 160 min to 240 min) was 51.7±0.37 mg/d*l*.

The ribose concentration in the plasma rose to around 10 mg/d*l* during the infusion, but fell rapidly after the infusion was stopped. The plasma FFA concentration showed a marked individual variation, but appeared to decrease transiently during the first 20 min of the infusion.

In mixed venous blood, the plasma IRI concentration rose immediately after the infusion was started. It reached its peak within 5 to 10 min and then began to decline, reaching near the basal level before the end of the infusion. The plasma CGH concentration determined in 4 dogs did not change significantly. The hem-

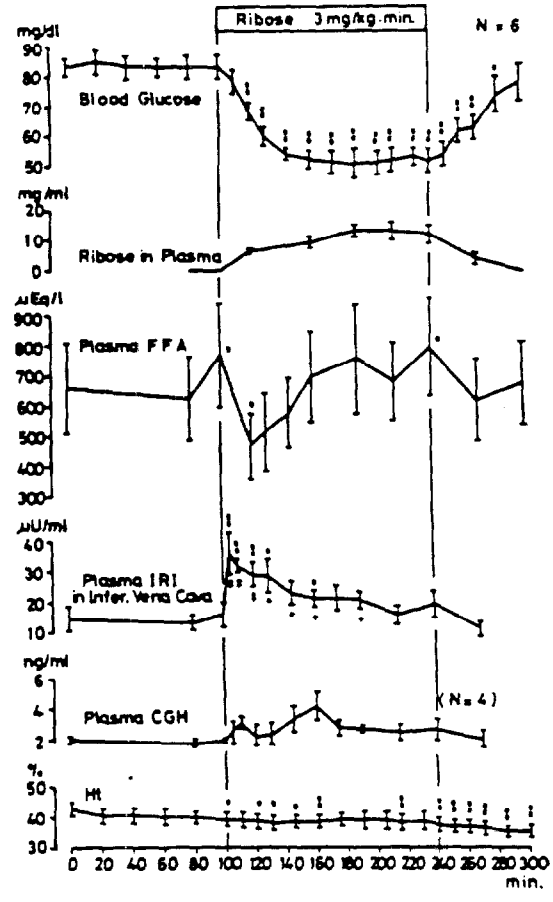

Fig. 1. Effects of the intravenous infusion of D-ribose at a rate of 3 mg/kg·min on blood glucose, plasma FFA, IRI and CGH, and hematocrit in nonanesthetized dogs. All values represent the mean±S.E.M. Significantly different from the value at 80 min, * at $P<0.05$, ‡ at $P<0.01$. Significantly different from the value at 270 min; † at $P<0.05$, ‡ at $P<0.01$.

atocrit decreased slightly during the experiment.

Effects of the intravenous infusion of saline in anesthetized dogs

Saline was infused for 360 min into 6 anesthetized dogs. Fig. 2 summarizes the results. The blood glucose concentration fell slightly during the initial 80 min, but returned to its basal level by 100 min. The concentrations of plasma FFA, plasma IRI and IRG (determined in 4 dogs) in pancreatic venous blood, and the hematocrit followed similar courses. The plasma IRI concentration in mixed venous blood did not show any change during this period.

After the first 100 min of the infusion the plasma FFA concentration began to rise and continued to rise until the end of the experiment, but no other components showed any appreciable changes.

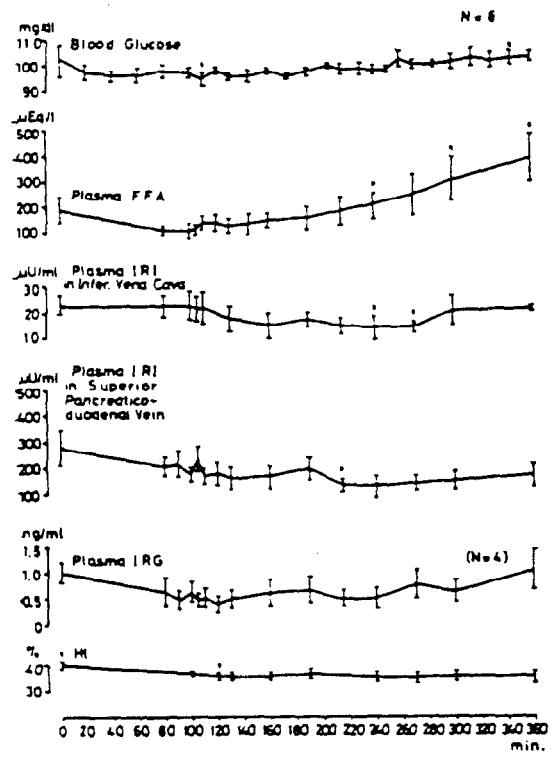

Fig. 2. Changes in blood glucose, plasma FFA, IRI and IRG, and hematocrit during the intravenous infusion of physiological saline in anesthetized dogs. All values represent the mean±S.E.M. Significantly different from the value at 80 min (or 100 min for hematocrit) * at $P<0.05$.

Effects of the intravenous infusion of D-ribose in anesthetized dogs

The effects of the intravenous infusion of D-ribose were followed in 12 dogs (Figs. 3, 4). The concentrations of blood glucose, plasma ribose, plasma FFA, plasma IRI, and the hematocrit were followed in all 12 dogs. In 4 of the first 6 dogs, the plasma CGH content in mixed venous blood was also followed (Fig. 3). In the other 6 dogs, the plasma IRG content was determined in pancreatic venous blood and experiments were carried out for a longer period after stopping D-ribose infusion (Fig. 4).

Changes in the concentration of blood glucose, plasma ribose, and plasma IRI in mixed venous blood and the hematocrit were similar to those observed in nonanesthetized dogs. The mean fasting blood glucose concentration ($\bar{x}\pm$S.E.M., the mean at 60 min to 100 min) was 90.9±0.23 mg/dl and the mean blood glucose concentration during the sustained hypoglycemia ($\bar{x}\pm$S.E.M. at 160 min to 240 min) was 59.1±0.18 mg/dl in 12 dogs. The blood glucose was restored to a slightly higher level than the preinfusion level from the sustained hypoglycemia at the end of the experiments carried out for the longer period (Fig. 4).

The plasma FFA concentration fell slightly around 30 to 40 min after the initiation of D-ribose infusion, then it began to rise until the end of the experiments (Figs. 3, 4).

The plasma IRI concentration in mixed venous blood and pancreatic venous blood rose immediately after the infusion of D-ribose was started and reached its peak within 10 min (Figs. 3, 4). The plasma IRI concentration in pancreatic venous blood in the first 6 dogs (Fig. 3) had already begun to rise at the time of initiation of D-ribose infusion. The rise was due to the untoward

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A lesser but apparent elevation of the plasma IRI concentration in both mixed and pancreatic venous blood was seen during the latter half of D-ribose infusion.

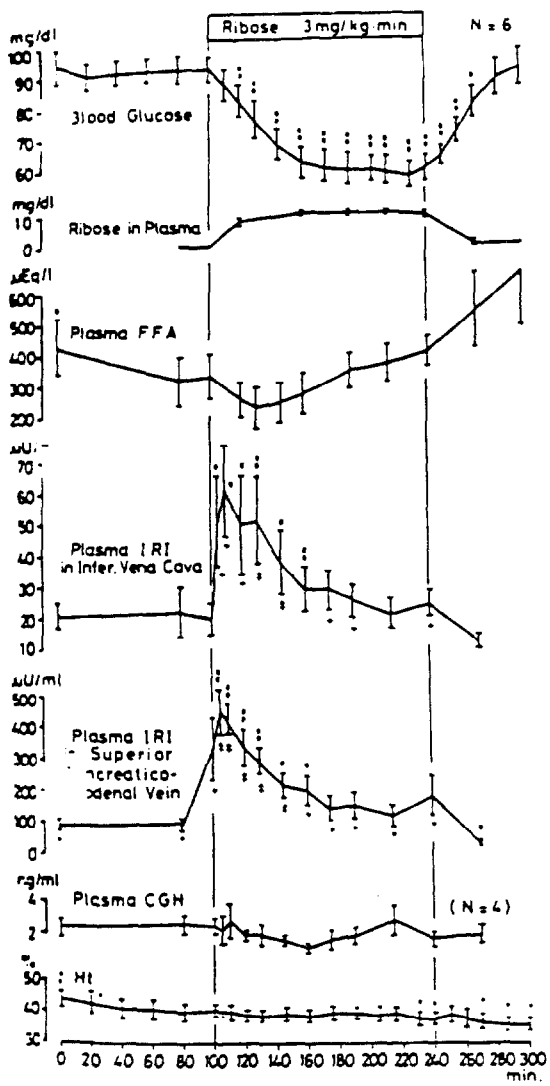

Fig. 3. Effects of the intravenous infusion of D-ribose at a rate of 3 mg/kg·min on blood glucose, plasma FFA, IRI and CGH, and hematocrit in anesthetized dogs (I). All values represent the mean±S.E.M. Significantly different from the value at 80 min * at P<0.05, ‡ at P<0.01. Significantly different from the value at 270 min, † at P<0.05, ‡ at P<0.01.

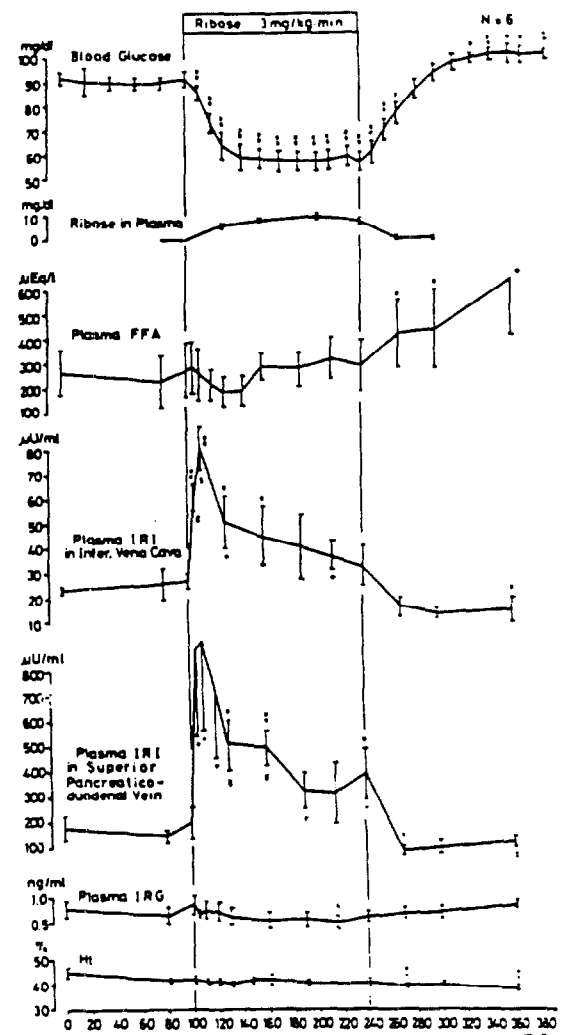

Fig. 4. Effects of the intravenous infusion of D-ribose at a rate of 3 mg/kg·min on blood glucose, plasma FFA, IRI and IRG, and hematocrit in anesthetized dogs (II). All values represent the mean±S.E.M. Significantly different from the value at 80 min * at P<0.05, ‡ P<0.01. Significantly different from the value at 270 min, † at P<0.05, ‡ at P<0.01.

After the termination of D-ribose infusion, the plasma IRI concentration in both mixed and pancreatic venous blood was decreased and then, seemed to fall further to the lower levels than those before D-ribose infusion. The plasma CGH and plasma IRG concentration did not change significantly throughout the experiments.

Discussion

It was confirmed in both nonanesthetized and anesthetized dogs that the intravenous infusion of D-ribose at a rate of 3 mg/kg·min promptly produced a sustained hypoglycemia.

The restoration of the blood glucose to a slightly higher level than the preinfusion level from the sustained hypoglycemia (Fig. 4) may be an apparent one caused by a decrease in the hematocrit, since the blood glucose concentration was measured with whole blood.

The hypoglycemia seemed to be produced by the release of insulin, because of the invariable occurrence of hyperinsulinemia in both pancreatic effluent blood and mixed blood during the period of progressive fall of blood glucose and sustained hypoglycemia. This was also supported by the fact that the recovery from hypoglycemia was associated with the decrease of plasma IRI in mixed blood and in pancreatic effluent blood. The discrepancy in insulin responses in the mixed blood between this report and the first report (Hetenyi and Ishiwata, 1968) may be explained by the difference in the sensitivity of the radioimmunoassay for insulin.

The fall of IRI release after the initial phase of D-ribose infusion may be caused by the fall of blood glucose. The concentration of plasma ribose (the primary stimulus of insulin release) remained at the same level or rose slightly higher during this period. It has been reported that the fall of blood glucose inhibits the endogenous insulin secretion and also the glucose-stimulated secretion of insulin during fish-insulin-induced hypoglycemia (Sando *et al.*, 1970). There is another possibility that this pattern of insulin release is produced by the mechanism similar to that which produces the biphasic response to a rise in blood glucose (Kanazawa *et al.*, 1968; Grodsky *et al.*, 1969), if the pancreatic β-cells respond to D-ribose as they respond to glucose. However, the progressive suppression of insulin release caused by the progressive fall of blood glucose seems to better explain the establishment of a sustained hypoglycemia at a certain level. Namely, the steady hypoglycemic level attained during D-ribose infusion may be determined by a balance between the secretagogue stimulus of D-ribose and the inhibitory effect of hypoglycemia on the pancreatic β-cells.

FFA seemed to have no apparent role in the regulatory response to hypoglycemia. The course and degree of hypoglycemia was the same in nonanesthetized and anesthetized groups, despite the differences in FFA changes in these groups.

Growth hormone and glucagon did not appear to be mobilized by D-ribose-induced hypoglycemia. These findings seem to indicate that the recovery from hypoglycemia of this degree may occur without increasing these hormones. In contrast, these hormones are reported to rise in severe insulin-induced hypoglycemia in dogs (Tsushima *et al.*, 1971; Ohneda *et al.*, 1969). Whether the lack of CGH and IRG responses merely means that the degree of hypoglycemia was insufficient or is specific to D-ribose-induced hypoglycemia remains to be elucidated.

Although it has been established that D-ribose releases insulin, controversy still remains as to whether or not the release of extra insulin is the sole cause of the hypoglycemia. The conclusion that the amount of insulin released was insufficient to account for the hypoglycemia has been drawn mostly from the observations of the

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changes in the insulin concentration in peripheral blood (Ginsburg *et al.*, 1970; Goodman and Goetz, 1970; Steinberg *et al.*, 1967 and 1970; Hetenyi and Ishiwata, 1968; Sloviter and Petkovic, 1969) which do not seem to be sensitive enough to provide satisfactory information concerning the small amount of insulin release from the pancreas. Moreover, it seems to have been influenced by a tendency to underestimate the efficacy of insulin released into the pancreatic vein so that it can act effectively on the liver to diminish its glucose production. The discrepancy in timing beween insulin release and hypoglycemia seems to be explained by the above reasons and also by the suppression of insulin release in response to hypoglycemia. After these considerations our results seem to support the view that the release of insulin can be the sole cause of the hypoglycemia.

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References

Elphick, M. C. (1968). *J. Clin. Path.* 21, 567.
Ginsburg, J., B. Boucher and P. Beaconsfield (1970). *Diabetes* 19, 23.
Goodman, C. and F. C. Goetz (1970). *Metabolism* 19, 1094.
Grodsky, G. M., D. L. Curry, H. Landahl and L. Bennet (1969). *Acta Diabetol. Latina* 6, Suppl. 1, 554.
Hetenyi, G. Jr. and K. Ishiwata (1968). *Amer. J. Physiol.* 214, 1333.
Ishiwata, K., G. Jr. Hetenyi and M. Vranic (1969). *Diabetes* 18, 820.
Kajinuma, H., T. Kuzuya, T. Ide and J. M. Tyler (1975). *Endocrinol. Japon.* 22, 517.
Kanazawa, Y., T. Kuzuya and T. Ide (1968). *Amer. J. Physiol.* 215, 620.
Kanazawa, Y., T. Kuzuya, T. Ide and K. Kosaka (1966). *ibid.* 211, 422.
Kaneto, A. and K. Kosaka (1971). *Endocrinology* 88, 1239.
Kuzuya, T. and Y. Kanazawa (1969). *Diabetologia* 5, 248.
Ohneda, A., E. Aguilar-Parada, A. M. Eisentraut and R. H. Unger (1969). *Diabetes* 18, 1.
Roe, J. H. and E. W. Rice (1948). *J. Biol. Chem.* 173, 507.
Sando, H., Y. Kanazawa and T. Kuzuya (1970). *Amer. J. Physiol.* 218, 1357.
Sloviter, H. A. and M. R. Petkovic (1969). *Nature* 221, 371.
Somogyi, M. (1945). *J. Biol. Chem.* 160, 69.
Steinberg, T., P. Ortman, R. Poucher, B. Cochran, Jr. and G. Gwinup (1967). *Metabolism* 16, 40.
Steinberg, T., R. L. Poucher, R. K. Sarin, R. B. Rees and G. Gwinup (1970). *Diabetes* 19, 11.
Tsushima, T., M. Irie and M. Sakuma (1971). *Endocrinology* 89, 685.

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Effect of D-glucose or D-ribose on the Turnover of Glucose in Pancreatectomized Dogs Maintained on a Matched Intraportal Infusion of Insulin

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SUMMARY

It was found previously that in normal dogs D-glucose or D-(-)-ribose decrease the rate of endogenous (hepatic) glucose production. To investigate whether this effect is due to the release of extra insulin only, or to other factors as well, the following experiments were performed: Endogenous insulin secretion was replaced by intraportal porcine or canine insulin infusion of 200 μU./kg./min. in conscious dogs. Such an insulin infusion commenced at time of removal of the remnant pancreatic autograft was able to maintain unchanged the plasma glucose level and the tracer determined glucose production and utilization. Three main conclusions emerged from this study: (1) Since an infusion of D-(-)-ribose did not affect concentration and turnover rates of glucose, it was concluded that ribose affects glucose metabolism only through an increase of the rate of insulin secretion. (2) The endogenous glucose production did not decrease in response to a glucose infusion at two thirds to twice the basal normal glucose production, even when the concentration of glucose in plasma exceeded 200 mg./100 ml. It is concluded that the release of extra insulin is indispensable for the regulation of glucose production in the normal dog. (3) The utilization of glucose rose in proportion to its concentration in the plasma in the absence of any change in the rate of insulin supply. DIABETES *18*:820-27, December, 1969.

