STUDIES OF RIBOSE METABOLISM. V. FACTORS INFLUENCING IN VIVO RIBOSE SYNTHESIS IN THE RAT ^{1,2}

By HOWARD H. HIATT

(From the Department of Medical Research, Beth Israel Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.)

(Submitted for publication May 14, 1958; accepted June 12, 1958)

Ribose synthesis in animal tissue (1-3) has been demonstrated to take place via the oxidative and the nonoxidative reactions of the pentose phosphate pathway (4). The levels of the enzymes which catalyze the oxidative reactions of the pathway have been shown to be affected by hormonal alterations (5), and evidence has been presented indicating depression of the nonoxidative reactions in the tissues of thiamine-deficient animals (6). However, a systematic study of those factors influencing ribose production has not been possible because of the absence of a satisfactory method for obtaining this pentose from the living animal.

We have recently reported a convenient technique for inducing the urinary excretion of ribose by the rat, and have presented evidence that urinary ribose and that isolated from nucleic acid are synthesized via the same or similar pathways (7). This ribose "trapping" procedure is based on the observation of Tabor and Hayaishi (8) and of Karjala (9) that the administration of imidazoleacetic acid (ImAA) or of histamine to the rat is followed by the urinary excretion of ImAA riboside.

We have now used the ImAA riboside technique to study the effects of thiamine deficiency, partial hepatectomy, tumor growth, diabetes, hypopituitarism, and the administration of thyroxine, glucagon and adrenocorticotrophic hormone (ACTH) on ribose synthesis. We have also obtained evidence for the direct incorporation of exogenous ribose into ImAA riboside. The results of these investigations are the subject of the present report.

¹ These studies were aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research and the United States Public Health Service (C-3151).

² Presented in part at the forty-ninth annual meeting of the American Society for Clinical Investigation, Atlantic City, N. J., May 6, 1957.

Most of the studies to be reported were carried out with glucose-2-C¹⁴ as the administered radioactive precursor of ribose. Hexose specifically labeled in its second carbon atom is useful in distinguishing ribose produced by the two reaction sequences of the pentose phosphate pathway. Ribose derived from the oxidative removal of carbon 1 of hexose-2-C¹⁴ phosphate (the C-1 oxidation pathway) is labeled in its first position:

On the other hand, ribose-2-C¹⁴ is produced in the nonoxidative reactions via the transketolase-catalyzed transfer of the first two carbons of hexose-2-C¹⁴ phosphate to an appropriate acceptor such as triose phosphate ³:

³ Ribose-2-C¹⁴ is also the product following the oxidative removal of carbon atom 6 of hexose-2-C¹⁴ (the C-6 oxidation pathway). However, recent studies have demonstrated that little if any pentose is synthesized from hexose via this mechanism (10).

In the normal rat ribose synthesized from glucose-2-C¹⁴ is predominantly labeled in carbon atoms 1 and 2, with the latter more radioactive than the former (2, 7). Carbon 4 is also labeled to an appreciable extent, presumably as a result of aldolase cleavage of hexose-2-C¹⁴ diphosphate and equilibration of the triose phosphates, followed by participation of triose-2-C¹⁴ phosphate in the transketolase reaction.

METHODS

ImAA riboside ribose isolation. The methods employed for the administration of imidazoleacetic acid and for the isolation and hydrolysis of ImAA riboside have been described in detail elsewhere (7). Briefly, each rat received three intraperitoneal injections at two hour intervals, except where otherwise noted. The injected fluid contained imidazoleacetic acid dihydrochloride, or histamine, sufficient sodium bicarbonate to effect neutrality, and a radioactive substrate, and was brought to a total volume of 15 ml. with isotonic saline. Rat 2 received ImAA and glucose-2-C¹⁴ by stomach tube. Urine was collected during the period of the injections and for the succeeding 16 hours. ImAA riboside was isolated by column chromatography, and hydrolyzed to free ribose and imidazoleacetic acid by incubation with an enzyme prepared from *Lactobacillus delbrückii* (11). The ribose was purified by column and paper chromatography, and then degraded by methods which have been summarized elsewhere (3), and which permit the isolation of each carbon atom of the pentose molecule as barium carbonate. The radioactivity of these samples was determined with a "micromil" end window counter with a counting efficiency of 13 per cent. Glucose was determined by the Nelson-Somogyi procedure (12, 13) and ribose by the orcinol method (14).

