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. Horecker, Gibbs, Klenow, and Smyrniatis (9) have 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 of these studies have been published (13), as well as 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 glucose had been destroyed by glucose oxidase. Serum inorganic phosphate was determined by the method

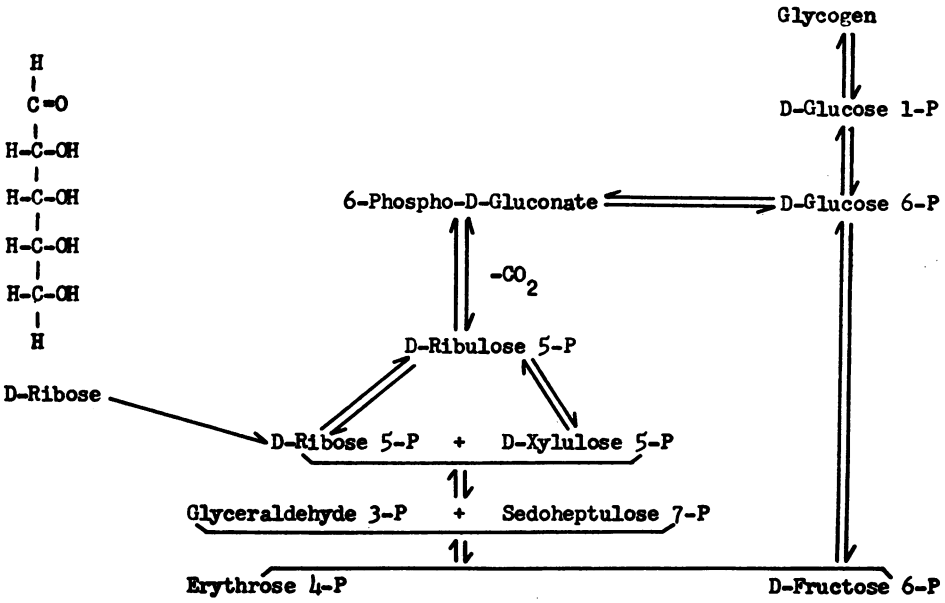

FIG. 1. THE SEQUENCE OF REACTIONS FOR THE ENTRANCE OF RIBOSE INTO THE PENTOSE PHOSPHATE PATHWAY AND ITS CONVERSION TO GLUCOSE

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

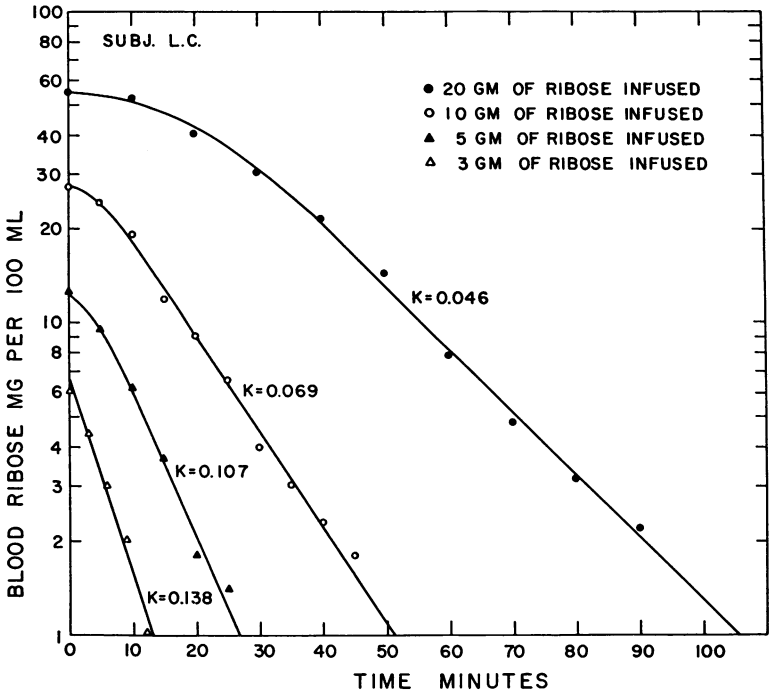

FIG. 2. SEMILOGARITHMIC DISAPPEARANCE CURVES OF RIBOSE FROM BLOOD AFTER 15 MINUTE INFUSION OF VARIOUS AMOUNTS OF PENTOSE (SUBJECT L. C.)