The intravenous injection or infusion of D-ribose has been shown to decrease the concentration of glucose in the blood plasma of normal animals[1,2] and humans,[3] but not in pancreatectomized dogs[1] or diabetic patients.[4] This finding has been interpreted as an effect of ribose on the rate of release of insulin from the beta cells of the islets of Langerhans. This interpretation has been corroborated by the results of the experiments of Steinberg et al.[5] who found an increase in the plasma insulin level during the infusion of ribose in dogs, and also by the demonstration of an effect of ribose on isolated beta cells in vitro by Montague et al.[6] Whereas there is little doubt that ribose indeed releases insulin from the pancreas in the intact animal, a discrepancy between the apparent amount of the insulin released and the extent and duration of the ensuing hypoglycemia has been noticed.[3,5] Although the effects of ribose on the time course of the changes in rates of endogenous glucose production and over-all glucose utilization have been successfully simulated by an intraportal infusion of canine insulin in dogs,[1] considerable doubt remained about the existence of a second effect of ribose interfering with the compensatory increase of (hepatic) glucose production during hypoglycemia.

The aim of the experiments to be described was to investigate the effect of the infusion of ribose on the plasma concentration and the turnover of glucose in dogs in which the pancreas has been replaced by an infusion of insulin into the portal vein at a matched physiological rate.[7] If ribose has the same effects in this preparation as in normal dogs, this proves the existence of a mechanism dependent on the presence of physiological amounts of insulin but not upon the release of extra insulin. If, on the other hand, ribose has no effects, (as it has no effect in diabetic animals), its entire effect could be accounted for by the release of extra insulin. If such be the case, the reported[3,5] discrepancy between the rate of insulin secretion and hypoglycemic activity has to be due to differences between the effects of endogenous insulin secreted into the portal

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vein and of heterologous insulins injected by other routes.

Since the injection or infusion of glucose has been reported to decrease endogenous (hepatic) glucose production in both normal[5,9] and pancreatectomized[9] dogs, the effect of an intravenous infusion of glucose has also been investigated on all dogs on which the effect of ribose has been tested. In two additional experiments it has been attempted to obtain more detailed information about the effect of glucose; in this way we expected to obtain an answer to the question of whether glucose infused intravenously at relatively moderate rates is able to decrease the rate of hepatic glucose production without the release of extra insulin.

METHODS

Animal and surgical procedures

All experiments were carried out on nonanesthetized dogs trained to stand or lie quietly in a Pavlov stand during the experiment. Partial pancreatectomy and the exteriorization of the uncinate process of the pancreas into a subcutaneous pouch was carried out under general anesthesia six days prior to the experiment as described by Rappaport et al.[10] At the same time a vinyl catheter filled with heparin was inserted into a tributary of the splenic vein pointing towards the portal vein. The postoperative care and diet of the operated dogs has been described previously.[7] On the day of the experiment three more plastic catheters were inserted under local (procain) anesthesia, two of these into each one of the cephalic veins for the infusion of the tracer and the sugars respectively, the third introduced into the vena cava (with the tip below the entry of the hepatic veins) via the saphenous vein. This catheter was used for the collection of blood samples.

After the insertion of the catheters the uncinate process was removed under local anesthesia and an infusion of insulin* at the approximate rate of 200 μU./kg./min. into the portal circulation was started immediately.[7]

Porcine insulin was used in the first six, and canine in the additional two experiments. The animal was then transferred into the Pavlov stand and the experiment started after a sixty-minute period of equilibration.

Experimental design

All but the two experiments to be described subsequently strictly followed the same design. They consisted of four sequential periods of ninety, seventy, eighty and sixty minutes, in this order. During the first and third period 0.9 per cent NaCl was infused, during the second and fourth the infusion was changed to ribose (3 mg./kg./min.) or glucose (1.8 mg./kg./min.). In the first three experiments ribose was infused in the second, and glucose in the fourth periods. In the fourth, fifth and sixth experiment this sequence was reversed. Minor adjustments in the rate of the infusion of insulin were carried out early in the first period if the blood sugar level, estimated by a Dextrostix (Ames) test, warranted it. All infusions were given at a constant rate with help of Harvard or Sage infusors.

In two experiments 0.9 per cent saline was infused during the first and fourth periods and glucose in the second and third. (In these experiments both middle periods lasted eighty minutes. The rate of glucose infusion was 1.9 and 2.6 mg./kg./min. respectively in the second periods and was doubled in the third periods in both experiments.

Tracer methods and calculations

Rates of endogenous (hepatic) glucose production ("rate of appearance" R_a) and utilization ("rate of disappearance" R_d) were calculated by the method of primed infusion of tracer according to Wall et al.[11] as simplified by de Bodo et al.[12] Metabolic clearance of glucose was calculated as the ratio of R_d and the concentration of plasma glucose.[1] The time of the priming injection of tracer was taken as $t = 0$. R_a, R_d and metabolic clearance values were not calculated prior to $t =$ fifty minutes. All calculations were carried out on a CGE time sharing computer system. When the effect of glucose was tested on the rates of production and utilization, a mixture of C-14-labeled and ordinary C-12 glucose of a specific activity (SA) close to the one of plasma glucose was infused in order to avoid artefacts arising from the slow mixing of the glucose load with the labeled glucose pool in the body.[8] The calculated R_a was corrected for differences between the SA's of plasma and infused glucose.

In the additional two experiments the use of glucose-1-C-14 as tracer permitted the correction of the calculated rates of appearance and disappearance and also the metabolic clearance for the small inaccuracies arising from the incorporation of C-14 into the newly released glucose from the metabolized molecules of the infused tracer.

Processing of blood samples

The collection and processing of blood samples have been described.[1,9] The heparin solution was withdrawn

*The solution infused was 40 mU./ml. insulin and 5 mg./ml. of gelatin (Upjohn Co., Kalamazoo) in an acid-saline solution of pH 3.9-4.0. Thus the binding of insulin to glassware and tubing was prevented.

from the portal cannula prior to the experiment. A solution of heparin (5 I.U./ml.) was used only during the first forty-five minutes of the experiment to keep the sampling catheter patent. During later stages this catheter was filled with saline.

The isolation of glucose with help of an ion exchange resin (IRA-410, Rohm and Haas) and determination of glucose (Huggett and Nixon[13]), as well as the counting procedures, have been described.[1] In the additional two experiments the incorporation of C-14 into the sixth carbon atom was determined and the proper correction for recirculation of C-14 into newly formed glucose was carried out as described by Reichard et al.[14] Free fatty acid (FFA) in plasma was measured according to Dole and Meinertz[15] and the concentration of ribose according to Roe and Rice.[16]

MATERIALS

Glucose labeled uniformly with C-14 was used as tracer in the first six experiments, and glucose-1-C-14 in the additional two. The tracers were obtained from the Radiochemical Center, Amersham, England. D-(-)-ribose and D-glucose (Analar grade) were obtained from Eastman Organic Chemicals and from the British Drug Houses, respectively.

RESULTS

The plasma concentration and the rates of production and utilization of glucose

The results of the first six experiments are summarized in tables 1 and 2 showing the effects of an infusion of D-ribose (3 mg./kg./min.) and D-glucose (1.8 mg./kg./min.) on the plasma concentration and on the rates of production (R_a), over-all utilization (R_d) and the metabolic clearance (M) of glucose. It appears that in dogs maintained in (or near) a dynamic steady state by a constant supply of intraportally infused insulin, the plasma concentration and the rate of production of glucose was increased marginally by the intravenous infusion of D-ribose. In the experiments shown in table 2 the infusion of glucose during the second period increased glucose concentration at an average rate of 0.21 mg./100 ml./min. This was changed to an average decrease of 0.41 mg./100 ml./min. in the third period during which saline was infused. In the experiments shown in table 1 the average increase in concentration was 0.47 mg./100 ml./min. during the final period of glucose infusion.

When the data were tested by variance analysis, no evidence was found that infusion of ribose exerted any effect on the rate of glucose production (R_a). In this analysis the rates before and during the infusion of glucose were compared: "before" referring to the rates calculated between the 180th to 240th minutes of the experiment in table 1, and the fiftieth to ninetieth in table 2. The results of the analysis are summarized in table 3. Neither did the differences in the rates of utilization prove to be of statistical significance when subjected to a similar analysis.

The results of one of the two additional experiments carried out with glucose-1-C-14 as tracer and yielding rates of production and utilization of glucose unaffected by the reincorporation of C-14 into endogenously produced glucose are shown in figure 1. The concentration of glucose in the plasma has been increased at a rate of 0.63 and 1.06 mg./100 ml./min. during the infusion of 1.9 and 2.6 mg./kg./min. glucose respectively. No effect on the rate of appearance of glucose has been observed. The increase in the rate of disappearance in the two middle periods is not entirely commensurable with the increase of the plasma glucose level as indicated by the somewhat increased metabolic clearance. The fall in the latter during the final period of saline infusion is noteworthy.

Plasma concentration of free fatty acids

As shown in table 4, the level of FFA in plasma tended to rise during the experiments of the first series. This is most evident if one compares the average levels of FFA of the first and third periods, during both of which saline was infused. Ribose did not interfere with this pattern, whereas during three of the six infusions of glucose plasma FFA was decreased (Dogs 30, 33, 34). A similar decrease was noticeable in the two additional experiments (see figure 1), in both of which the plasma FFA concentration increased during the final infusion of saline. This effect, however, was not consistent in view of the three negative experiments of the first series. A more consistent decrease of about 30 per cent in the concentration of FFA in plasma has been found by Vranic et al.[17] after the injection of 0.5 gm./kg. glucose in dogs maintained on matched infusion of insulin exactly similar to our experiments.

Plasma concentration of ribose

During the infusion of ribose the concentration of the pentose reached a value between 9.4 and 14.0 mg./100 ml. Maximal levels have been closely approximated by forty minutes after the beginning of the infusion. After the cessation of the infusion, ribose was cleared from the plasma within twenty minutes.

DISCUSSION

In previous experiments, Vranic and Wrenshall[7]

TABLE 1

Effect of the intravenous infusion of D-ribose or D-glucose on plasma glucose concentration and glucose utilization and the production and the metabolic clearance of glucose

Infusion:	Saline				Ribose 3 mg./kg./min.					
Time (min.)	35-50	50-65	65-80	80-90	90-100	100-110	110-125	125-140	140-150	150-160
Expt. #										
				Plasma glucose concentration mg., 100 ml.						
28	225	235	227	229	213	217	212	239	256	254
30	111	106	106	100	99	96	95	99	99	103
32	119	111	115	112	107	106	116	112	110	119
Mean		151	149	147	140	140	141	150	155	159
				R_d (glucose utilization) mg./kg., min.						
28	4.22	4.34	4.07	3.88	5.12	2.90	4.27	3.50	4.90	5.40
30	2.96	2.95	3.04	2.59	2.13	3.27	3.05	2.14	3.32	2.40
32	3.23	3.60	2.58	3.15	3.68	3.60	2.22	3.46	3.72	2.11
Mean		3.60	3.23	3.21	3.64	3.26	3.18	3.03	3.98	3.30
				R_a (glucose production) mg./kg., min.						
28	5.31	5.00	3.53	4.05	3.51	3.33	3.95	5.32	6.63	5.19
30	2.38	2.36	3.07	1.58	1.95	2.87	2.93	2.51	3.42	2.98
32	2.94	2.91	2.96	2.63	3.11	3.36	3.12	3.15	3.41	3.43
Mean		3.42	3.19	2.75	2.86	3.19	3.33	3.66	4.49	3.87
				Metabolic clearance of glucose ml./kg., min.						
28		2.01	1.76	1.70	2.31	1.34	1.98	1.55	1.97	2.11
30	2.61	2.72	2.87	2.52	2.15	3.35	3.18	2.20	3.35	2.37
32	2.68	3.13	2.28	2.77	3.37	3.38	2.01	3.04	3.36	1.84
Mean		2.62	2.30	2.33	2.61	2.69	2.39	2.26	2.89	2.11

Infusion:	Saline						D-glucose 1.8 mg./kg./min.			
Time (min.)	160-170	170-180	180-195	195-210	210-225	225-240	240-250	250-265	265-280	280-300
Expt. #										
				Plasma glucose concentration mg., 100 ml.						
28	260	260	283	284	286	258	279	279	281	292
30	96	102	92	89	84	86	90	90	103	105
32	118	121	116	110	116	117	119	124	133	142
Mean	158	161	164	161	162	154	163	164	172	180
				R_d (glucose utilization) mg./kg., min.						
28	3.80	5.56	8.93	2.04	7.14	5.88	8.01	7.81	5.86	5.59
30	4.77	2.80	4.45	2.47	3.56	2.27	3.00	3.90	1.89	3.44
32	3.03	2.01	3.20	3.18	2.23	2.17	4.05	3.81	2.87	3.36
Mean	3.87	3.46	5.52	2.56	3.23	3.44	5.02	5.17	3.54	4.13
				R_a (glucose production) mg./kg., min.						
28	4.39	5.52	6.82	5.80	7.26	4.00	8.35	5.95	4.17	4.32
30	3.81	3.83	3.38	2.13	2.94	2.57	1.74	2.09	1.53	1.73
32	2.87	2.38	2.71	2.68	2.74	2.30	2.59	2.43	1.88	2.15
Mean	3.69	3.91	4.30	3.54	4.31	2.96	4.22	3.49	2.53	2.73
				Metabolic clearance of glucose ml./kg./min.						
28	1.47	2.13	3.64	0.79	2.49	2.15	2.97	2.78	2.08	1.98
30	4.79	2.82	4.58	2.73	4.11	2.67	3.41	4.35	1.95	3.31
32	2.25	1.68	2.71	2.82	1.97	1.91	3.44	3.13	2.24	2.45
Mean	2.84	2.21	3.64	2.11	2.86	2.24	3.27	3.42	2.09	2.58

TABLE 2

Effect of intravenous infusion of D-glucose or D-ribose on plasma glucose concentration and glucose utilization and production and the metabolic clearance of glucose

Infusion:	Saline				D-glucose 1.8 mg./kg./min.					
Time (min.)	35-50	50-65	65-80	80-90	90-100	100-110	110-125	125-140	140-150	150-160
Expt. #										
				Plasma glucose concentration mg./100 ml.						
33	103	92	77	81	80	86	87	86	80	86
34	93	96	85	85	88	97	101	100	108	111
35	89	82	88	77	77	79	79	86	89	95
Mean	95	90	83	81	82	87	89	91	92	97
				R_d (glucose utilization) mg./kg./min.						
33	3.47	3.08	4.40	1.92	3.06	2.28	3.92	3.22	5.06	1.53
34	4.06	2.54	3.73	2.66	3.06	3.43	3.67	4.06	3.32	2.68
35		2.70	3.82	2.53	5.61	4.18	4.21	5.07	3.32	4.81
Mean		2.77	3.98	2.37	3.91	3.30	3.93	4.11	3.90	3.01
				R_a (glucose production) mg./kg./min.						
33	3.59	1.53	2.36	2.67	1.10	1.72	2.15	1.26	2.07	0.91
34	2.47	2.90	2.24	2.75	1.67	3.49	2.52	2.10	3.02	2.06
35		3.28	2.77	2.52	3.76	2.06	2.68	3.07	2.09	2.58
Mean		2.57	2.46	2.65	2.18	2.42	2.45	2.14	2.39	1.85
				Metabolic clearance of glucose ml./kg./min.						
33	3.37	3.15	5.20	2.43	3.81	2.74	4.54	3.74	6.10	1.84
34	4.11	2.69	4.13	3.13	3.54	3.73	3.71	4.03	3.19	2.46
35		3.17	4.63	3.29	7.31	5.31	5.35	5.89	3.75	5.04
Mean		3.00	4.65	2.95	4.89	3.93	4.53	4.55	4.35	3.11

Infusion:	Saline						D-ribose 3 mg./kg./min.			
Time (min.)	160-170	170-180	180-195	195-210	210-225	225-240	240-250	250-265	265-280	280-300
Expt. #										
				Plasma glucose concentration mg./100 ml.						
33	76	74	64	57	51	50	51	56	57	58
34	97	98	87	76	75	68	66	72	70	70
35	96	92	87	80	85	70	72	68	72	72
Mean	90	88	79	71	70	63	63	65	66	67
				R_d (glucose utilization) mg./kg./min.						
33	3.93	2.23	3.38	2.77	2.50	2.08	1.91	1.86	2.56	2.37
34	4.65	3.20	3.32	3.39	2.88	2.90	2.53	2.15	3.19	3.16
35	3.90	2.84	3.56	2.23	3.95	2.52	3.75	2.92	3.43	3.76
Mean	4.16	2.76	3.42	2.80	3.11	2.50	2.73	2.31	3.06	3.10
				R_a (glucose production) mg./kg./min.						
33	1.95	1.75	1.99	1.94	1.65	1.96	2.14	2.42	2.74	2.45
34	1.80	3.31	1.77	1.94	2.62	2.02	2.04	2.94	2.91	3.20
35	3.33	2.24	2.85	2.72	2.50	2.74	3.13	2.92	3.43	3.76
Mean	2.36	2.43	2.21	2.20	2.26	2.24	2.44	2.76	3.03	3.14
				Metabolic clearance of glucose ml./kg./min.						
33	4.85	2.98	4.92	4.57	4.59	4.10	3.76	3.48	4.56	4.13
34	4.46	3.28	3.59	4.16	3.82	4.06	3.77	3.13	4.52	4.54
35	4.17	3.17	4.25	2.70	5.08	3.54	5.34	3.71	4.66	4.25
Mean	4.49	3.14	4.25	3.81	4.50	3.90	4.29	3.44	4.58	4.31

TABLE 3

The effect of the infusion of glucose on the rate of endogenous glucose production (R_a). Summary of the analysis of variance of the data shown in tables 1 and 2.

	table 1				table 2			
Source of variation	df	SS	MS	F*	df	SS	MS	F*
Total	23	86.247	—	—	26	12.865	—	—
Between rows (dogs)	2	64.869	32.434	2.12	2	4.988	2.494	6.22
Between columns (times)	7	10.997	—	—	8	1.463	0.183	—
Before vs. during infusion	1	1.707	1.707	1.10	1	0.608	0.608	5.04
Discrepancy	6	9.290	1.5484	—	7	0.855	0.121	—
Error	14	10.381	0.7415	—	16	6.414	0.401	—

*None of the F values shown indicate a statistically significant difference.