Animal preparation. Male rats of the Wistar or Sprague-Dawley strain with initial weights approximating 250 grams were used for these studies, except where otherwise noted. Most animals were maintained on Purina chow until the time of the first injection; Rats 13 and 14 were fasted for 72 hours prior to the study. Throughout the period of injections and for the ensuing 18 hours all of the rats were offered water *ad libitum* but were fasted.

Thiamine deficiency. In Experiment 3 the rat was maintained on a thiamine-free diet for six weeks, during which time it lost approximately 90 grams in weight. A fall in pulse rate, as measured electrocardiographically, from control levels of 550 beats per minute to 400 at the time of the study was considered evidence of profound thiamine deficiency (15). Rats 30 and 32 were young animals with initial weights approximating 100 grams, and received the thiamine-deficient diet and intraperitoneal injections containing 100 micrograms of oxythiamine daily for two weeks prior to the ImAA study. These animals gained 11 and 4 grams during the period of de-

veloping thiamine deficiency, as compared to weight gains of about 35 grams in litter mates receiving Purina chow.

Partial hepatectomy (Rats 4 and 5). Nine grams of liver was removed from Rat 4 under light ether anesthesia. ImAA administration and urine collection were begun 24 hours later. At the time of sacrifice 72 hours following partial hepatectomy, this animal's liver weighed 8.5 grams. The entire liver of a control unoperated rat of equal weight weighed 10.5 grams. Five grams of liver was removed from Rat 5, 72 hours prior to the study.

Diabetes mellitus (Rats 6, 7 and 8). Following a 48 hour fast these rats were injected subcutaneously with a freshly prepared, charcoal-treated solution containing 200 mg. of alloxan monohydrate per Kg. of body weight. Rat 6 was studied 48 hours, and Rats 7 and 8 seven days following alloxan administration. Rats 6 and 7 appeared very sick and had blood sugars of 650 and 800 mg. per 100 ml. The diabetes in Rat 8 was very mild (fasting blood sugar, at the conclusion of the study, was 150 mg. per 100 ml.).

Hormonal alterations (Rats 9, 10, 11 and 31). Rat 9 was made hyperthyroid by the daily subcutaneous injection of 0.5 mg. of sodium L-thyroxine pentahydrate for seven days prior to the study. During this period the animal lost 34 grams in body weight. Rat 10 was injected intraperitoneally with 4 units of adrenocorticotrophic "gel" daily for eight days prior to the experiment. Rat 11 received 1 unit of crystalline glucagon (1 mg.) subcutaneously one-half hour before, and at hourly intervals during the period of imidazoleacetic acid administration, for a total of five injections. Rat 31 was hypophysectomized (Charles River Laboratories, Boston) one week prior to the study and received 5 per cent glucose in its drinking water. After the study the animal was sacrificed and autopsied; no tissue was found in the sella turcica.

Lactate and acetate injections. Rat 12 received 5, and Rat 13, 2 millimoles of sodium lactate, and Rat 14, 3.3 millimoles of sodium acetate in each of the three intraperitoneal injections containing the imidazoleacetic acid and glucose-2-C¹⁴.

Tumor study. Rats 16 and 17 were each injected intraperitoneally with 1 ml. of the Yoshida hepatoma in the ascitic form. Rat 16 was studied 48 hours and 7 days later, and Rat 17, 10 days later. Massive ascites was present both in Rat 17 and in Rat 16 at the time of the second study. Both animals were given ImAA intraperitoneally, and Rat 17 received the glucose-2-C¹⁴ by the same route. To obviate complete glycolytic cleavage of the glucose-C¹⁴ by the tumor cells (16), Rat 16 was given the labeled sugar subcutaneously immediately following each intraperitoneal injection of ImAA.