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 clearance system, can be derived. The K_S is 10 mg. 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

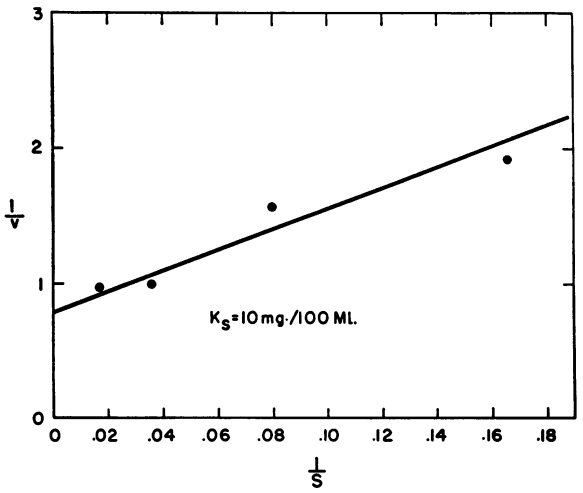

FIG. 3. GRAPH OF THE RECIPROCAL OF THE INITIAL VELOCITY IN MILLIGRAMS PER 100 MILLILITERS PER MINUTE VERSUS THE RECIPROCAL OF THE INITIAL CONCENTRATION IN MILLIGRAMS PER 100 MILLILITERS OBSERVED AFTER THE INFUSION OF VARIOUS DOSES OF RIBOSE IN SUBJECT L. C.

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.

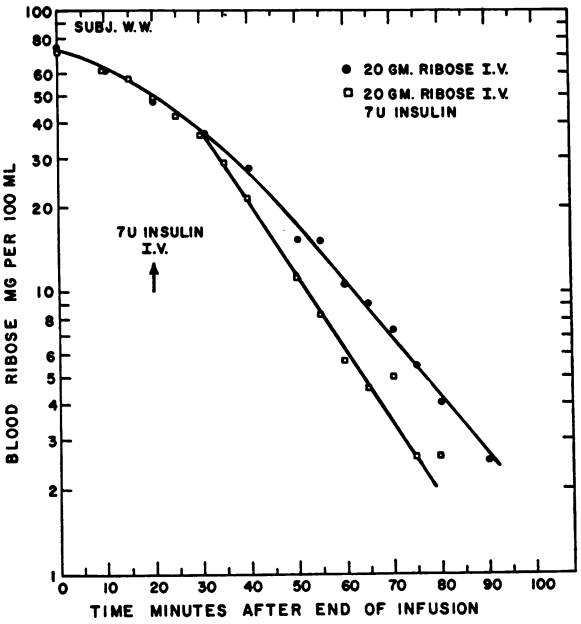

FIG. 4. THE EFFECT OF INTRAVENOUS INSULIN ON THE DISAPPEARANCE OF RIBOSE FROM BLOOD

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.

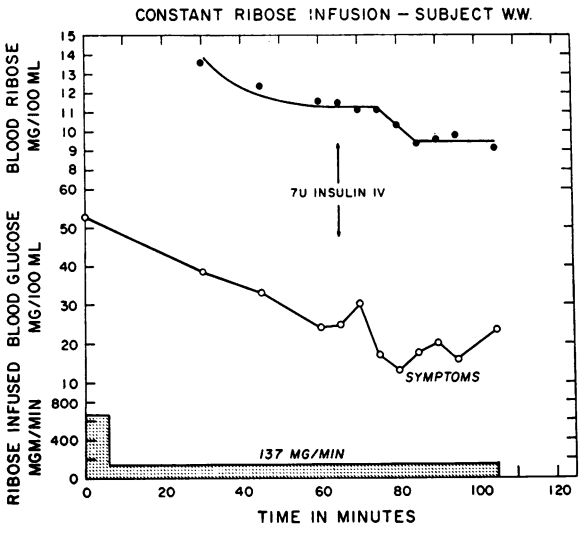

FIG. 5. BLOOD LEVELS OF RIBOSE AND GLUCOSE OBSERVED DURING THE CONSTANT INFUSION OF RIBOSE

Effects of intravenous insulin administration.