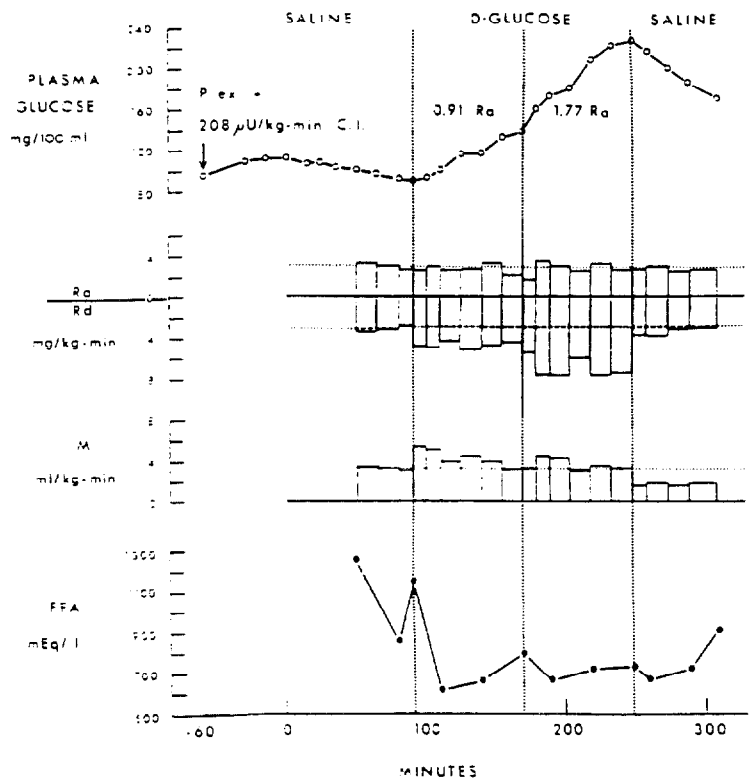

FIG. 1. Effect of an infusion of D-glucose on the concentration of glucose and FAA in the plasma and the calculated rates of appearance and disappearance and metabolic clearance of glucose. Glucose was infused between t = 90 and 170 at a rate of 2.6 mg./kg./min. and between t = 170 and 250 min. at a rate of 5.2 mg./kg./min. These rates correspond to 0.91 and 1.77 times R_a respectively. Abscissa: time in minutes. Uppermost ordinate: concentration of glucose in plasma as mg./100 ml. Second ordinate: rates of appearance R_a (above zero) and rates of disappearance R_d (below zero) of glucose as mg./kg./min. Third ordinate: metabolic clearance (M) of glucose as ml./kg./min. Lowest ordinate: concentration of FFA in plasma as mEq./L.

The intravenous infusions are indicated at the top of the figure. P_{ex} indicates the removal of the pancreatic graft and the beginning of the intraportal infusion of canine insulin (C.I.).

demonstrated that a portal infusion of insulin at a rate of 200 μU./kg./min. matched the endogenous secretion of insulin in the fasted dog. Such an infusion commenced at the time of the removal of the remnant pancreatic autograft was able to maintain the plasma glucose level and the rates of glucose production and utilization at normal levels. This rate of insulin infusion was considered to be "basal." In spite of the normoglycemia and normal turnover of glucose, dogs maintained on a basal infusion of insulin exhibited a lower than normal tolerance (smaller exponential decay constant) to intravenously injected glucose.[17]

From the data of the experiments presented in this paper three conclusions emerge:

(a) *D-ribose* does not decrease either the plasma concentration or the rate of production of glucose in dogs in which glucose homeostasis is maintained at a normal level by a basal infusion of insulin into the portal vein. Thus the hypoglycemic effect of ribose in the normal animal can be accounted for entirely by the release of extra insulin.

(b) *D-glucose*, when infused at a rate of about two thirds to twice the basal normal R_a to dogs in which the pancreas has been replaced by the intraportal infusion of insulin, was also ineffective in altering R_a significantly. This finding corroborates the conclusion of

TABLE 4

Changes in plasma FFA concentration during the infusion of D-glucose (1.8 mg./kg./min.) or D-ribose (3 mg./kg./min.) in normal dogs

Expt.	Infusion: time	Saline 65	90	Ribose 110	160	Saline 180	240	Glucose 250	280	300
28		1,122	1,107	1,076	1,123	—	1,499	1,578	1,280	1,248
30		1,030	999	1,094	1,189	1,030	1,078	919	650	713
32		682	666	1,141	1,094	1,094	999	1,062	1,411	1,411
Mean		945	924	1,104	1,135	1,062	1,192	1,186	1,113	1,124
± S.E.M.		134	133	19	28	—	155	200	235	211

	Infusion: time	Saline 65	90	Glucose 110	150	160	Saline 180	240	Ribose 250	280	300
33		920	780	499	749	764	849	1,264	1,139	952	—
34		655	764	718	640	686	780	976	936	983	976
35		864	769	729	895	1,075	1,035	1,283	1,283	1,140	1,190
Mean		813	771	649	761	841	886	1,174	1,119	1,073	1,083
± S.E.M.		81	5	75	74	118	77	99	101	58	—

Time refers to the injection of the priming dose of the tracer (t = 0) and is in minutes.

Steele et al.[5] who ascribed the effect of an intravenous glucose infusion of about this magnitude on R_a in normal dogs to a release of extra insulin. On the other hand, Hetenyi and Wrenshall[9] have demonstrated that an intravenous infusion of glucose decreased R_a in both normal and pancreatectomized dogs deprived of insulin for forty-eight hours. More recent experiments, however, carried out with more refined technics by Cowan and Hetenyi,[15] demonstrated a marked decrease of a very consistent pattern in R_a after or during the injection or infusion of glucose in normal dogs, but only a small and rather inconsistent one in insulin-deprived diabetic animals.

Thus it appears that in normal dogs the release of extra insulin is essential for an adaptation in endogenous glucose production during an infusion of glucose at a moderate rate. This finding is in agreement with those of Wrenshall et al.[19] who demonstrated that a sudden interruption of the endogenous insulin secretion results in a large increase in hepatic glucose production in spite of a rapidly rising level of plasma glucose, indicating thereby a sudden change in the normal relation between the plasma level and hepatic production of glucose. It is possible that when high glucose levels are being reached and maintained in the diabetic dog, the liver may regain its ability to respond with a decreased rate of production to an elevation of plasma glucose concentration. This could represent a second, not necessarily physiological, mechanism in the adaptation of endogenous production to exogenously injected glucose. It is also possible that the decrease of net glucose release observed on the perfused rat liver[20] in response to a raised glucose level in the perfusing fluid also represents a mechanism different from the physiological, insulin-dependent one that has been demonstrated to operate the fine balance in normal dogs. In this preparation the decrease of the net glucose output might be the result of an increased glucose uptake by the perfused liver, a mechanism entirely different from any change in the actual release of glucose. Tracer methods in general measure this latter one.

(c) The rate of glucose disappearance (R_d) increased with an increased glucose concentration in the absence of any change in insulin secretion (figure 1). The metabolic clearance of glucose was calculated to illustrate this relationship between the concentration and uptake of glucose. A relatively unaltered clearance indicates a linear relationship between the two parameters, i.e., when the concentration of glucose in plasma is doubled, its over-all uptake (R_d) is doubled as well. More detailed studies are being planned to investigate this correlation in the absence of variations in the rate of the secretion of insulin. As shown on figure 1, however, the metabolic clearance is higher at increasing plasma glucose concentration than it is at a decreasing one. This might be due to a nonlinear relationship between the concentration and uptake of glucose or, alternatively, to an artefact inherent in the tracer method used, causing an underestimation of the true size of the glucose pool. Evidently, if the pool size of glucose is underestimated some glucose being distributed into the pool contributes to the calculated "disappearance" during a rising concentration of glucose. Conversely when the concentration of glucose in the plasma is

falling, an underestimated pool size will lead to an underestimation of R_d.

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REFERENCES

[1] Hetenyi, G., Jr., and Ishiwata, K: Effect of D-(-)-ribose on the turnover of glucose in dogs. Amer. J. Physiol. 214:1333-39, 1968.

[2] Sloviter, H., Ikeda, M., and Sakata, K.: Hypoglycemic action of D-ribose in the rabbit. Amer. J. Physiol. 207:407-10, 1964.

[3] Segal, S., Foley, J., and Wyngaarden, B.: Hypoglycemic effect of D-ribose in man. Proc. Soc. Exp. Biol. Med. 95:551-55, 1957.

[4] Bierman, E. L., Barker, M., and Plough, I. C.: Metabolism of D-ribose in diabetes mellitus. Diabetes 8:455-58, 1959.

[5] Steinberg, T., Ortman, P., Poucher, R., Cochran, B., and Gwinup, G.: The effect of D-ribose infusion on serum immunoreactive insulin and glucose concentration. Metabolism 16:4046, 1967.

[6] Montague, W., Howell, S. L., and Taylor, K. W.: Pentitols and the mechanism of insulin release. Nature 215:1088, 1967.

[7] Vranic, M., and Wrenshall, G. A.: Matched rates of insulin infusion and secretion and concurrent tracer-determined rates of glucose appearance and disappearance in fasting dogs. Canad. J. Physiol. Pharmacol. 46:383-90, 1968.

[8] Steele, R., Bishop, J., Dunn, A., Altszuler, N., Rathgeb, I., and de Bodo, R. C.: Inhibition of hepatic glucose production in the normal dog. Amer. J. Physiol. 208:301-06, 1965.

[9] Hetenyi, G., Jr., and Wrenshall, G. A.: Adaptive changes in rates of appearance and disappearance of glucose in dogs following step changes in the rate of glucose infusion. Canad. J. Physiol. Pharmacol. 46:391-98, 1968.

[10] Rappaport, A. M., Vranic, M., and Wrenshall, G. A.: A pedunculated subcutaneous autotransplant of an isolated pancreatic remnant for the temporary deprivation of internal pancreatic secretion in the dog. Surgery 59:792-98, 1966.

[11] Wall, J. S., Steele, R., de Bodo, R. C., and Altszuler, N.: Effect of insulin on utilization and production of circulating glucose. Amer. J. Physiol. 189:43-50, 1957.

[12] de Bodo, R. C., Steele, R., Altszuler, N., Dunn, A., and Bishop, J. S.: The hormonal regulation of carbohydrate metabolism; studies with C-14 glucose. Recent Prog. Hormone Res. 19:445-82, 1963.

[13] Huggett, A. St. G., and Nixon, D. A.: Enzymic determination of glucose. Biochem. J. 66:12P, 1957.

[14] Reichard, G. A., Jr., Moury, N. F., Hochella, N. J., Patterson, A. L., and Weinhouse, S.: Quantitative estimation of the Cori cycle in the human. J. Biol. Chem. 238:495-501, 1963.

[15] Dole, V. P., and Meinertz, H.: Microdetermination of long chain fatty acids in plasma and tissues. J. Biol. Chem. 235:2595-99, 1960.

[16] Roe, J. H., and Rice, E. W.: A photometric method for the determination of free pentoses in animal tissues. J. Biol. Chem. 173:507-19, 1948.

[17] Vranic, M., Fono, P., Kuksis, A., and Lin, B.: The tracer determined metabolic clearance and glucose tolerance in dogs with matched insulin secretion rate. Diabetes 17:348, 1968 (Abstract).

[18] Cowan, J. S., and Hetenyi, G., Jr.: Continuous determination of glucose turnover rates in normal and diabetic dogs before and after the injection of glucose or insulin. Fed. Proc. 28:507, 1969 (Abstract).

[19] Wrenshall, G. A., Vranic, M., Cowan, J. S., and Rappaport, A. M.: Effects of sudden deprivation and restoration of insulin secretion on glucose metabolism in dogs. Diabetes 14:689-95, 1965.

[20] Herrera, M. G., Kamm, D., Ruderman, N., and Cahill, G. F., Jr.: Nonhormonal factors in the control of gluconeogenesis. Advances Enzym. Regulat. 4:225-36, 1966.

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Functional and metabolic effects of ribose in combination with prazosin, verapamil and metoprolol in rats in vivo

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ABSTRACT Ribose improves the function of the rat heart in various pathological conditions through its effects on cardiac energy metabolism, while having no direct haemodynamic actions. We therefore studied its functional and metabolic effects in closed chest rats when given in combination with prazosin, verapamil or metoprolol, all of which have direct effects on the circulation. Ribose administration for 24 h at 200 $mg \cdot kg^{-1} \cdot h^{-1}$ did not affect heart function but increased the available pool of 5-phosphoribosyl-1-pyrophosphate in heart (four fold) and skeletal muscle (1.7-fold), as assessed by the incorporation of ^{14}C-adenine into the adenine nucleotides. The utilisation of adenine for adenine nucleotide synthesis, expressed as the ratio of adenine nucleotide radioactivity to tissue extract radioactivity, was 70% in heart and 20% in skeletal muscle under control conditions, and 97% and 88% after 24 h of ribose administration. Ribose decreased the ^{14}C-adenine incorporation into the adenine nucleotides in kidney, lungs and liver. After 24 h infusion of prazosin (100 $\mu g \cdot kg^{-1} \cdot h^{-1}$), heart rate and $LVdP/dt_{max}$ were not changed, but LVSP (−20%), mean aortic pressure (−16%) and peripheral resistance (−40%) were decreased. Cardiac output was enhanced (+40%). Verapamil (2$mg \cdot kg^{-1} \cdot h^{-1}$) and metoprolol (2$mg \cdot kg^{-1} \cdot h^{-1}$) infused for 24 h decreased the pressure-rate and pressure-volume product of the left ventricle to the same extent (−40%). Verapamil had no influence on cardiac output, while metoprolol depressed it (−30%). Simultaneous administration of prazosin, verapamil or metoprolol with ribose did not affect the ribose induced increase in the myocardial 5-phosphoribosyl-1-pyrophosphate pool. On the other hand, ribose did not alter the characteristic haemodynamic changes induced by these three drugs. We conclude that it is feasible to combine ribose with prazosin, verapamil or metoprolol.

It is well documented that, in a number of pathological conditions such as ischaemia and heart failure, the decrease of oxygen delivery to the heart is associated with degradation of cardiac adenine nucleotides. The degradation products are released from the heart so the content of myocardial adenine nucleotides is diminished and this may be detrimental to heart function and cellular integrity.

Most pharmacological agents used in conventional cardiac therapy may indirectly affect cardiac energy processes by decreasing heart function or increasing blood supply. For example, it has been shown[1-3] that the calcium antagonist verapamil protects the heart against the deleterious effects of ischaemia. It reduces total peripheral resistance and thereby left ventricular afterload,[4] increases coronary blood flow[5,6] and antagonises coronary spasm. The β blocker metoprolol decreases the inotropy and the chronotropy of the heart, and reduces blood pressure and cardiac output. It is therefore beneficial in many pathological situations.[4,7,8] The α blocker prazosin decreases total

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peripheral resistance and mean blood pressure[8] and has been shown to prevent ventricular arrhythmias associated with acute ischaemia in dogs[9] and to be beneficial in patients with heart failure.[10] In addition to these haemodynamic effects, verapamil also has an influence on cardiac metabolism. It has been shown to reduce the loss of adenine nucleotide metabolites from the heart during hypoxia and subsequent re-oxygenation, thus helping to preserve cardiac adenine nucleotides.[11]

The mechanism of action of the cardioprotective agent ribose is quite different. Ribose directly affects cardiac adenine nucleotide metabolism by stimulating the production of 5-phosphoribosyl-1-pyrophosphate (PRPP).[12] PRPP then becomes available both for the salvage of adenine nucleotides and for their de novo synthesis. Ribose has been shown to have beneficial effects on the metabolism and function of the rat heart in several pathological conditions.[13 14] In contrast to the pharmacological agents mentioned, ribose has no haemodynamic effects. It could therefore be ideal to combine drugs which have direct haemodynamic effects with ribose which has direct effects on cardiac energy metabolism.

It was therefore the purpose of this study to see whether ribose could be administered in combination with the α blocker prazosin, the calcium antagonist verapamil and the β blocker metoprolol. A previous study[4] described the haemodynamic effects of verapamil and metoprolol in the rat heart in vivo and showed that the stimulation of de novo synthesis of myocardial adenine nucleotides brought about by ribose was not modified when verapamil and metoprolol were added. In the experiments described here, we have studied the haemodynamic effects of prazosin and have sought to determine whether the increase in PRPP availability (measured by the incorporation of ^{14}C-adenine into adenine nucleotides) is maintained when ribose is given in combination with prazosin, verapamil and metoprolol. This study is not confined to the heart but has been extended to the liver, kidney, lungs and skeletal muscle.

Methods

The animals used in these experiments were Sprague Dawley rats weighing approximately 250 g. They were fed Altromin pellets and had free access to water. The rats were handled in accordance with the law on the care and use of laboratory animals (BG Bl I, 1320–9, 1986).

D(-)Ribose was purchased from Sigma Chemie München. Verapamil was a gift from Knoll A G, Ludwigshafen. Metoprolol tartrate was provided by Astra Chemicals, Wedel. Prazosin was donated by Pfizer, Karlsruhe.