Materials. Imidazoleacetic acid dihydrochloride was prepared according to the method of Bauer and Tabor (17). Glucose-2-C¹⁴ and ribose-1-C¹⁴ were obtained from the National Bureau of Standards through the courtesy of Dr. H. S. Isbell. Both the glucose (18) and the ribose

were degraded to their constituent carbon atoms and were found to contain radioactivity only in the carbon atom specified. Ribose, randomly labeled with C^{14} ("ribose- $R-C^{14}$ "), was purchased from Schwarz Laboratories, and randomly- C^{14} -labeled glucose ("glucose- $R-C^{14}$ ") from New England Nuclear Corporation. The isotope patterns in the administered ribose and glucose are summarized in Tables III and IV, respectively. The specific molar activities of the radioactive sugars approximated 2 μ c. per mg. A partial purification of *Lactobacillus delbrückii* riboside hydrolase was carried out according to the procedure of Takagi and Horecker (11). The thiamine-free diet and oxythiamine were purchased from Nutritional Biochemicals Company. A rat with the Yoshida hepatoma in ascites form was kindly provided by Dr. M. L. Stephenson of the Huntington Laboratories, Boston.

RESULTS

The data summarizing the fraction of administered imidazoleacetic acid or histamine excreted as ImAA riboside are presented in Table I. The administration of a total of 250 to 300 micromoles of ImAA to normal animals in three injections two hours apart was followed by the excretion of 22 to 31 per cent of ImAA as the riboside (Rats 1, 12 and 18). In a single experiment (Rat 2) in which ImAA was administered orally, 19 per cent was excreted as the riboside. Considerably less riboside was excreted by hepatectomized rats (Rats 4 and 5) and by severely diabetic animals (6 and 7). No untoward effects were observed following

TABLE I
Urinary riboside ribose excreted by rats given imidazoleacetic acid (ImAA) or histamine and C^{14} -labeled sugar

"Experiment"	Animal status	ImAA administered	Urinary ribose	Per cent administered ImAA	C^{14} administered		Molar activity of ribose	Total radioactivity in ribose	Per cent administered C^{14} in ribose
					Compound	Cpm $\times 10^4$			
		μM	μM				$cpm/\mu M$	cpm	
1	Normal	250	77	31	Glucose-2- C^{14}	2.86	202	15,600	0.54
2	Normal	250*	47	19	Glucose-2- C^{14}	1.43	166	7,800	0.55
3	Thiamine-deficient	250	60	24	Glucose-2- C^{14}	2.86	185	11,100	0.39
32	Thiamine-deficient (oxythiamine)	150	42	28	Glucose-2- C^{14}	0.95	188	7,850	0.82
4	24 hrs. after subtotal hepatectomy	150	15	10	Glucose-2- C^{14}	5.83	150	2,250	0.04
4	48 hrs. after subtotal hepatectomy	167	9.5	6	Glucose-2- C^{14}	2.94	100	950	0.03
5	72 hrs. after 50% hepatectomy	400	45	11	Glucose-2- C^{14}	5.83	167	7,500	0.13
6	Alloxan diabetes	250	8	3	Glucose-2- C^{14}	2.86	295	2,400	0.08
7	Alloxan diabetes	500	30	6	Glucose-2- C^{14}	8.59	635	19,000	0.22
8	Alloxan diabetes, mild	220	53	24	Glucose-2- C^{14}	5.29	368	19,500	0.37
9	Glucagon-treated	500	72	14	Glucose-2- C^{14}	7.15	666	48,000	0.67
10	ACTH-treated	250	53	21	Glucose-2- C^{14}	2.01	153	8,100	0.40
11	Hyperthyroid	300	40	13	Glucose-2- C^{14}	2.86	237	9,500	0.33
12	Normal, lactate-injected (15 mM)	250	55	22	Glucose-2- C^{14}	4.29	127	7,000	0.16
13	Normal, fasted 72 hrs., then injected with 6 mM lactate	500	69	14	Glucose-2- C^{14}	5.72	455	31,400	0.55
14	Normal, fasted 72 hrs., then injected with 10 mM acetate	500	101	20	Glucose-2- C^{14}	5.72	485	49,000	0.86
16	Yoshida ascites tumor, 48 hrs.	150	29	19	Glucose-2- C^{14}	4.17	158	4,500	0.11
16	Yoshida ascites tumor, 7 days	165	39	24	Glucose-2- C^{14}	5.72	282	11,000	0.19
17	Yoshida ascites tumor, 10 days	250	50	20	Glucose-2- C^{14}	2.86	150	7,500	0.26
18	Normal	300	84	28	Ribose- $R-C^{14}$	0.95	613	51,500	5.40
		Histamine administered (μM)		Per cent administered histamine					
19	Normal	313	66	20	Glucose-2- C^{14}	2.86	115	7,600	0.27
20	Normal	272	32	12	Ribose-1- C^{14}	1.14	1,420	45,500	4.00

* Imidazoleacetic acid given orally.