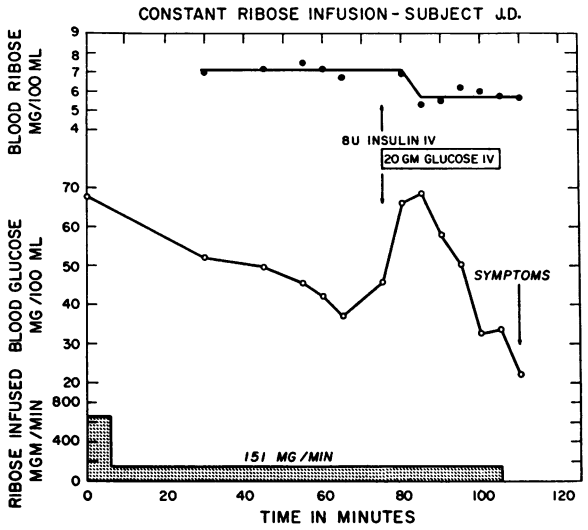

FIG. 6. BLOOD LEVELS OF RIBOSE AND GLUCOSE OBSERVED DURING THE CONSTANT INFUSION OF RIBOSE

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 constant infusion of ribose at a slow rate.

Table 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). In four normals receiving 20 Gm. the decrease ranged 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

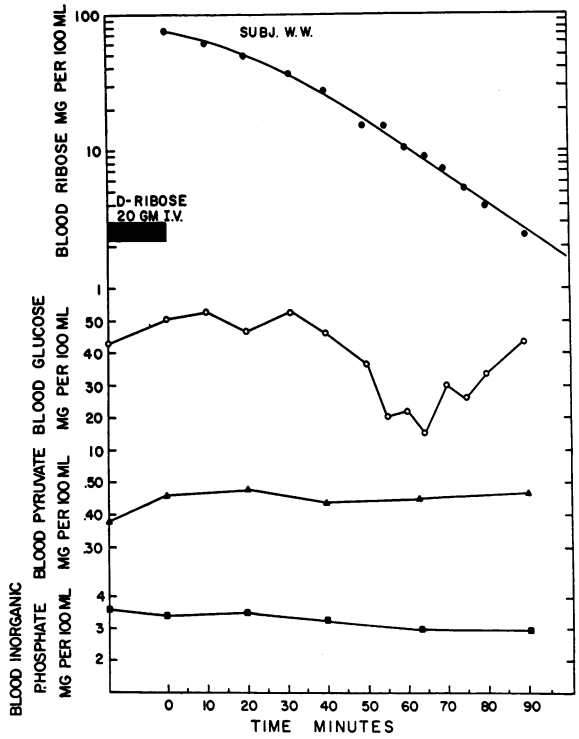

FIG. 7. THE EFFECT OF THE ADMINISTRATION OF RIBOSE ON BLOOD GLUCOSE, PYRUVATE AND SERUM INORGANIC PHOSPHATE LEVELS

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.