Drugs were given to non-restrained rats as a continuous intravenous infusion over 24 h. For this purpose, a steriflex catheter of 1 mm outside diameter (Vygon Company, Aachen) was placed in the left jugular vein under ether anaesthesia, tunnelled under the skin and brought out at the back of the neck, where it was connected to a 20 ml syringe in an Infors infusion pump (type 5003, Infors AG, Hofstetten). The catheters were covered with plastic shields to prevent them from being bitten by the rats. Control animals received 0.9% NaCl. Ribose was

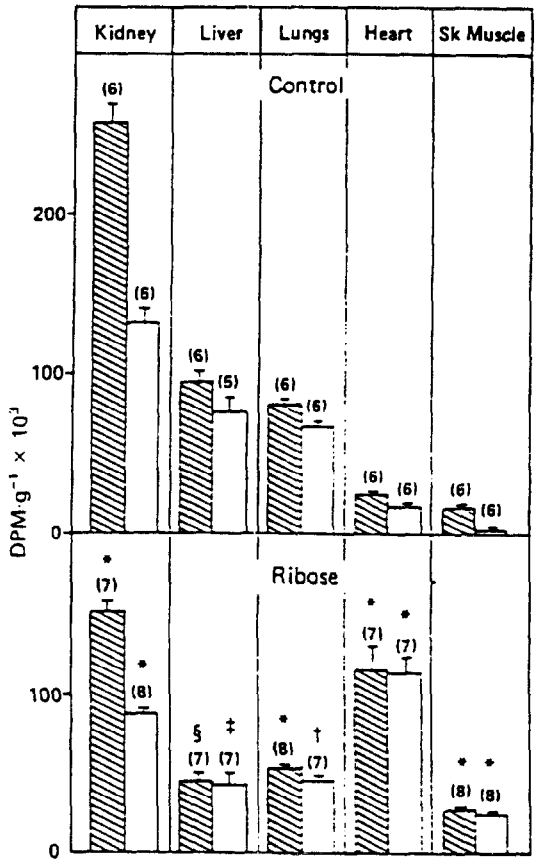

FIG 1 *Uptake of ^{14}C-adenine into different tissues and adenine nucleotide synthesis from ^{14}C-adenine. Uptake of ^{14}C-adenine (shaded columns) was assessed by measuring the tissue extract radioactivity per gram tissue and the synthesis of adenine nucleotides (blank columns) on the basis of the radioactivity of adenine nucleotides per gram tissue due to incorporation of ^{14}C-adenine. After 24 h infusion of NaCl 0.9% (control) or ribose (200 mg·kg^{-1}·h^{-1}) each rat received an intravenous injection of 10 μmol·litre^{-1} adenine containing 5 μCi ^{14}C-adenine. Columns are means, bars = SEM. Number of experiments in parentheses. *p=0.0005, †p=0.005, ‡p=0.01, §p=0.0125 v control value.*

administered in a concentration of 200 $mg \cdot kg^{-1} \cdot h^{-1}$ alone or in combination with prazosin (100 $\mu g \cdot kg^{-1} \cdot h^{-1}$), verapamil (2 $mg \cdot kg^{-1} \cdot h^{-1}$) or metoprolol (2 $mg \cdot kg^{-1} \cdot h^{-1}$). The effects of these three drugs were also tested without ribose, in the same concentrations.

Left ventricular function was measured after 24 h in closed chest rats, anaesthetised with Inactin Byk, 80 $mg \cdot kg^{-1}$ intraperitoneally.[15] Spontaneous respiration was facilitated by placing a tracheal cannula after tracheotomy. An ultraminiature catheter pressure transducer (Model PR-249, Millar Instruments Inc, Houston, Texas) was inserted into the right carotid artery and advanced into the left ventricle for measurement of systolic pressure and maximal rate of rise in pressure ($LVdP/dt_{max}$). It was then withdrawn into the aorta for the measurement of aortic pressure. This catheter was attached to a Millar transducer control unit (model TC-100) which was connected to an HSE electromanometer and an electronic differentiation system (Hugo Sachs Electronik, March-Hugstetten). Calibration of pressure and dP/dt_{max} was done using a mercury manometer (type 376, Hugo Sachs Electronik).

Cardiac output was measured using the thermodilution method. For this purpose, the Millar catheter was replaced by the thermodilution microprobe (1.5 French gauge, Columbus Instruments Inc, Columbus, Ohio), and positioned in the ascending aorta. The thermodilution curve obtained after injection of 100 μl saline into the right atrium via the right jugular vein was analysed by a computer (Cardiomax II, Columbus Instruments Inc).

The incorporation of ^{14}C-adenine into the adenine nucleotides was measured 24 h after the intravenous infusion was begun, as already described.[12] The rats were anaesthetised with ether and then received an intravenous injection (via the tail vein) of 10 $\mu mol \cdot litre^{-1}$ adenine containing 5 μCi of ^{14}C-adenine (235 $mCi \cdot mmol^{-1}$, Amersham Int) in the prazosin experiments, and 20 μCi in the verapamil and metoprolol experiments. Exactly 15 min later, heart,

TABLE 1 *^{14}C-adenine incorporation into the adenine nucleotides (adenine nucleotide synthesis) of kidney, liver, lungs, heart and skeletal muscle after a 24 h intravenous infusion of NaCl 0.9% (control), prazosin (100 $\mu g \cdot kg^{-1} \cdot h^{-1}$), ribose (200 $mg \cdot kg^{-1} \cdot h^{-1}$) and the combination of prazosin and ribose in the same concentrations. Each animal received an intravenous injection of 10 $\mu mol \cdot litre^{-1}$ of adenine containing 5 μCi of ^{14}C-adenine. Data expressed as $DPM \cdot g^{-1}$ tissue (see methods) are given as means(SEM).*

	Kidney	*Liver*	*Lungs*	*Heart*	*Skeletal muscle*
Control	131 413(8817)	76 117(9465)	67 445(4103)	18 016(888)	3343(328)
n	6	5	6	6	6
Prazosin	143 281(13 335)	102 360(9805)	62 275(5767)	21 146(2234)	3972(326)
n	7	7	6	7	5
Ribose	87 829(4407)*	44 210(7433)‡	46 577(2383)*	114 372(12198)*	25 277(2204)*
n	8	8	8	7	8
Prazosin + ribose	79 116(6691)*	41 694(6014)§	47 053(4378)†	99 656(8863)*	28 828(1071)*
n	7	6	7	7	7

*p=0.0005, †p=0.005, ‡p=0.0125, §p=0.01 *v* control.

TABLE 2 *Haemodynamic variables after prazosin, ribose and prazosin plus ribose treatment. NaCl 0.9% (control), prazosin 100 $\mu g \cdot kg^{-1} \cdot h^{-1}$, ribose 200 $mg \cdot kg^{-1} \cdot h^{-1}$, or the combined treatment of prazosin and ribose in the same concentration, were given intravenously for 24 h. Data are means(SEM).*

	LVSP (mm Hg)	*$LVdP/dt_{max}$ ($mm\ Hg \cdot s^{-1}$)*	*MAP (mm Hg)*	*COI ($ml \cdot min^{-1} \cdot kg^{-1}$)*	*TPR ($Pa \cdot l^{-1} \cdot min^{-1}$)*	*HR ($beats \cdot min^{-1}$)*
Control	139(4)	9200(497)	121(4)	316(10)	0.384(0.023)	352(12)
n	14	14	14	11	11	14
Prazosin	112(2)*	10 651(6639)	102(2)†	438(18)*	0.230(0.01)*	375(19)
n	7	7	7	6	6	7
Ribose	130(4)	8503(531)	109(5)	295(12)	0.380(0.029)	357(13)
n	7	7	7	6	6	7
Prazosin + ribose	112(8)*	9234(345)	97(3)*	394(21)†	0.252(0.018)*	394(21)
n	7	7	7	7	7	7

LVSP = Left ventricular (LV) systolic pressure; $LVdP/dt_{max}$ = the maximum rate of rise of the LV pressure; MAP = mean aortic pressure; HR = heart rate; COI = cardiac output index; TPR = total peripheral resistance (MAP/COI).
*p=0.0005; †p=0.0025 *v* control.

liver, kidney, skeletal muscle and the lungs were quickly removed and frozen in liquid nitrogen. Adenine nucleotides were then extracted, and their specific radioactivity was determined.[12] The radioactivity of tissue extract served as a measure for the uptake of ^{14}C-adenine into the respective tissues. The total radioactivity of adenine nucleotides per gram tissue (DPM·g^{-1}) was taken as a measure of adenine nucleotide synthesis and an indirect measure of the available pool of PRPP. The ratio of adenine nucleotide radioactivity/tissue radioactivity represented the utilisation of adenine for adenine nucleotide synthesis.

All data are presented as means (SEM). Statistical evaluation was performed using analysis of variance and unpaired *t* test.

Results

The metabolic effect of ribose was studied by measuring the incorporation of ^{14}C-adenine into kidney, liver, lungs, heart and skeletal muscle. The results presented in fig 1 show that, in control conditions, the uptake of ^{14}C-adenine into the heart and skeletal muscle, as assessed by the radioactivity present in tissue extract, was very low compared with the three other organs studied. In these conditions, ^{14}C-adenine was mainly taken up by the kidney (50% of the extract radioactivity of the five organs) and to a lesser extent by the liver and the lungs. Most of the radioactivity of the heart tissue extract was present in the adenine nucleotides (70%). In contrast, in the skeletal muscle only 20% of the radioactivity of the extract could be accounted for by the radioactivity of adenine nucleotides which served as an indirect measure of the PRPP availability. Thus the utilisable PRPP pool was very low in heart and skeletal muscle.[12]

In the presence of ribose, the uptake of ^{14}C-adenine was markedly increased in heart and skeletal muscle. Most of the radioactivity was recovered in adenine nucleotides (97% in heart and 88% in skeletal muscle). This shows that the synthesis of adenine nucleotides from adenine and therefore the PRPP availability are markedly increased by ribose. The uptake of ^{14}C-adenine into kidney, liver and lungs was lowered under the influence of ribose, but the percentage of radioactivity present in adenine nucleotides was unchanged.

To study the feasibility of combining prazosin with ribose, we first investigated the effect of prazosin on the metabolic changes induced by ribose (table 1). Prazosin had no effect on adenine nucleotide synthesis from ^{14}C-adenine in the different organs and did not modify the ribose induced increase in ^{14}C-adenine incorporation into the adenine nucleotides of heart and skeletal muscle and the decrease in kidney, liver and lungs.

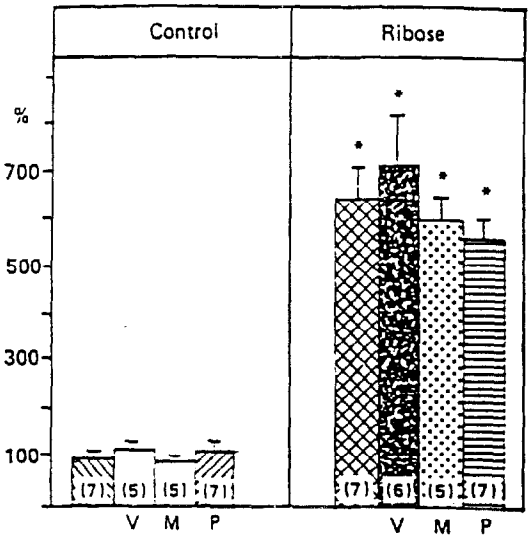

FIG 2 *^{14}C-adenine incorporation into the myocardial adenine nucleotides of rats after continuous intravenous infusion for 24 h with 0.9% NaCl (control) or ribose, 200 mg·kg^{-1}·h^{-1}, each one alone or in combination with verapamil, 2 mg·kg^{-1}·h^{-1} (V), metoprolol, 2 mg·kg^{-1}·h^{-1} (M) or prazosin, 100 µg·kg·h^{-1} (P). After the 24 h infusion period, each rat received an intravenous injection of 10 µmol·litre^{-1} adenine containing 20 µCi ^{14}C-adenine in the verapamil and metoprolol experiments, and with 5 µCi of ^{14}C-adenine in the prazosin experiments. Results are expressed as percentage of the radioactivity present in adenine nucleotides of control. Columns are means, bars = SEM. Number of experiments in parentheses. *p=0.0005 v control.*

Further experiments were performed to study the haemodynamic effects of prazosin. Table 2 shows that the intravenous infusion of prazosin elicited a decrease in LVSP (−20%), in mean aortic pressure (−16%) and peripheral resistance (−40%). Cardiac output index was markedly increased (+40%). Heart rate and $LVdP/dt_{max}$ were not significantly changed as compared to controls. Ribose alone had no effect on haemodynamic variables and the combination of ribose with prazosin did not change the prazosin induced haemodynamic effects.

In the final series of experiments, the effects of the combination of ribose with prazosin, verapamil and metoprolol were compared. Figure 2 shows that verapamil, metoprolol and prazosin had no metabolic effect: the incorporation of ^{14}C-adenine into myocardial adenine nucleotides was no different from the control value; and the stimulation of ^{14}C-adenine incorporation into adenine nucleotides elicited by

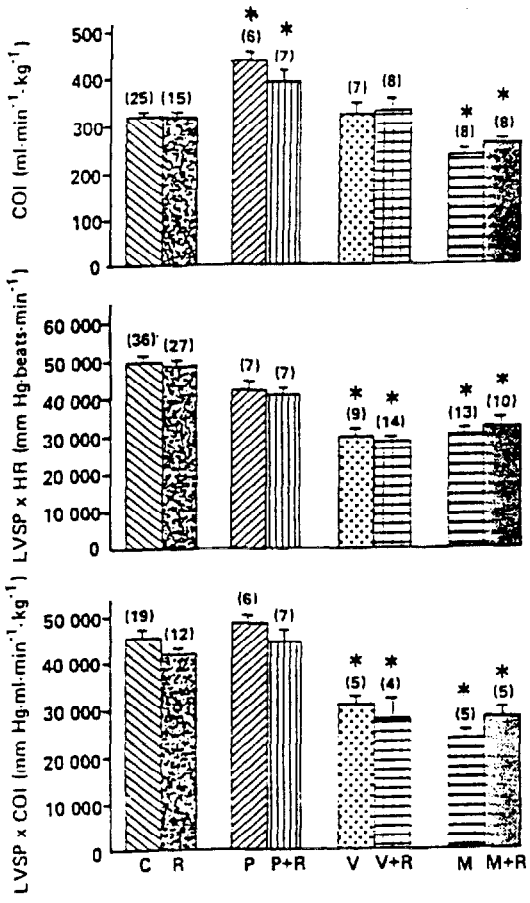

FIG 3 *Cardiac output index (COI), pressure-rate product of the left ventricle (LVSP.HR) and left heart external work (LVSP.COI) of rats infused for 24 h with 0.9% NaCl (C), ribose 200 mg·kg⁻¹·h⁻¹ (R), prazosin 100 μg·kg⁻¹·h⁻¹ (P), verapamil 2 mg·kg⁻¹·h⁻¹ (V), metoprolol 2 mg·kg⁻¹·h⁻¹ (M) or with the combination of ribose with prazosin (P+R), verapamil (V+R), and metoprolol (M+R) in the same concentrations. Columns are means, bars = SEM. Number of experiments in parentheses.*
**p=0.0005 v control.*

ribose was not changed when verapamil, metoprolol or prazosin were added to ribose treatment.

To assess the functional effects of prazosin, verapamil and metoprolol, three haemodynamic variables were selected (fig 3): cardiac output index, the pressure-rate product of the left ventricle (which gives an estimation of oxygen consumption), and the cardiac output-pressure product of the left ventricle (which can be considered as an index of ventricular work). Cardiac output index, measured in rats treated with prazosin, was increased (+36%). It was not significantly different from the control value in rats treated with verapamil, and it was depressed in rats treated with metoprolol (−30%). Neither the pressure-rate product of the left ventricle nor the cardiac output-pressure product were significantly changed by prazosin, while verapamil and metoprolol both reduced these products to nearly the same extent. The co-administration of ribose with prazosin, verapamil or metoprolol did not affect the haemodynamic alterations brought about by these drugs.

Discussion

In heart and skeletal muscle, the low capacity of the oxidative pentose phosphate pathway,[16] as estimated by the activity of the first and regulating enzyme glucose-6-phosphate dehydrogenase,[17] leads to a low production of ribose-5-phosphate, a precursor of PRPP synthesis. As a result, the available pool of PRPP is low in these organs, limiting the de novo synthesis of adenine nucleotides and the salvage utilisation of purine bases. Ribose bypasses this oxidative pentose phosphate pathway and increases the available PRPP pool in heart and skeletal muscle.[12] A proportional increase in de novo synthesis of adenine nucleotides and in the salvage utilisation of adenine has been shown.[18]

In the experiments described here, we have studied the effect of ribose by measuring the salvage utilisation of exogenous radioactive adenine. Within the cell, this base can react with PRPP to give AMP. Further phosphorylations lead to ADP and ATP. Thus the radioactivity present in all adenine nucleotides is an estimation of the salvage utilisation of adenine. Furthermore, since the first step of this pathway is limited by PRPP availability, and provided that adenine concentration is not limiting, this value gives an estimation of the intracellular availability of PRPP.

Our results confirm that ribose increases PRPP availability in heart and skeletal muscle. As a result of the increased uptake of adenine by these two muscular organs, the amount of adenine taken up by kidney, liver and lungs is decreased.

From these results, we can conclude that ribose may have two beneficial effects for the heart under pathological conditions: (1) by increasing myocardial PRPP availability, ribose may stimulate the synthesis of adenine nucleotides, either de novo or by the salvage pathway; (2) in hypoxic or post-ischaemic conditions, most of the degradation products of adenine nucleotides may be reutilised by the heart and incorporated into the other organs to a lesser extent.

The vasodilator effect of the α_1 receptor blockade is well known and this is confirmed in our in vivo experiments: peripheral resistance is decreased and cardiac output is increased. These receptors have also been identified in the rat heart, but their

pathophysiological role in this tissue is still unclear.[19] It has been shown that their stimulation increases the inotropic state of the heart[20] and can provoke a hypertrophic response in isolated cardiac myocytes.[21 22] Prazosin is a competitive α_1 adrenergic antagonist with a very high affinity for the heart.[23] Nevertheless, although LVSP is significantly reduced by prazosin, in our in vivo experiments this drug had no significant effect on the pressure-rate product of the left ventricle, which has been shown to be correlated with myocardial oxygen consumption.[24 25] Likewise it had no effect on cardiac contractility (estimated by $LVdP/dt_{max}$) another determinant of myocardial oxygen consumption,[26] or on the external work peformed by the left ventricle, estimated by the product of LVSP and cardiac output.

The two other drugs studied, verapamil and metoprolol, have quite different effects, since they act mainly on the myocardium. Both diminish myocardial oxygen consumption and left ventricular external work. These effects are accompanied by a decrease in cardiac output in the case of metoprolol, while cardiac output is unchanged in the case of verapamil, which also has a vasodilator effect.

From these results it is evident that prazosin may have beneficial effects in the treatment of heart failure, since cardiac output increases though the pressure-volume work of the left heart is unchanged. One can assume that this improvement in cardiac output will not increase the energy requirement of the heart. On the other hand, increasing the oxygen delivery to the heart (which may be limited in cases of severely depressed blood flow) may have beneficial effects on myocardial ATP content.

Verapamil and metoprolol are used clinically in the treatment of patients with cardiac disorders. In particular, myocardial ischaemia may benefit from these drugs since they decrease the oxygen consumption and the external work of the left heart. In the case of calcium antagonists,[27 28] it has already been shown that the cardiac ATP content is preserved in several experimental conditions.

It is interesting to note that prazosin and verapamil have some haemodynamic features in common that distinguish them from the β receptor blocker metoprolol. Both prazosin (table 2) and verapamil[4] reduce total peripheral resistance. As a result, cardiac output is enhanced with prazosin (table 2) and maintained at a constant level with verapamil, which depresses heart rate.[4] The prazosin induced increase in cardiac output occurs with no alteration in oxygen consumption (pressure-rate product) and in the external heart work (pressure-volume product). In the case of verapamil, cardiac output is kept normal despite a reduction in oxygen consumption and in pressure-volume work. In contrast, under the influence of metoprolol, cardiac output is depressed and this reduction is paralleled by a decrease in oxygen consumption and pressure-volume work (fig 3). Thus heart performance may be more efficient with prazosin and verapamil. These different drug induced haemodynamic alterations are not influenced at all by ribose (fig 3).

In summary, our study shows that: (1) the increase in PRPP availability brought about by ribose is not altered when prazosin, verapamil or metoprolol are added to the treatment; (2) the haemodynamic effects of prazosin, verapamil or metoprolol are unchanged in the presence of ribose, which has no haemodynamic effect on its own. We can therefore conclude that the co-administration of ribose with prazosin, verapamil or metoprolol is feasible. Under pathological conditions, and in addition to their haemodynamic effects, these treatments are likely to maintain the myocardial ATP level at or near the normal value by increasing the rate of adenine nucleotide synthesis and at the same time decreasing ATP utilisation.

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References

1 Nayler WG, Ferrari R, Williams A. Protective effect of pretreatment with verapamil, nifedipine and propranolol on mitochondrial function in the ischemic and reperfused myocardium. *Am J Cardiol* 1980;46:242–8.
2 Fleckenstein A. History of calcium antagonists. *Circ Res* 1983:52(suppl I):3–16.
3 Przyklenk K, Kloner RA. Effect of verapamil on postischemic "stunned" myocardium: importance of the timing of treatment. *J Am Coll Cardiol* 1988;11:614–23.
4 Zimmer H-G, Zierhut W, Marschner G. Combination of ribose with calcium antagonist and beta-blocker treatment in closed-chest rats. *J Mol Cell Cardiol* 1987;19:635–9.
5 Van Amsterdam FTM, Haas M, Zaagsma J. Dynamic and kinetic differences of the vascular and myocardial effects of calcium antagonists in the rat heart. *J Cardiovasc Pharmacol* 1987;9:570–7.
6 Clozel J-P. Haemodynamic effects of tiapamil and verapamil in conscious rats following chronic myocardial infarction. *Cardiology* 1988:75:161–9.
7 Wirtzfeld A, Klein G. Beta-receptor antagonists in acute myocardial infarction. In: Kuemmerle HP, ed. *Progress in clinical pharmacology: The effects of beta-receptor antagonists on the myocardium*. Munich-Diesenhofen: Dustri-Verlag Dr Karl Feistle, 1982:64–76.
8 Van Zwieten PA. Antihypertensive drugs interacting with α and beta adrenoreceptors. A review of basic pharmacology. *Drugs* 1988:35(suppl 6):6–19.
9 Wilber DJ, Lynch JJ, Montgomery DG, Lucchesi BR. α-Adrenergic influences in canine ischemic sudden death: effects of α_1-adrenoceptor blockade with prazosin. *J Cardiovasc Pharmacol* 1987;10:96–106.
10 Colucci WS, Wynne J, Holman BL, Braunwald E. Long-term therapy of heart failure with prazosin: a

randomized double blind trial. *Am J Cardiol* 1980; 45:337–44.
11 Takeo S, Tanonaka K, Tazuma Y, Fukao N, Yoshikawa C, Fukumoto T, Tanaka T. Diltiazem and verapamil reduce the loss of adenine nucleotide metabolites from hypoxic hearts. *J Mol Cell Cardiol* 1988;20:443–56.
12 Zimmer H-G, Gerlach E. Stimulation of myocardial adenine nucleotide biosynthesis by pentoses and pentitols. *Pflugers Arch* 1978;376:223–7.
13 Zimmer H-G, Ibel H, Steinkopff G, Korb G. Reduction of the isoproterenol-induced alterations in cardiac adenine nucleotides and morphology by ribose. *Science* 1980; 207:319–21.
14 Zimmer H-G. Normalization of depressed heart function in rats by ribose. *Science* 1983;220:81–2.
15 Zimmer H-G. Measurement of left ventricular hemodynamic parameters in closed-chest rats under control and various pathophysiologic conditions. *Basic Res Cardiol* 1983; 78:77–84.
16 Glock GE, McLean P. Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in mammalian tissues and tumors. *Biochem J* 1954;56:171–5.
17 Eggleston LV, Krebs HA. Regulation of the pentose phosphate cycle. *Biochem J* 1974;138:425–35.
18 Zimmer H-G. Acceleration of adenine nucleotide biosynthesis after ischemic insult. In: De Jong JW, ed. *Myocardial energy metabolism*. Amsterdam: Martinus Nijhoff, 1988:105–14.
19 Brückner R, Mügge A, Scholz H. Existence and functional role of alpha$_1$-adrenoceptors in the mammalian heart. *J Mol Cell Cardiol* 1985;17:639–45.
20 Otani H, Otani H, Das DK. α_1-Adrenoceptor-mediated phosphoinositide breakdown and inotropic response in rat left ventricular papillary muscles. *Circ Res* 1988;62:8–17.
21 Simpson P, McGrath A. Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha$_1$-adrenergic response. *J Clin Invest* 1983;72:732–8.
22 Simpson P. Stimulation of hypertrophy of cultured neonatal rat heart cells through an α_1-adrenergic receptor and induction of beating through an α_1 and beta$_1$-adrenergic receptor interaction. *Circ Res* 1985;56:884–94.
23 Skomedal T, Osnes J-B, Oye I. Competitive blockade of α-adrenergic receptors in rat heart by prazosin. *Acta Pharmacol Toxicol* 1980;47:217–22.
24 Feinberg H, Katz LN, Boyd E. Determinants of coronary flow and myocardial oxygen consumption. *Am J Physiol* 1962;202:45–52.
25 Neill WA, Levine HJ, Wagman RJ, Gorlin R. Left ventricular oxgyen utilization in intact dogs: effect of systemic hemodynamic factors. *Circ Res* 1963;12:163–9.
26 Braunwald E. Control of myocardial oxygen consumption. Physiologic and clinical considerations. *Am J Cardiol* 1971;27:416–32.
27 De Jong JW, Harmsen E, De Tombe PP, Keijzer E. Nifedipine reduces adenine nucleotide breakdown in ischemic rat heart. *Eur J Pharmacol* 1982;81:89–96.
28 De Jong JW. Timely administration of nisoldipine essential for prevention of myocardial ATP catabolism. *Eur J Pharmacol* 1985;118:53–9.

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RAPID COMMUNICATION

Combination of Ribose with Calcium Antagonist and β-Blocker Treatment in Closed-chest Rats*

(*Received 14 August 1986, accepted in revised form 16 March 1987*)

In previous studies ribose has been recognized as a substrate that has beneficial effects on myocardial metabolism. It leads to an elevation of the available 5-phosphoribosyl-1-pyrophosphate pool and stimulates adenine nucleotide *de novo* synthesis in the rat heart under control and various pathophysiological conditions (Zimmer and Gerlach, 1978; Zimmer and Ibel, 1983, 1984). When the clinical application of ribose in patients with heart disease is envisaged, it is understood that conventional cardiac therapy must be continued. It is therefore necessary to demonstrate in animal experiments that ribose retains its stimulating metabolic effect when administered in conjunction with therapeutically used drugs. In this study we have selected representatives of two pharmacological principles, the calcium antagonist verapamil and the β_1-specific adrenoceptor blocker metoprolol. Measurements of functional parameters in closed-chest rats revealed that i.v. administration of ribose alone for 24 h (200 mg/kg/h) had no hemodynamic or vasoactive influence, whereas verapamil and metoprolol (i.v. infusion of 2 mg/kg/h each for 24 h) induced negative chronotropic and negative inotropic effects. Cardiac output was reduced by metoprolol, but not by verapamil. Ribose did not affect these drug-induced hemodynamic alterations, and verapamil as well as metoprolol did not interfere with the characteristic metabolic effect of ribose, the stimulation of cardiac adenine nucleotide *de novo* synthesis. Administration of ribose in combination with these pharmacological agents is therefore compatible.

All experiments were done on female Sprague–Dawley rats (220 to 240 g body weight) fed Altromin® pellets with free access to tap water. D(-)Ribose was purchased from Sigma Chemie München. Verapamil was a gift from Knoll AG, Ludwigshafen. Metoprolol-tartrate was provided by Astra Chemicals, Wedel. After 24 h of continuous i.v. infusion of ribose and the drugs in the conscious, unrestrained animals, left ventricular function was measured with the ultraminiature catheter pressure transducer (model PR–249, Millar Instruments, Inc., Houston, TX, USA). The catheter was attached to a Millar transducer control unit (model TC–100) which was connected to a HSE electromanometer (Hugo Sachs Elektronik, March-Hugstetten). The maximal rate of rise in left ventricular pressure (LV dP/dt_{max}) was obtained with an electronic differentiation system (Physio–Differentiator, Hugo Sachs Elektronik). The recording system was a Gould Brush 2600 recorder. Calibration of pressure and of dP/dt_{max} was done using a mercury manometer (type 367, Hugo Sachs Elektronik) and the calibration device that is

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* Dedicated to Professor Dr E. Gerlach on his 60th birthday.

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TABLE 1. Heart rate, left ventricular systolic pressure (LVSP), maximal rate of rise of left ventricular pressure (LV dP/dt_{max}), and the rates of myocardial adenine nucleotide (AN) *de novo* synthesis in rats that had received continuous i.v. infusion of 0.9% NaCl (controls), D(-)-ribose (200 mg/kg/h), verapamil (2 mg/kg/h), metoprolol (2 mg/kg/h), and the combination with ribose (200 mg/kg/h) for 24 h.

	Heart rate (beats/min)	LVSP (mmHg)	LV dP/dt_{max} (mmHg/s)	AN de novo synthesis (nmols/g/h)
Controls	363 ± 7 (27)	138 ± 3 (22)	9533 ± 308 (22)	8.0 ± 1.0 (11)
Ribose	361 ± 7 (23)	136 ± 3 (20)	9440 ± 368 (20)	35.4 ± 2.6 (9)[a]
Verapamil	287 ± 10 (9)[a]	103 ± 4 (9)[a]	6516 ± 560 (9)[a]	10.4 ± 0.9 (8)
Verapamil + Ribose	284 ± 9 (18)[a]	101 ± 2 (14)[a]	6986 ± 439 (13)[a]	33.0 ± 4.2 (11)[a]
Metoprolol	270 ± 8 (16)[a]	116 ± 5 (13)[ab]	5726 ± 488 (13)[a]	6.9 ± 1.2 (4)
Metoprolol + Ribose	265 ± 8 (15)[a]	120 ± 4 (12)[ac]	6130 ± 229 (12)[a]	39.2 ± 4.2 (5)[a]

[a] $P < 0.0005$ vs. controls; [b] $P < 0.05$, [c] $P < 0.0025$ vs. Verapamil + Ribose, respectively.
Student's *t*-test for unpaired data.
The functional parameters were measured after catheterization of the left ventricle with the Millar ultraminiature catheter pressure transducer (model PR-249). Data are mean values ± S.E.M.; number of experiments in parentheses.

built into the Physio-Differentiator. The ultraminiature catheter pressure transducer was inserted into the right carotid artery and quickly advanced into the left ventricle in the closed-chest rats anesthetized with thiobutabarbital sodium (Inactin® Byk, 80 mg/kg, i.p.). The depth of anesthesia was tested by eliciting reflexes in the legs. When the measurements of left ventricular function had been completed, the catheter was withdrawn from the left heart and positioned in the aorta so that blood pressure was obtained.

In another series of experiments, cardiac output index was measured using the thermodilution technique. To do this, the ultraminiature catheter pressure transducer was replaced by a thermodilution microprobe (1.5 French, Columbus Instruments, Inc., Columbus, OH, USA) which was positioned in the ascending aorta. A bolus of 100 μl saline (18°C) was injected into the right atrium via the right jugular vein. From the recorded temperature change, cardiac output was determined using a computer (Cardiomax II, Columbus Instruments, Inc.). Stroke volume index was calculated by dividing cardiac output index by heart rate, and total peripheral resistance index was calculated by dividing mean blood pressure by cardiac output index.

Rates of myocardial adenine nucleotide *de novo* synthesis (nmols/g/h) were determined in a separate group of animals by relating the radioactivity of cardiac adenine nucleotides due to the incorporation of [1-^{14}C]-glycine (specific activity: 54 mCi/mmol, Amersham International, 250 μCi/kg body weight) after an *in vivo*-exposure time of 60 min to the mean specific activity of the tissue glycine precursor pool as described previously (Zimmer *et al.* 1973).

In Table 1, the effects of ribose, of verapamil as well as of metoprolol alone and in combination with ribose on functional parameters and on adenine nucleotide *de novo* synthesis are shown. Continuous i.v. infusion of ribose (200 mg/kg/h) for 24 h had no effect at all on heart rate, LVSP and LV dP/dt_{max}. In these experiments LV dP/dt_{max} was higher than in previous studies (Zimmer, 1983; Zimmer and Ibel, 1983). This is most likely due to the younger age of the rats and to the better controlled anesthesia. In contrast, verapamil and metoprolol depressed all hemodynamic parameters significantly, the effect on LVSP being more pronounced with verapamil. Ribose had no influence on this depression.

It was therefore of interest to examine whether ribose, in the presence of verapamil or metoprolol, may retain its stimulating metabolic effect. Ribose induced an increase in myocardial adenine nucleotide *de novo* synthesis. After the same period of infusion time verapamil as well as metoprolol did not al

ximal rate of rise of left ventricular
ine nucleotide (AN) *de novo* synthe-
% NaCl (controls), D(-)ribose (200
h), and the combination with ribose

V dP/dt_{max} (mmHg/s)	AN de novo synthesis (nmols/g/h)
3 ± 308 (22)	8.0 ± 1.0 (11)
0 ± 368 (20)	35.4 ± 2.6 (9)[a]
6 ± 560 (9)[a]	10.4 ± 0.9 (8)
16 ± 439 (13)[a]	33.0 ± 4.2 (11)[a]
26 ± 488 (13)[a]	6.9 ± 1.2 (4)
30 ± 229 (12)[a]	39.2 ± 4.2 (5)[a]

se, respectively.

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.; number of experiments in parentheses.

TABLE 2. Hemodynamic parameters in rats after 24 h of continuous i.v. infusion of 0.9% NaCl (controls), D-ribose (200 mg/kg/h), verapamil (2 mg/kg/h), metoprolol (2 mg/kg/h), and the combination with ribose (200 mg/kg/h).

	n	Cardiac output index (ml/kg/min)	Stroke volume index (ml/kg)	Total peripheral resistance index (mmHg · kg · min/ml)
Controls	13	325 ± 13	0.907 ± 0.048	0.410 ± 0.022
Ribose	9	334 ± 14	0.912 ± 0.042	0.371 ± 0.035
Verapamil	7	321 ± 27	1.120 ± 0.104[a]	0.280 ± 0.030[b]
Verapamil + Ribose	8	327 ± 28	1.134 ± 0.098[a]	0.271 ± 0.038[b]
Metoprolol	8	233 ± 14[c]	0.861 ± 0.050	0.377 ± 0.027
Metoprolol + Ribose	8	255 ± 11[b]	0.967 ± 0.060	0.372 ± 0.034

$P < 0.025$; [b] $P < 0.0025$; [c] $P < 0.0005$ vs. controls as determined with the Student's t-test for unpaired data.
Cardiac output index was measured with the thermodilution technique. The stroke volume index and the total peripheral resistance index were calculated.
Data are mean values ± S.E.M.
n = number of experiments.

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well as metoprolol did not alter appreciably this biosynthetic process. When verapamil or metoprolol were administered in combination with ribose, myocardial adenine nucleotide *de novo* synthesis was increased to about the same extent as with ribose alone. Essentially similar results were obtained with respect to the available pool of 5-phosphoribosyl-1-pyrophosphate, which was increased by ribose. Again, this elevation was not affected by verapamil or metoprolol (data not shown).

While verapamil and metoprolol had similar effects on heart rate and left ventricular functional parameters (Table 1), there was a marked difference as to the effect on cardiac output (Table 2). Cardiac output index was maintained after 24 h of continuous i.v. infusion of verapamil due to the increase in stroke volume index. Total peripheral resistance index was significantly reduced. Ribose had no influence on these changes. On the other hand, cardiac output index was markedly depressed by metoprolol, and total peripheral resistance index was unchanged irrespective of whether ribose was co-infused or not. Thus, ribose does not affect the hemodynamic changes induced by verapamil and metoprolol, and these drugs do not interfere with the marked metabolic stimulation elicited by ribose.

The hemodynamic effects of metoprolol were clearly dose-dependent. When it was administered as continuous i.v. infusion in a dose of 1 mg/kg/h over 24 h, heart rate was reduced to 282 ± 9 beats/min, LV dP/dt_{max} was depressed to 7643 ± 928 mmHg/s, and LVSP was not affected ($n = 5$). Again, ribose had no influence on these alterations. The metabolic effect of ribose, however, was independent of the dose of and the exposure to the drugs. For instance, the rate of cardiac adenine nucleotide *de novo* synthesis was increased to 36.5 ± 4.8 nmols/g/h ($n = 6$), when ribose was administered for 5 h in combination with verapamil in a dose of 1 mg/kg/h and to 37.0 ± 3.1 nmols/g/h ($n = 4$), when it was given for 5 h in conjunction with verapamil in a dose of 2 mg/kg/h.

The ribose-induced stimulation of cardiac adenine nucleotide *de novo* synthesis is also maintained when ribose is administered in combination with catecholamines. It has been shown in previous studies that ribose even exaggerated the stimulating effect of isoproterenol (Zimmer and Ibel, 1983). This is due to the fact that the ATP content is reduced by isoproterenol so that release of feedback inhibition of 5-phosphoribosyl-1-pyrophosphate amidotransferase, the first enzyme of the purine biosynthesis pathway, is brought about, thus resulting in a further stimulation of adenine nucleotide synthesis with ribose. As a consequence of this, the isoproterenol-induced decline of the cardiac ATP level is

prevented by ribose (Zimmer and Gerlach, 1978; Zimmer and Ibel, 1983). It is interesting to note in the present study that even after 24 h of continuous application, the stimulating effect of ribose on adenine nucleotide *de novo* synthesis can be demonstrated both in the heart with normal and reduced contractility. It can be expected that the myocardial ATP content will rise with continuous ribose application, although adenine nucleotide *de novo* synthesis is tightly regulated. An increase in adenine nucleotide synthesis can be regarded to be beneficial, since prevention of the cardiac ATP decline with ribose was associated with normalization of depressed heart function (Zimmer, 1983).