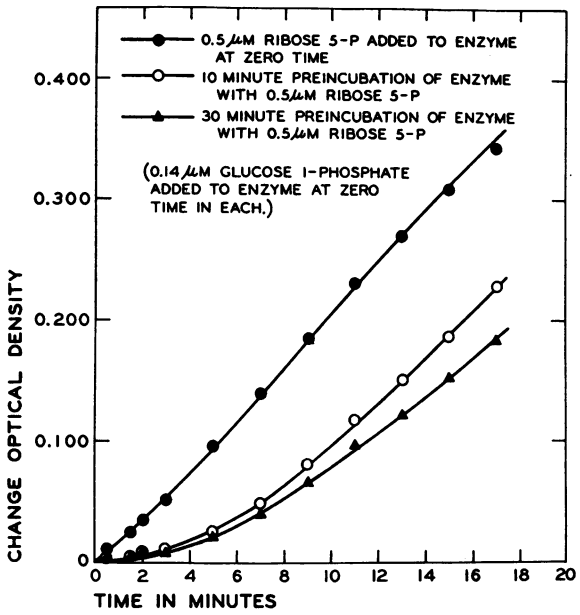

FIG. 8. THE INHIBITION OF PHOSPHOGLUCOMUTASE BY RIBOSE 5-PHOSPHATE

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

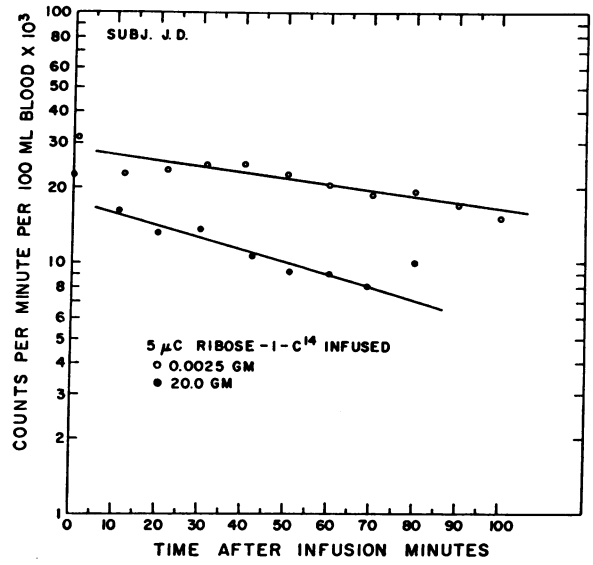

FIG. 9. A SEMILOGARITHMIC PLOT OF THE C^{14} DISAPPEARANCE FROM BLOOD AFTER RIBOSE-1-C^{14} INFUSION BOTH AS TRACER AND LOAD DOSES

were carried out in one normal individual in which the fate 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.

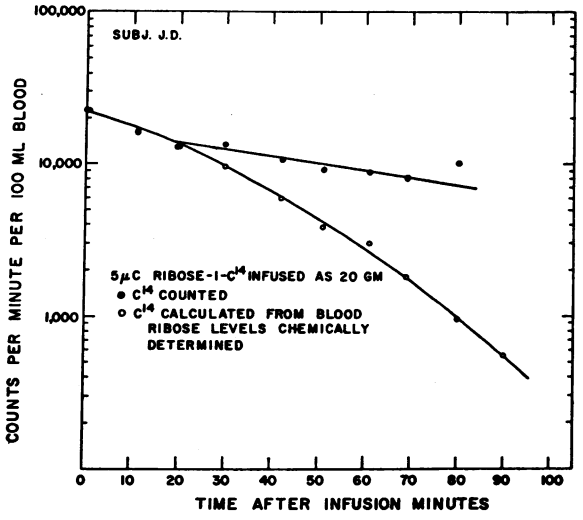

FIG. 10. A COMPARISON OF THE OBSERVED DISAPPEARANCE OF C^{14} FROM BLOOD AND THAT CALCULATED FROM CHEMICALLY DETERMINED RIBOSE LEVELS

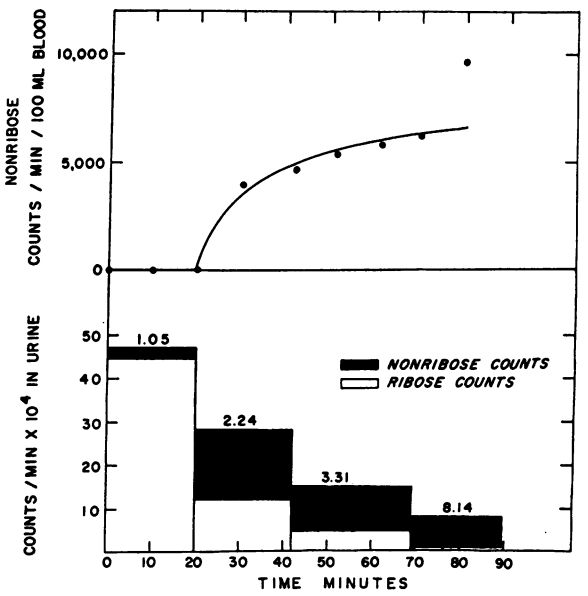

FIG. 11. THE SIMULTANEOUS APPEARANCE OF NONRIBOSE C^{14} IN BLOOD AND URINE

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

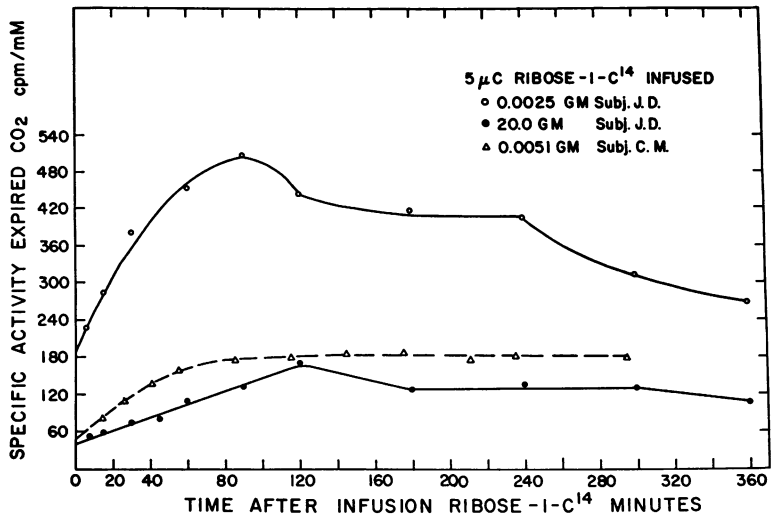

FIG. 12. THE SPECIFIC ACTIVITY OF EXPIRED $C^{14}O_2$ AFTER THE INFUSION OF RIBOSE-1-C^{14}

diabetic, the labeling of CO_2 reaches a low maximum and then remains constant throughout the period of observation. In five hours, the cumulative 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 obtained for the first 5 hours. Twenty-eight per cent of the C^{14} administered was excreted via this route 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 in the diabetic urine is shown in Figure 13. Five hours 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 constants observed after the infusion of small amounts of ribose are larger than those observed after the infusion of various hexoses and other pentoses (15). Only D-galactose disappears from blood at a rate similar to that of ribose (47). At comparable dose levels, the rate constant of ribose removal from blood is four to five times as large as that of other pentoses (15). Helmreich and Cori (48) have similarly reported that injected ribose disappears from the body of the nephrectomized rat at rates much greater than those of other pentoses.

The average total urinary excretion of ribose after a 15 minute infusion was 21 per cent of the dose. A much smaller amount was excreted after constant infusion of the sugar at a slow rate. None of our studies was designed to determine accurately the renal mechanism for excreting ribose, and we are not aware of any careful studies of the renal handling of this pentose comparable to those reported for xylose (49). One difference between the renal handling of ribose and xylose is 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 of 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 a 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).

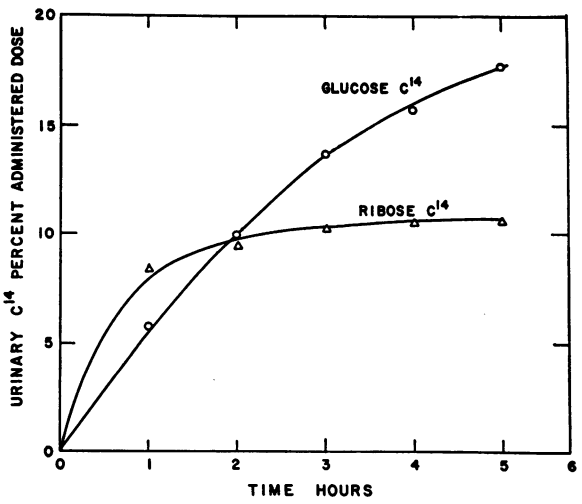

FIG. 13. URINARY EXCRETION OF C^{14} AS RIBOSE AND GLUCOSE AFTER INFUSION OF LABELED RIBOSE TO A DIABETIC SUBJECT

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

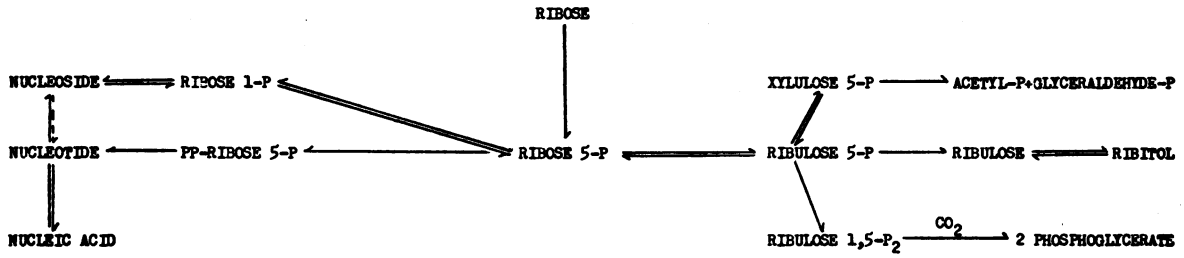

FIG. 14. FATES OF RIBOSE OTHER THAN ENTRANCE INTO THE PENTOSE PHOSPHATE PATHWAY OF METABOLISM

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

occurs (in bacteria) to form an acetyl phosphate and glyceraldehyde phosphate (66). Free ribulose has been reported to be present in normal human urine (67), suggesting that ribulose phosphate is dephosphorylated. Recently Hollmann and 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 two 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.

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