Another interesting feature of this study is the fact that long-term application of metoprolol did not affect appreciably adenine nucleotide *de novo* synthesis and that it did not impair the ribose-elicited stimulation. These findings indicate that endogenous catecholamines are not involved in the maintenance of this biosynthetic process under control conditions and in the ribose-induced enhancement.

A final aspect of our results is that ribose appears to fulfill the essential criteria that should be met by a good cardioprotective substrate. (i) The mechanism of action on cardiac metabolism must be known; (ii) the effects should be specific for the heart; (iii) there must be a marked and long-term beneficial effect on myocardial metabolism in several pathophysiological situations, and this must outweigh the risk of adverse side effects; (iv) it should be effective in man; (v) it must not have hemodynamic or vasoactive properties; (vi) combination with conventional cardiac therapy must be possible. We have demonstrated in the present *in vivo* experiments that ribose meets the last two of these specifications.

As to the other criteria, it has been shown previously (i) that the mechanism of action of ribose is based on the low capacity of the oxidative pentose phosphate pathway, as evaluated by the activity of the first and regulating enzyme glucose-6-phosphate dehydrogenase (Eggleston and Krebs, 1974) in heart and skeletal muscle (Glock and McLean, 1954). Thus, ribose-5-phosphate, an end-product of this pathway, becomes limiting for the production of 5-phosphoribosyl-1-pyrophosphate, an essential precursor substrate fo both the synthesis of purine and pyrimidin nucleotides. Ribose bypasses the critical ste and stimulates the distal segment of the oxid tive pentose phosphate pathway leading to a expansion of the available 5-phosphoribosy 1-pyrophosphate pool. The increased avai able 5-phosphoribosyl-1-pyrophosphate ca then be utilized for the salvage of purine bas when present in a pathophysiological sit ation or when supplied exogenousl (Schneider *et al.* 1985) as well as for the *de no* synthesis of purine and pyrimidine nucle tides. This has been demonstrated both in is lated mature rat cardiac myocytes (Dow *et* 1985) and in the intact heart *in vivo* (Zimm and Gerlach, 1978). (ii) Because of the lo activity of glucose-6-phosphate dehydrog nase in muscular organs (Glock and McLea 1954), these effects are confined to heart a skeletal muscle. (iii) Ribose stimulat adenine nucleotide *de novo* synthesis in ma pathophysiological conditions over a lo period of time to such an extent that the A decline was prevented or greatly attenuat For instance, during recovery from reversi regional ischemia, the ribose-mediated res ration of the cardiac ATP pool was compl already after 12 h of reperfusion (Zimmer a Ibel, 1984), whereas about 72 or more ho were needed without any intervention for almost complete recovery (Reimer *et* 1981). Moreover, the depression of glo heart function induced by pressure overl and isoproterenol administration was p vented by ribose (Zimmer, 1983). (vi) T ribose may be effective in the human hea suggested by the fact that the activity glucose-6-phosphate dehydrogenase is of same order of magnitude in the human a the rat and guinea-pig heart in which beneficial effects have been demonstr (Zimmer *et al.*, 1984). Recently it has b shown in patients with myoadenylate de nase deficiency in skeletal muscle that ri prevents the characteristic post-exerti aches, cramps and pain (Patten, Zöllner *et al.*, 1986). In this disorder there disruption of the purine nucleotide (Goodman and Lowenstein, 1977) resulting degradation of adenine nucleo to adenosine (Sabina *et al.* 1984) similar t situation in the heart. Thus, if ribose or

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pentoses and pentitols are effective in human skeletal muscle, one may expect also effects on the human heart.

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KEY WORDS: Ribose; Adenine nucleotides; Metoprolol; Verapamil.

References

Dow, J. W., Nigdikar, S., Bowditch, J. (1985) Adenine nucleotide synthesis *de novo* in mature rat cardiac myocytes. Biochim Biophys Acta **847**, 223–227.

Eggleston, L. V., Krebs, H. A. (1974) Regulation of the pentose phosphate cycle. Biochem J **138**, 425–435.

Glock, G. E., McLean, P. (1954) Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in mammalian tissues and tumours. Biochem J **56**, 171–175.

Goodman, M. N., Lowenstein, J. M. (1977) The purine nucleotide cycle. Studies of ammonia production by skeletal muscle in situ and in perfused preparations. J Biol Chem **252**, 5054–5060.

Patten, B. M. (1982) Beneficial effect of D-ribose in patient with myoadenylate deaminase deficiency. Lancet **1**, 1071.

Reimer, K. A., Hill, M. L., Jennings, R. B. (1981) Prolonged depletion of ATP and of the adenine nucleotide pool due to delayed resynthesis of adenine nucleotides following reversible myocardial ischemic injury in dogs. J Mol Cell Cardiol **13**, 229–239.

Sabina, R. L., Swain, J. L., Olanow, C. W., Bradley, W. G., Fishbein, W. N., DiMauro, S., Holmes, E. W. (1984) Myoadenylate deaminase deficiency. Functional and metabolic abnormalities associated with disruption of the purine nucleotide cycle. J Clin Invest **73**, 720–730.

Schneider, J. R., St. Cyr, J. A., Mahoney, J. R., Bianco, R. W., Ring, W. S., Foker, J. E. (1985) Recovery of ATP and return of function after global ischemia. [Abstract] Circulation **72** [Suppl. III], 1192.

Zimmer, H.-G. (1983) Normalization of depressed heart function in rats by ribose. Science **220**, 81–82.

Zimmer, H.-G., Trendelenburg, C., Kammermeier, H., Gerlach, E. (1973) De novo synthesis of myocardial adenine nucleotides in the rat. Acceleration during recovery from oxygen deficiency. Circ Res **32**, 635–642.

Zimmer, H.-G., Gerlach, E. (1978) Stimulation of myocardial adenine nucleotide biosynthesis by pentoses and pentitols. Pflügers Arch **376**, 223–227.

Zimmer, H.-G., Ibel, H. (1983) Effects of ribose on cardiac metabolism and function in isoproterenol-treated rats. Am J Physiol **245**, H880–H886.

Zimmer, H.-G., Ibel, H. (1984) Ribose accelerates the repletion of the ATP pool during recovery from reversible ischemia of the rat myocardium. J Mol Cell Cardiol **16**, 863–866.

Zimmer, H.-G., Ibel, H., Suchner, U., Schad, H. (1984) Ribose intervention in the cardiac pentose phosphate pathway is not species-specific. Science **223**, 712–714.

Zöllner, N., Reiter, S., Gross, M., Pongratz, D., Reimers, C. D., Gerbitz, K., Paetzke, I., Deufel, T., Hübner, G. (1986) Myoadenylate deaminase deficiency: Successful symptomatic therapy by high dose oral administration of ribose. Klin Wschr **64**, 1281–1290.

FILE COPY #3347

MUNICATIONS

FEBS Lett.

FEBS Lett.

Acta, in press.
est. 45,

27.
1982) Eur.

197, 117-126.
) Biochim.

51) J. Biol.

H. (1984)

.G., Malaisse-
759-764.
786.
1976) Biochim.

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July 31, 1986 Pages 673-678

D-RIBOSE INHIBITS DNA REPAIR SYNTHESIS IN HUMAN LYMPHOCYTES

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Summary. D-ribose is cytotoxic for quiescent human lymphocytes and severely inhibits their PHA-induced proliferation at concentrations (25-50 mM) at which other simple sugars are ineffective. In order to explain these effects, DNA repair synthesis was evaluated in PHA-stimulated human lymphocytes treated with hydroxyurea and irradiated. D-ribose, in contrast to other reducing sugars, did not induce repair synthesis and therefore did not apparently damage DNA in a direct way, although it markedly inhibited gamma ray-induced repair. Taking into account that lymphocytes must rejoin physiologically-formed DNA strand breaks in order to enter the cell cycle, we suggest that D-ribose exerts its cytotoxic activity by interfering with metabolic pathways critical for the repair of DNA breaks. © 1986 Academic Press, Inc.

Introduction. The administration of D-ribose at the onset of mitogen-stimulated human and murine T and B lymphocyte cultures severely inhibits DNA, RNA, and protein synthesis at concentrations (25-50 mM) at which other simple sugars are ineffective (1). D-ribose is also toxic for non-dividing cells, such as unstimulated lymphocytes and semi-confluent fibroblasts, and leads to cell death.

It has recently been reported (2, 3) that some reducing sugars, as D-glucose, at similar concentrations, are able to kill cells by damaging their DNA, thus inducing DNA repair synthesis (unscheduled DNA synthesis, UDS).

In the present paper, we report experiments aimed at ascertaining whether D-ribose, a reducing sugar, exerts its cytotoxic effect in a similar way.

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Materials and Methods. Blood samples were collected aseptically from healthy volunteers. Lymphocytes were prepared as previously described (1).

100 µl of the cell suspension, containing 1x10 cells, was seeded in 96 wells tissue culture microplates and 100 µl of PHA-P (Difco) was added (final concentration 1µl/ml). 25 µl of D-ribose (Calbiochem) or D-arabinose (Sigma) were added to the cultures at the final concentration of 12.5, 25, or 50 mM at the onset or at the 24th, 48th, or 66th hr of culture. The purity of the sugars had been ascertained (1). Plates were incubated for 72 hrs at 37° C in a humidified atmosphere of 5% CO_2 and 95% air. Six hrs before the end of the cultures, each well was pulsed with 0.5 µCi of tritiated thymidine [(^{3}H)-TdR, Amersham, spec. act. 5 Ci/mmol].

UDS was evaluated according to the method of Lavin and Kidson (4), with slight modifications (5). PHA-stimulated lymphocytes were plated in microwells as described above. Eight wells were prepared for each sugar concentration and time of sugar addition. At the 66th hr of culture, 25 µl of hydroxyurea (HU, Sigma, 10 mM final concentration) were added to each well. After 30 min of incubation the microplate was irradiated with 300 Gy in a gamma cell, using a source of ^{60}Co. In each experiment, two control microplates were cultured and processed at the same time. In the first, the cells were treated with HU, but not irradiated, and in the second the cells were neither treated with HU nor irradiated. All the microplates were then pulsed with [^{3}H]-TdR as described above, and incubated at 37° C for 4 hrs.

[^{3}H]-TdR incorporation into DNA was evaluated as previously described (1).

Results

i- Time course of the effect exerted by D-ribose on PHA-induced [^{3}H]-TdR incorporation in human lymphocytes.

[^{3}H]-TdR incorporation in PHA-stimulated lymphocytes, which received 12.5, 25, or 50 mM D-ribose or D-arabinose at the onset or at the 24th, 48th, or 66th hr of culture, is reported in fig. 1.

50 mM D-ribose inhibited [^{3}H]-TdR incorporation, if administered at the onset or at the 24th hr of culture; its effect decreased as the time of contact with cells became shorter. Similar results, even if the inhibition was less pronounced, were obtained using 12.5 and 25 mM D-ribose (data not shown). D-arabinose, an epimer of D-ribose, did not appreciably affect [^{3}H]-TdR incorporation.

ii- Effect of D-ribose on DNA repair synthesis (UDS).

Table 1 shows that a 99% inhibition of DNA synthesis was obtained after addition of 10 mM HU to PHA-stimulated lymphocytes The exposure of lymphocytes to gamma rays gave rise to a marked and significant ($P < 0.01$) [^{3}H]-TdR incorporation, which is

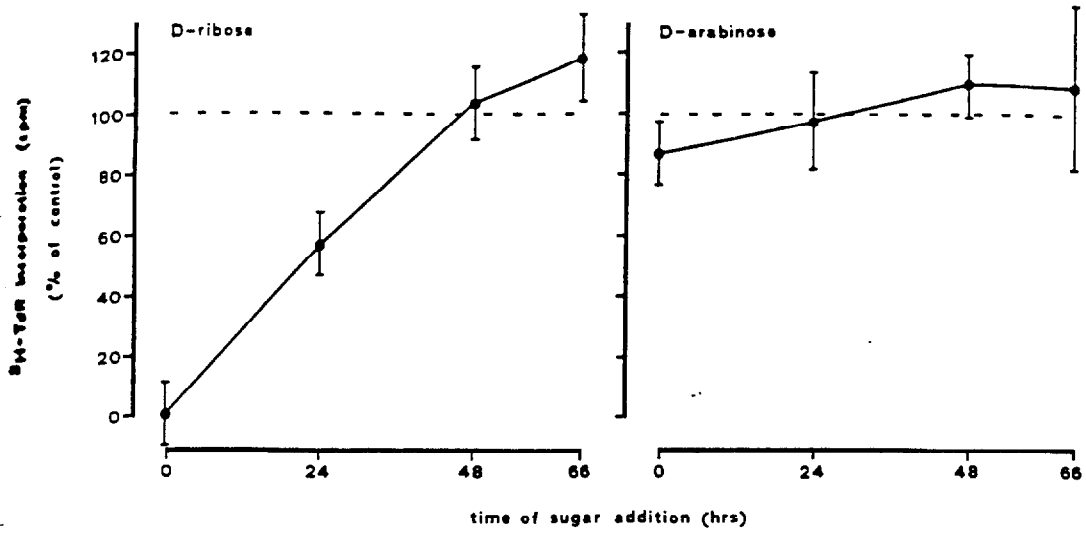

Fig.1. Time-course of the effect exerted by D-ribose and D-arabinose on PHA-induced [^{3}H]-TdR incorporation in human lymphocytes. Sugars were added at different times from the beginning of the cultures. Data are expressed as percentages +/- S.E.M. of control cultures (no sugar added) (42067 c.p.m. +/- 2625). Each point is the mean of at least three independent experiments.

ascribable to UDS. In these experimental conditions, no UDS was observed when D-ribose or D-arabinose was added to HU-treated lymphocytes, in order to test DNA damaging activity, if any.

However, D-ribose was able to interfere with gamma ray-induced UDS. Because of the high toxicity of D-ribose, when administered at the onset of lymphocyte cultures (fig.1), the sugar was

Table 1 - Effect of D-ribose and D-arabinose on DNA repair in PHA-stimulated human lymphocytes

	[^{3}H]-TdR incorporation (c.p.m.)*
lymphocytes	195 +/- 12
lymphocytes + PHA	64,682 +/- 5434
lymphocytes + PHA + HU	695 +/- 29
lymphocytes + PHA + HU + gamma rays	1,334 +/- 41
lymphocytes + PHA + HU + D-ribose	537 +/- 11
lymphocytes + PHA + HU + D-arabinose	521 +/- 23

* Mean of octuplicate samples +/- S.E. For details see 'Materials and Methods'.

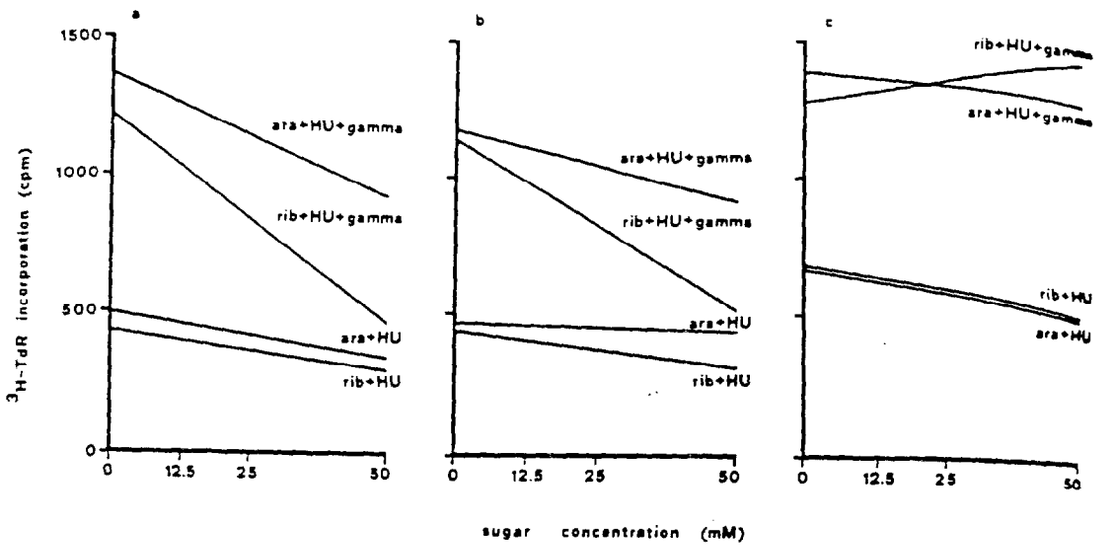

Fig.2. Effect of different concentrations of D-ribose (rib) and D-arabinose (ara) on gamma ray-induced DNA repair synthesis in PHA-stimulated human lymphocytes. Sugars were added at the 24th (a), 48th (b), or 66th (c) hr from the beginning of the cultures. Data are reported as regression lines, calculated on the basis of [^{3}H]-TdR incorporation of at least three independent experiments. For details see 'Materials and Methods'.

addded at the 24th, 48th, or 66th hr of culture. The addition of D-ribose caused a dose-dependent inhibition of [^{3}H]-TdR incorporation, but only when added at the 24th or 48th hr of culture (fig. 2a and 2b). The correlation among the experimental points was highly significant ($P < 0.01$) for both experimental times used, as revealed by Student's t test of regression coefficient. The control sugar D-arabinose had minimal effect and the correlation among the experimental points was not significant ($P > 0.05$).

Discussion. In the present paper we have confirmed and extended previous observations. We have shown that D-ribose severely inhibits lymphocyte division when added at the onset or at the 24th hr of culture, while it is ineffective when added at the 48th hr or later. These data suggest that D-ribose interferes with early events, which are critical for lymphocyte activation (6). The lack of effectiveness of the sugar, when administered at the 66th hr of culture, confirms that it takes several hours to exert its inhibitory effect, which is not due to competition with the entry of [^{3}H]-TdR (1).

Our results show that two reducing sugars, D-ribose and D-arabinose, did not induce UDS, in contrast to D-glucose (3). Therefore, the cytotoxic effect of D-ribose is probably not due to direct DNA damage. However, D-ribose, but not D-arabinose, inhibited gamma ray-induced DNA repair synthesis in a dose-dependent fashion, when administered to PHA-stimulated lymphocytes at the 24th or 48th hr of culture.

The inhibition of DNA repair synthesis by D-ribose may explain both the inhibitory effect exerted by the sugar on mitogen-stimulated lymphocytes and its toxic effect on non-dividing cells. Lymphocyte proliferation and DNA repair are intimately connected. In fact, quiescent lymphocytes, as well as other cells, contain physiologically-formed DNA strand breaks, which seem to be the net steady-state result of a dynamic equilibrium of breaking and ligation (7). Lymphocytes must rejoin these breaks in order to enter the cell cycle (8, 9). The inhibition of DNA repair synthesis by D-ribose may prevent their duplication; moreover, accumulation of DNA breaks due to the presence of D-ribose may be fatal to G_0 -cells.

As far as the mechanism of action of D-ribose is concerned, several targets can be hypothesized. The adenosine diphosphoribosyl transferase (ADPRT) and DNA ligase reactions (10), the intracellular levels of NADP/NAD (11, 12) and the nucleotide pools (13), are possible candidates. These metabolic pathways and reactions are deeply interconnected and related to lymphocyte immunological functions, cell proliferation, cell viability and DNA repair (14). However, a direct inhibition of ADPRT by D-ribose is unlikely, taking into account the time required for the sugar to inhibit DNA repair synthesis (fig. 2c). Work is still in progress to examine the remaining hypotheses.

Acknowledgments. This work has been supported by C.N.R. grant no. 83.2099 and Public Instruction Ministry funds (40%, 1984) to M.A.B. and by C.N.R. grants no. 84.00587.44 and 85.02781 to C.F. within the Special Project "Oncologia". We are grateful to Dr. C.M. Betts for rewieving the manuscript.

References

(1) Marini,M., Zunica,G., and Franceschi,C. (1985) Proc. Soc. Exp.Biol.Med. 180,246-257.
(2) Bucala,R., Model,P., and Cerami,A. (1984) Proc.Natl.Acad.Sci. US 81,105-109.

(3) Lorenzi,M., Montisano,D.F., Toleco,S., and Barrieux,E. (1985) J.Clin.Invest. 77,322-325.
(4) Lavin,M.F., and Kidson,C. (1977) Nucleic Acid Res. 4,4015-4022.
(5) Licastro,F., Franceschi,C., Chiricolo,M., Battelli,M.G., Tabacchi,P., Cenci,M., Barboni,F., and Pallenzona,D. (1982) Carcinogenesis 3,45-48.
(6) Ashman,R.F. (1984) in: Fundamental Immunology.pp.267-300, Raven Press, New York.
(7) Farzaneh,F., Shall,S., and Johnstone,A.P. (1985) FEBS lett. 189,62-66.
(8) Johnstone,A.P., and Williams,G.T. (1982) Nature 300,368-370.
(9) Farzaneh,F., Zalin,R., Brill,D., and Shall,S. (1982) Nature 300,362-366.
(10) Wintersberger,U., and Wintersberger,E. (1985) FEBS lett. 188,189-191.
(11) Berger,N.A., and Sikorski,G.M. (1980) Biochem.Biophys. Res.Commun. 95,67-72.
(12) Seto,S., Carrera,C.J., Kubota,M., Wasson,D.B., and Carson,D. A. (1985) J.Clin.Invest. 75,377-383.
(13) Strauss,P.R., Henderson,J.F., and Goodman,M.G. (1985) Proc.Soc.Exp.Biol.Med. 179,413-418.
(14) Carson,D.A., Seto,S., and Wasson,D.B. (1986) J.Exp.Med. 163,746-751.

FILE COPY # 3292

HYSICAL RESEARCH COMMUNICATIONS

, Watanabe, S., Itoh, N., Shibuya, M., 595.
K., Dawson, T., and Knopf, J. (1990)

, Suzuki, K.(1989) Biochem Biophys.

eil, G., Melnick, D., Fleit, H. B., and 666.
gy 4, 257-264.
R. P., Unkeless, J. C. (1991) Science

.., Schreiber, A. D. and Brugge, J. S.

zie, I. F. C. and Hogarth, P. M. (1988)

eiber, A. D. (1993) J. Immunol. 150,

V. H., and Bonnerot, C. (1992) Nature

A. D. (1993) J. Clin. Invest. 92, 1967-

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D-RIBOSE AND DEOXY-D-RIBOSE INDUCE APOPTOSIS IN HUMAN QUIESCENT PERIPHERAL BLOOD MONONUCLEAR CELLS

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SUMMARY: In previous papers we reported that D(-)-ribose and 2-deoxy-D-ribose, which rank at the top among reducing sugars, kill a variety of human and animal cells and cell lines [1, 2]. Here we demonstrate that these two sugars induce apoptosis in human quiescent peripheral blood mononuclear cells which are relatively insensitive to apoptosis. Apoptosis was assessed by morphological changes, DNA fragmentation by agarose gel electrophoresis and the appearance of an hypodiploid peak by flow cytometry. 2-deoxy-D-ribose was more potent than D(-)-ribose and apoptosis was evident from 48 hours of culture onwards. 2-deoxy-D-ribose-induced apoptosis was inibited by N-acetyl-L-cysteine, suggesting that glutathione metabolism and/or oxidative stress are involved in this type of apoptosis. Thus, D(-)-ribose and 2-deoxy-D-ribose can be useful tools to study the cellular and molecular events of apoptosis in human quiescent lymphocytes. © 1994 Academic Press, Inc.

We previously reported that D(-)-ribose (Rib) and 2-deoxy-D-ribose (dRib) induce cell death in a wide variety of cells (dividing and nondividing, normal and neoplastic, freely floating and substrate adhering, human and murine) at concentrations at which other monosaccharides have little or no effect [1, 2]. In these studies the effect of Rib and dRib was analysed on various cell types by measuring the inhibition of cell proliferation after different mitogenic stimuli. On the basis of these data, we decided to analyze if the death induced by these sugars was of the apoptotic type.

Apoptosis (programmed death) is a peculiar type of cell death that has been described as the opposite of necrosis (accidental death). It can be initiated by a number of external signals like glucocorticoids, irradiation or withdrawal of growth factors [3]. Apoptosis is characterized by several morphological and biochemical events, such as cellular shrinkage, chromatin condensation, DNA fragmentation and loss of apoptotic bodies, in which fragmented chromatin is contained [4, 5].

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Abbreviations used: Rib, D(-)-ribose; dRib, 2-deoxy-D-ribose; Ara, D(-)-arabinose; PBMC, peripheral blood mononuclear cells; PI, propidium iodide; NAC, N-acetyl-L-cisteine.

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Apoptosis in Rib- and dRib-treated human peripheral blood mononuclear cells (PBMC) was assessed by evaluating morphological changes by light microscopy, DNA fragmentation by agarose gel electrophoresis and the appearance of an hypodiploid peak by flow cytometry. The results obtained from these techniques suggest that indeed both sugars, when compared with a control sugar such as D(-)-arabinose (Ara), a D(-)ribose isomer, induce apoptosis in quiescent human PBMC, in which apoptosis is rather difficult to induce.

MATERIALS AND METHODS

Materials. D(-)-Ribose, 2-deoxy-D-ribose, D(-)-arabinose, N-acetyl-L-cysteine (NAC) and propidium iodide (PI) were purchased from Sigma Chemical Co., St. Louis, USA. Lymphoprep was obtained from Nycored Pharma AS, Oslo, Norway. RPMI 1640, Hanks' Balanced Salts (HBSS), L-Glutamine and Penicillin-Streptomycin were from Gibco, Paisley, Scotland, U.K.
Lymphocyte cultures. Peripheral blood from healthy volunteers was collected aseptically; mononuclear cells were separated by centrifugation in Lymphoprep gradient [6], and cultured as previously described [7]. Sugars were added in order to reach a final concentration ranging from 1 mM to 50 mM. Cultures were incubated for 24, 48, 72 and 96 hs in a humidified atmosphere of 5% CO_2 in air at 37°C, with or without sugars. At each time point, cells were harvested and analyzed by light microscopy, flow cytometry and gel electrophoresis.
Light microscopy. Cells were collected using a cytocentrifuge (Shandon, Cheshire, England) at 500 rpm for 5 min and cell morphology was evaluated on May-Grunwald/Giemsa stained preparations by examination in a Axioscope, Zeiss, Germany. Apoptotic cells were identified using previously defined criteria [4].
DNA fragmentation and agarose gel electrophoresis. DNA fragmentation was assessed by DNA agarose gel electrophoresis, performed as previously described [8].
Flow cytometry. Sugar-induced apoptosis was detected by reduced fluorescence of the PI - a DNA binding dye - in the apoptotic nuclei, as previously described [9].
Statistical analysis. Statistical analysis was performed by two tail paired Student's *t* Test. A *p* value lower than 0.05 was considered statistically significant.

RESULTS

PBMC were treated with 50 mM Rib and 10 mM dRib, i.e. concentrations which were highly effective in inhibiting cell proliferation and inducing cell death [1]. The morphology of Rib- and dRib-treated cells using light microscopy indicated that human quiescent PBMC were dying by apoptosis (Figs. 1a and 1b). In cell cultures to which Ara was added, the percentage of apoptotic cells was negligible and similar to that of control, untreated cells. Indeed, Rib- and dRib-treated cells showed characteristic cytoplasmic condensation, compaction of chromatin and fragmentation of the nucleus into one or several dense spheres.

These morphological changes were often accompanied by fragmentation of DNA at internucleosomal levels resulting in a characteristic ladder on gel electrophoresis. The DNA ladder was evident in Rib- and dRib-treated cells (Fig. 2). As expected, DNA fragmentation was practically absent in control untreated and Ara-treated cells. Either in Rib- or in dRib-treated cells, DNA fragmentation became evident 48 hs after sugar addition and increased in a time-dependent manner. Moreover, dRib was more effective as an apoptotic agent, as judged by the amount of fragmented DNA visible on the gel.

The presence of apoptotic cells in Rib- and dRib-treated cultures was confirmed using flow cytometry, as indicated by the red fluorescence intensity obtained after incubation of cells with propidium iodide. A decrease of fluorescence intensity indicates a decrease in chromatin

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Fig. 1. Human PBMC treated with Rib and dRib show the morphological changes of apoptosis. Morphological characteristics of PBMC treated for 48 hs with Rib 50 mM (a) and dRib 10 mM (b). Cells show classical features of apoptosis, such as condensed and fragmented state of the nuclear chromatin (arrows). Zeiss axioscope, x100. Bars represent 10 mm.

content due to the loss of apoptotic bodies [9]. The advantage of this technique is that it allows a quantitation of the phenomenon [9, 10]. Rib- and dRib-treated cells showed a time-dependent reduction in the percentage of nuclei with diploid DNA content and a concomitant increase in the

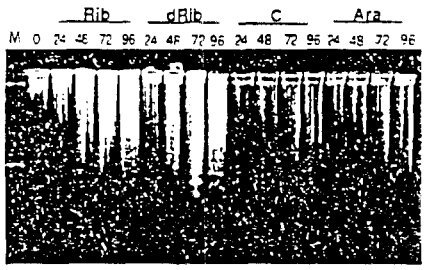

Fig. 2. DNA fragmentation in Rib- and dRib-treated human PBMC. Human PBMC ($5x10^6$ cells) were incubated with or without 50 mM Rib, 10 mM dRib or 50 mM Ara for 24, 48, 72 and 96 hs. M: molecular weight marker DNA (Lambda/174 DNA-Hind III/Hae II Digest, Pharmacia). 0: high molecular weight DNA from untreated PBMC, recovered immediately after lymphoprep separation. 24, 48, 72 and 96 refer to the time (hours) of treatment with sugars.

hypodiploid DNA peak (Fig. 3). As expected, these changes did not occur in control untreated nor in Ara-treated cells, where a typical diploid DNA peak was evident. A comparison between the data reported in Figures 2 and 3 shows that DNA fragmentation precedes the changes revealed by flow cytometry. Moreover, flow cytometry confirmed that dRib is more potent than

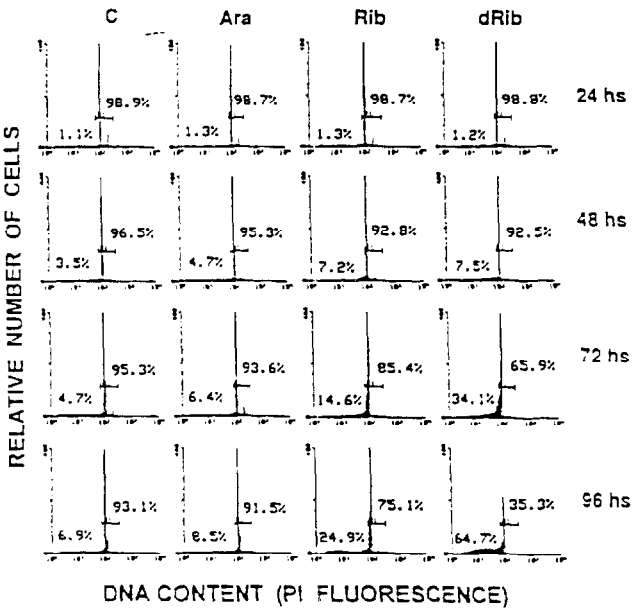

Fig. 3. Rib and dRib induce apoptosis in human PBMC, as assessed by flow cytometry. Cytofluorimetric analysis of human PBMC DNA content, as assessed by PI-staining technique. Cells were analyzed after 24, 48, 72 and 96 hs (rows) of incubation in the presence or absence of sugars. C = control untreated cells (first column); Ara = 50 mM Ara-treated cells (second column); Rib = 50 mM Rib-treated cells (third column); dRib = 10 mM dRib treated-cells (fourth column). In each panel, the numbers refer to the percentage of apoptotic cells (on the left) or of non-apoptotic cells (on the right).

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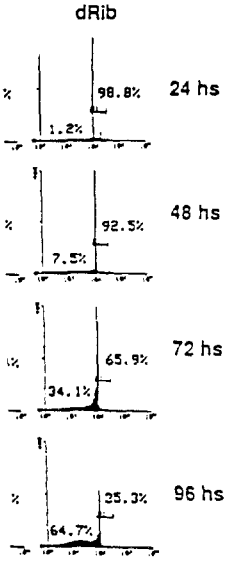

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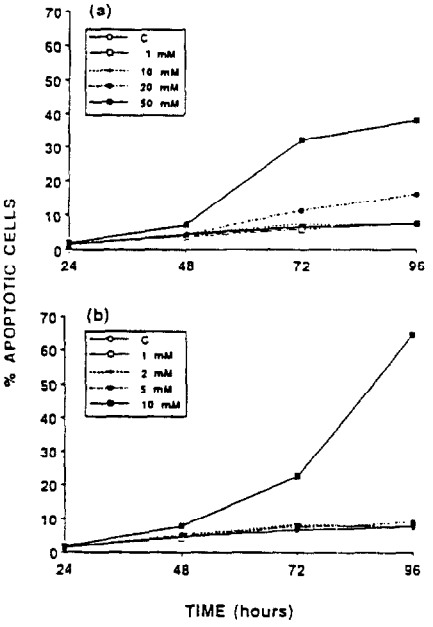

Fig. 4. dRib is about five times more powerful than Rib as an apoptotic agent. Cells were incubated for 24, 48, 72 and 96 hs as described in Materials and Methods, either alone (control) or with different concentrations of Rib (a) and dRib (b). Data represent the percentage apoptotic cells determined by flow cytometry following incubation with PI and after having gated out debris. One representative experiment is reported.

Rib in inducing apoptosis of human PBMC. The effect of both sugars was time-dependent. dRib was more potent than Rib (34.1% *vs* 14.6% at 72 hs; 64.7% *vs* 24.9% at 96 hs) at a five times lower concentration (10 mM dRib *vs* 50 mM Rib).

In order to establish the lowest concentration of Rib or dRib capable of exerting an apoptotic effect on human PBMC, time- and dose-response experiments were performed. Cells were cultured in the presence or in the absence of 1, 10, 20, 50 mM Rib, and 1, 2, 5, 10 mM dRib for 24, 48, 72, 96 hs. Cells were then harvested and DNA fragmentation and percentage of hypodiploid peaks were evaluated (Fig. 4). In the case of Rib the apoptotic effect was dose- and time-dependent. Indeed, the percentage of hypodiploid peak at 72 and 96 hs was slightly higher than that observed in control cultures when the concentration of 20 mM was tested, but became quite evident at the 50 mM concentration. The effect of dRib was evident when the concentration of 10 mM was used. Thus, as an apoptotic agent, dRib was about five times more powerful than Rib as a more massive loss of chromatin was obtained using a five times lower concentration.

On the basis of these results, 50 mM Rib and 10 mM dRib were used in five different experiments to test the individual variability. The results were highly reproducible in different individuals (Table I).

Table I: Reproducibility of Rib- and dRib-induced apoptosis in human PBMC

TIME (hs)	Control	Ara(50mM)	Rib(50mM)	dRib(10mM)
24	2.6 ± 0.9	2.7 ± 1.2	3.3 ± 0.4	2.9 ± 0.9
48	6.3 ± 1.7	6.0 ± 1.5	8.1 ± 1.6*	6.3 ± 1.3**
72	8.8 ± 2.3	9.8 ± 1.9	22.4 ± 5.2*	36.2 ± 6.8**
96	12.7 ± 3.1	14.2 ± 2.5	40.5 ± 8.1**	69.7 ± 6.6***

Cells were incubated for 24, 48, 72 and 96 hs as described in Materials and Methods, either alone (Control) or with 50 mM Ara, 50 mM Rib and 10 mM dRib. The percentage of apoptotic cells was determined by flow cytometry following incubation with PI and debris were gated out. Values are means ± SEM of five separate experiments. Statistical analysis was performed by two tail paired Student's *t* Test.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ *vs* control.

In order to understand the mechanism(s) by which Rib and dRib induce apoptosis, experiments were performed with several possible inhibitors. Taking into account that these two sugars have a high reducing activity and rank at the top among reducing sugars [11], thus having the possibility to interfere with the redox status of the cell, different antioxicants were tested [5, 12]. In Fig. 5 the protective effect of NAC is reported. Other substances had no protective effect (3-aminobenzamide, nicotinamide, acetyl-L-carnitine) (data not shown).

DISCUSSION

The main finding of this paper is that two simple sugars, such as Rib and dRib, are able to induce apoptosis in human quiescent PBMC, as assessed by morphological and biochemical analysis. The percentage of cells killed by Rib and dRib is quite high, involving about 70% of PBMC when the effect of dRib is assessed after 96 hs of culture (table I). Taking into account

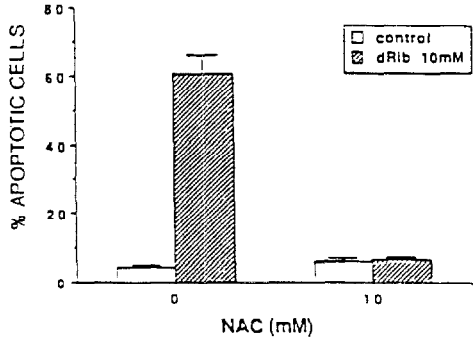

Fig. 5. N-acetyl-L-cysteine inhibits dRib-induced apoptosis. PBMC from 4 healthy subjects were incubated with or without 10 mM dRib for 96 hs as described in Materials and Methods, in presence or in absence of 10 mM NAC. The percentage of apoptotic cells (mean ± SEM) was assessed by PI-staining using flow cytometry.

that lymphocytes represent usually more than 70-80% of Ficoll-separated PBMC, we can reasonably assume that lymphocytes are likely the major target of these sugars. However further studies are needed to carefully assess a possible different sensitivity of lymphocytes (T and B cells, and monocytes to the apoptotic effect exerted by Rib and dRib.

Despite the ever increasing number of papers devoted to apoptosis, the reports which describe an apoptotic cell death in resting normal human lymphocytes are scanty. Although poorly documented, mature lymphocytes appear to be relatively insensitive to apoptosis [13]. This fact seriously hampered cellular and molecular analysis of the apoptotic process in lymphocytes, which are the most accessible cells from humans. Human lymphocytes can be induced to undergo apoptosis by the addition of activating agents such as superantigens [14]. Damaging agents can induce apoptosis in human lymphocytes, but a preactivation of the cells is generally required [15-17]. However, an intriguing relationship exists between cell proliferation and apoptosis [18-21]. The model here presented avoids this confounding variable and allows the study of apoptosis in resting cells, without the necessity to trigger the proliferative program.

In more general terms, Rib and dRib can be added to the long list of agents capable of inducing cell death by apoptosis. Apoptosis caused by Rib or dRib requires at least 48 hs to be evident either by assessing DNA fragmentation or by flow cytometry. As an apoptotic agent, dRib was clearly much more effective than Rib. Even if the concentration of dRib required to kill more than 50% of the cells was relatively high, i.e. 10 mM, no apoptotic effect was exerted by a concentration five times higher using a Rib isomer, such as Ara. The reason why dRib is more powerful as an apoptotic agent in comparison to Rib is at present unknown.

The mechanism(s) by which these two sugars are able to induce apoptosis in human PBMC is/are at present unknown. A possible key to understand this/these mechanism(s) is offered by the protective effect exerted by NAC. These experiments, performed because a major characteristic of these two sugars is their high reducing activities [11], suggest that dRib can interfere with glutathione metabolism [22]. NAC is, under certain conditions, a direct scavenger of reactive oxygen intermediates [23]. Thus, it cannot be excluded that dRib induces apoptosis by producing an oxidative stress. Further studies are needed to clarify this point.

In conclusion, we propose that the model here presented will contribute in understanding apoptosis in human PBMC.

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REFERENCES

1. Marini, M., Zunica, G. and Franceschi, C. (1985) Proc. Soc. Exp. Biol. Med. 180, 246-257
2. Zunica, G., Marini, M., Brunelli, M.A., Chiricolo, M. and Franceschi, C. (1986) Biochem. Biophys. Res. Commun. 138, 673-678

3. Franceschi, C. (1989) Aging 1, 3-15
4. Kerr, J.F.R. and Harmon, B.V. (1991) in "Apoptosis: the molecular basis of cell death" (Tomei, L.D. and Cope, F.O. eds) Cold Spring Harbor Laboratory Press, New York, 5-29
5. Monti, D., Troiano, L., Tropea F., Grassilli, E., Cossarizza, A., Barozzi, D., Pelloni, M.C.,Tamassia, M.G., Bellomo, G. and Franceschi, C. (1992) Am. J. Clin. Nutr. 55, 1208-1214
6. Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, Suppl. 97, 77
7. Franceschi, C., Licastro, F., Chincolo, M., Bonetti, F., Zanotti, M., Fabris, N., Mocheggiani, E., Fantini, M.P., Paolucci, P. and Masi M. (1981) J. Immunol. 126, 2161-2164
8. Smith, C.A., Williams, G.T., Kingstone, R., Jenkinson, E.J. and Owen, J.J.T. (1989) Nature 337, 181-184
9. Barbieri, D., Troiano, L., Grassilli, E., Agnesini, C., Cristofalo, E.A., Monti, D., Capri, M., Cossarizza, A. and Franceschi, C. (1992) Biochem. Biophys. Res. Commun. 187, 1256-1261
10. Nicoletti, I., Migliorati, G., Pagliacci, M.C., Grignani F. and Riccardi C. (1991) J. Immunol. Methods, 139, 271-279
11. Monnier ,V.M. (1990) J. Gerontol. 45, B105-111
12. Marini, M., Zunica, G., Tamba, M., Cossarizza, A., Monti, D. and Franceschi, C. (1990) Int. J. Rad. Biol. 58, 279-291
13. Lucas, M., Solano, F. and Sanz, A. (1991) FEBS Lett. 279, 19-20
14. Kabelitz, D. and Wesselborg, S. (1992) Int. Immunol. 4, 1381-1388
15. Bazar, L.S. and Deeg, H.J. (1992) Exp. Hematol. 20, 80-86
16. Janssen, O. (1992) Transplantation, 53, 233-234
17. Owen-Schaub, L.B., Yonehara, S., Crump III, W.L. and Grimm, E.A. (1992) Cell. Immun. 40, 197-205
18. Monti, D., Grassilli, E., Troiano, L., Cossarizza, A., Salvioli, S., Barbieri, D., Agnesini, C., Bettuzzi, S., Ingletti, M.C., Corti, A. and Franceschi, C. (1992) Ann. N. Y.Acad.Sci. 673, 70-82
19. Grassilli, E., Bettuzzi, S., Monti, D., Ingletti, M.C., Franceschi, C. and Corti, A. (1992) Biochem. Biophys. Res. Commun. 180, 59-63
20. Grassilli, E., Carcereri de Prati, A., Monti, D.,Troiano, L., Menegazzi, M., Barbieri, D., Franceschi, C. and Suzuki, H. (1992) Biochem. Biophys. Res. Commun. 188, 1261-1266
21. Sikora, E., Grassilli, E., Bellesia, E. and Franceschi, C. (1993) Biochem. Biophys. Res. Commun. 192, 386-391
22. Staal, F.J.T, Ela, S.W, Roederer, M., Anderson ,M.T., Herzenberg, L.A., Herzenberg, L.A. (1992) Lancet 339, 909-912
23. Aruoma, O. I., Halliwell, B., Hoey, B.M., Butler, J. (1989) Free Radic. Biol. Med. 6, 593-597

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Effects of ribose on exercise-induced ischaemia in stable coronary artery disease

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There is no established treatment specifically aimed at protecting or restoring cardiac energy metabolism, which is greatly impaired by ischaemia. Even after reperfusion, myocardial content of ATP remains low for more than 72 h. Long-term post-ischaemic dysfunction and irreversibility of ischaemic damage have been associated with low ATP content. Evidence that the pentose sugar ribose stimulates ATP synthesis and improves cardiac function led us to test the possibility that ribose increases tolerance to myocardial ischaemia in patients with coronary artery disease (CAD).

20 men with documented severe CAD underwent two symptom-limited treadmill exercise tests on 2 consecutive days; we postulated that the ischaemia induced might bring about changes in ATP metabolism lasting for several days. Patients whose baseline tests showed reproducibility were randomly allocated 3 days of treatment with placebo or ribose 60 g daily in four doses by mouth. Exercise testing was repeated after treatment on day 5. At that time mean (95% confidence interval) treadmill walking time until 1 mm ST-segment depression was significantly greater in the ribose than in the placebo group (276 [220–331] *vs* 223 [188–259] s; $p = 0{\cdot}002$). The groups did not differ significantly in time to moderate angina. In the ribose-treated group the changes from baseline to day 5 in both time to ST depression and time to moderate angina were significant ($p < 0{\cdot}005$), but these changes were not significant in the placebo group.

In patients with CAD, administration of ribose by mouth for 3 days improved the heart's tolerance to ischaemia. The presumed effects on cardiac energy metabolism offer new possibilities for adjunctive medical treatment of myocardial ischaemia.

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Introduction

Although myocardial ischaemia has profound effects on the heart's energy metabolism, there is no available treatment specifically aimed at preventing or correcting the changes. This is an important limitation of current treatment, since normal heart function and tissue preservation constantly require large amounts of energy derived from the hydrolysis of ATP. ATP is believed to be the main intermediary between energy-creating and energy-requiring reactions in myocardial cells. ATP stores in the heart are very limited—sufficient for less than 1 min of contraction.[1] During myocardial ischaemia, content of ATP and creatine phosphate, the chief reserve source of high-energy phosphate, declines rapidly.[2,3] When reperfusion occurs (spontaneously or as a result of therapeutic measures) before the tissue is irreversibly damaged, creatine phosphate content returns to normal within minutes; thus, cellular energy-producing mechanisms are intact.[4] By contrast, despite reperfusion, ATP concentrations take 72–96 h to recover,[5,6] and a recovery time of more than 7 days has been reported after global ischaemia.[6] Low myocardial ATP content has been associated with extended post-ischaemic dysfunction such as is observed in stunned myocardium. In some studies, ATP concentrations correlate with irreversibility of ischaemia, cell death, and the preservation and return of contractile performance after ischaemia.[2,7]

Factors that prevent faster post-ischaemic restoration of ATP concentrations have been identified. Myocardial ischaemia leads to degradation of ATP, and the dephosphorylated derivatives diffuse out of the myocyte into the extracellular space where they are no longer available as precursors for immediate ATP resynthesis in the salvage pathway.[8,9] Spontaneous or therapeutically induced reperfusion augments such loss of ATP metabolites. Thus, when blood flow is re-established, restoration of ATP content depends primarily on de-novo synthesis. This process is slow in the heart[10] because the essential precursor 5-phosphoribosyl-1-pyrophosphate (PRPP) is in short supply.[11] Exogenous ribose, a pentose sugar, bypasses rate-limiting steps in the oxidative pentose phosphate pathway that generates this precursor. This sugar increases the myocardial pool of the precursor[12] and accelerates ATP synthesis.[12,13] Ribose-induced increases in ATP concentrations have been associated with improved post-ischaemic ventricular performance in animals,[14,15] and less severe ischaemic cell damage has been reported.[16] There is strong evidence that ribose would be able to have these beneficial effects in man too.[3,9,17–19] In this study we tested the hypothesis that ribose increases tolerance to myocardial ischaemia in patients with coronary artery disease (CAD).

Patients and methods

We studied 20 men, aged 45–69 years, who had CAD documented by coronary arteriography (≥75% narrowing of at least one main vessel) and a history of angina induced by normal daily activities (table). 3 patients had had myocardial infarctions previously. Before entry to the study each patient had to have a positive treadmill test. An exercise test was judged positive when there was ischaemic ST-segment depression (≥1 mm [0·1 mV], horizontal or downsloping, persisting for at least 80 ms after the J point in the electrocardiogram) and angina during the first 9 min of exercise according to the Bruce protocol. Results of exercise tests were accepted as reproducible when the times of onset of ST-segment depression and of moderate angina on day 2 were within 60 s of the respective times on day 1. 4 patients who met all other entry criteria did not show reproducibility and were therefore excluded. During the study, patients continued their usual medication (nitrates, calcium-channel antagonists, beta-blockers) provided it did not change during the 72 h before the first exercise

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BASELINE CLINICAL CHARACTERISTICS OF STUDY PATIENTS

Patient	Age (yr)	Stenosis*	Treatment
Ribose group			
1	68	75% LAD, 99% RCA	B, C
2†	53	99% LAD, 50–60% CX, 75% RCA	B, C, N
3	50	100% LAD, 50% CX, 99% RCA	B
4	61	90% LAD, 99% CX, 100% RCA	C, N
5†	56	100% RCA	B, C, N
6	53	99% CX, 100% RCA	C, N
7	64	75–90% LAD, 75–90% CX, 90% RCA	B, C, N
8	55	99% LAD, 40% CX, 50% RCA	C, N
9	65	75–99% LAD, 99% CX, 75% RCA	C, N
10	45	100% LAD, 50% CX, 100% RCA	B, N
Placebo group			
11	53	99% LAD	B
12	53	75–99% LAD, 75–99% CX, 75–99% RCA	B, N
13	62	70% LAD, 70% CX, 80% RCA	B
14	69	100% LAD, 50% CX, 99% RCA	B, N
15	45	90% LAD	B, N
16†	50	100% LAD, 75% CX, 90% RCA	C, N
17	52	75% LAD, 50% CX, 100% RCA	B, C, N
18	67	100% LAD, 75–100% CX, 100% RCA	B, N
19	60	90% LAD, 100% RCA	B, C, N
20	62	70% LAD, 50% CX, 90% RCA	B, N

LAD = left anterior descending artery; CX = circumflex artery; RCA = right coronary artery; B = beta-blockers; C = calcium-channel antagonists; N = nitrates.
*An artery with ≥ 70% stenosis was considered diseased.
†Had previously had myocardial infarction.

test. On each day of exercise testing, morning doses were withheld until after the test. Reasons for exclusion were unstable angina, myocardial infarction within the previous 3 months, previous heart surgery, evidence of heart failure, left main coronary artery stenosis, valvular heart disease, any other non-coronary heart disease, any disorder rendering the ST segment in the exercise electrocardiogram unclear (eg, left bundle branch block), and compromised gastrointestinal absorption. Study patients had to be able to perform treadmill walking exercise and had no symptomatic peripheral vascular disease. All patients gave informed written consent. The study protocol was approved by the University of Munich Medical School Ethics Committee.

Exercise tests were done on a motor-driven treadmill according to the Bruce protocol. All patients were familiar with the procedure. The skin was prepared for a 12-lead electrocardiogram by shaving, cleansing with acetone, and abrasion with emery paper. At the first exercise test, lead position was marked with permanent dye for precise application on subsequent tests. During exercise, 3 standard electrocardiographic leads were continuously monitored. ST-segment deviation was measured in all leads by a computerised system with subsequent manual over-reading. In cases of discrepancy between the computer-processed signal and real-time electrocardiography recordings, the latter were used for analysis. On each day with exercise tests scheduled, the patient came to the laboratory after an overnight fast without having taken any drugs that morning. All three exercise tests for each patient were done under identical external conditions, at the same time of the morning with constant room temperature and humidity. All exercise tests were carried out by the same physician. If ST-segment depression did not occur in post-treatment exercise tests before the exercise was stopped at the onset of moderate angina, total exercise time was used for time to ST depression. Patients were instructed to continue exercise until they had angina of moderate severity (chest pain of such severity that the patient would normally stop activity).

Because the carryover effects of ribose are unknown, a crossover design was judged inappropriate. If a patient showed reproducible exercise tests on days 1 and 2 he was randomly allocated, by means of a table of random numbers, treatment with ribose or placebo for 3 days (starting after the exercise test on day 2 and continuing to day 4). A final exercise test was done on day 5 to assess treatment effects. We postulated that two consecutive exercise tests to the point of myocardial ischaemia would induce changes in cardiac energy metabolism lasting for more than 72 h.

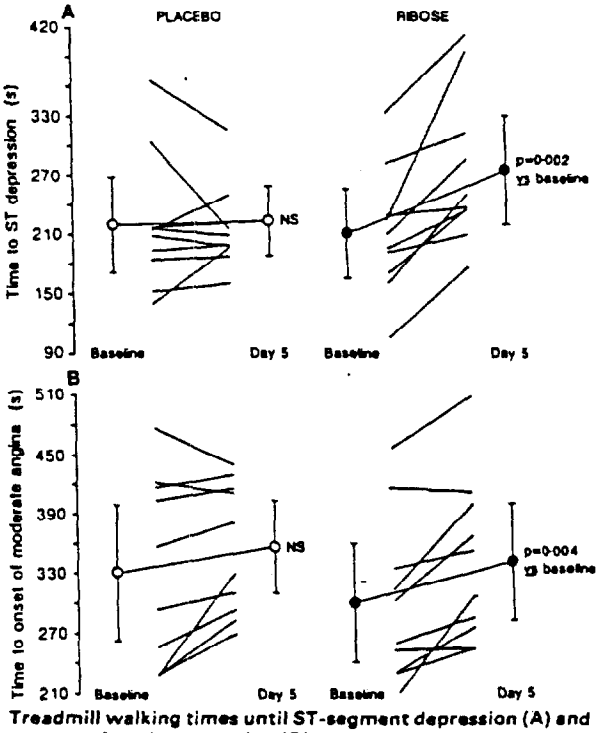
Patients in the ribose group received 60 g D-ribose daily, dissolved in water, in four doses of 15 g. Because of possible hypoglycaemic responses to ribose[20] patients were asked not to take ribose while fasting. The dosage was based on reports of animal experiments that showed the efficacy of high doses of ribose[14] and on human studies[21,22] showing that 60 g per day is well tolerated and not associated with gastrointestinal side-effects. The placebo solution contained mainly an equicaloric amount of glucose. It was identical to ribose solution in taste, colour, and appearance as assessed by a pretrial experiment with 10 hospital staff. Purity of substances was checked by the hospital pharmacy.

The sample size was prospectively chosen on the grounds of clinical practicability. The paired *t* test was used for within-group comparisons. Analysis of covariance was used to adjust for baseline measurement in between-group comparisons. $p < 0.05$ (two-tailed) was considered to indicate significance.

Results

In the ribose-treated group, the mean walking time until 1 mm ST-segment depression was significantly greater on day 5 than at baseline (figure, A), whereas there was no significant change in time to ST depression in the placebo group ($p = 0.67$). 2 ribose-treated patients did not show ST depression on day 5, so time to the onset of moderate angina was used in the analysis. After treatment, the time to ST depression (day 5) was significantly longer in the group that had received ribose than in the placebo-treated group (mean and 95% confidence interval [CI] 276 [220–331] *vs* 223 [188–259] s; $p = 0.002$).

The time to onset of moderate angina also increased significantly from baseline to day 5 in the ribose group (figure, B); there was an increase in this variable in the placebo group but it did not achieve significance ($p = 0.062$). There was no significant difference between the ribose and placebo groups in mean time to onset of moderate angina on



Treadmill walking times until ST-segment depression (A) and onset of moderate angina (B).

Baseline = average of results on days 1 and 2 for each patient; day 5 = after 3 days' treatment. Mean and 95% confidence intervals given for each group.