TABLE II
Isotope distribution in urinary ribose following administration of glucose-2-C¹⁴

Experiment	Animal status	Relative radioactivity in ribose carbon atoms*				
		C-1	C-2	C-3	C-4	C-5
1	Normal	54	100	15	38	20
2	Normal	67	100	16	37	21
19	Normal (received histamine)	59	100	42	66	28
3	Thiamine-deficient	264	100		44	34
30	Thiamine-deficient (oxythiamine)	314	100	44	88	50
32	Thiamine-deficient (oxythiamine)	295	100			
4	24 hrs. after subtotal hepatectomy	68	100		45	18
4	48 hrs. after subtotal hepatectomy	90	100	16	48	13
5	72 hrs. after partial hepatectomy	97	100	16	40	23
7	Alloxan diabetes	64	100	17	38	14
8	Alloxan diabetes (mild)	49	100	16	20	9
9	Glucagon-treated	61	100			
10	ACTH-treated	82	100	19	40	20
11	Hyperthyroid	58	100		22	11
12	Lactate-treated	71	100	19	28	18
13	Lactate-treated	80	100			
14	Acetate-treated	63	100			
16	Yoshida ascites tumor, 2 days; C ¹⁴ -glucose subcutaneously	84	100	20	38	12
16	Yoshida ascites tumor, 7 days; C ¹⁴ -glucose subcutaneously	144	100		47	20
17	Yoshida ascites tumor, 10 days; C ¹⁴ -glucose intraperitoneally	80	100	51	101	77
31	Hypophysectomized	65	100			

* Radioactivity in carbon 2 is given an arbitrary value of 100.

the administration of ImAA to any of these animals. Histamine administration (Rats 19 and 20) was also followed by substantial ImAA riboside excretion. The animals injected with histamine, however, were markedly somnolent for several hours following injection.

Table I also summarizes the data concerning isotope incorporation into ribose following the administration of C¹⁴-labeled substrates. Ribose excreted by the normal rats given glucose-C¹⁴ contained 0.16 to 0.86 per cent of the administered radioactivity. In marked contrast, the two rats given ribose-C¹⁴ (18 and 20) incorporated 5.40 and 4.00 per cent of the injected C¹⁴ into the urinary pentose.

Table II depicts the isotope distribution in the urinary ImAA riboside ribose of the animals given glucose-2-C¹⁴. There was a striking consistency in the radioactivity pattern in the pentose of all of

the normal animals, with the C¹⁴ predominantly in carbon atoms 1 and 2, and the latter more active than the former. A variable fraction of the total radioactivity was distributed throughout the remainder of the ribose molecule with carbon 4 containing more isotope than positions 3 and 5. A striking departure from the normal carbon 1 to carbon 2 isotope ratio was seen in the thiamine-deficient animals (Rats 3, 30 and 32) where carbon 1 was as much as three times as radioactive as carbon 2. In Rats 4 (48 hour study) and 5, the rats with regenerating liver, carbon 1 approached carbon 2 in isotope content, and in one animal with massive ascites (16, seven day study), the activity in carbon 1 exceeded that in position 2. In Rat 17, the ascites tumor-bearing animal given glucose-C¹⁴ intraperitoneally, there was extensive isotope distribution throughout the ribose molecule.

TABLE III
Isotope distribution in urinary ribose isolated following administration of ribose-C¹⁴

Experiment	Administered sugar	Relative radioactivity in isolated ribose carbon atoms*					Relative radioactivity in administered ribose carbon atoms*				
		C-1	C-2	C-3	C-4	C-5	C-1	C-2	C-3	C-4	C-5
20	Ribose-1-C ¹⁴	100	4	2	1	5	100	0	0	0	0
18	Ribose-R-C ¹⁴	80	100	109	68	46	100	100	122	78	54

* In Experiment 20 carbon 1, and in No. 18 carbon 2, are given arbitrary values of 100.

TABLE IV

Isotope distribution in urinary ribose isolated following administration of glucose randomly-labeled with C¹⁴

Material	Relative radioactivity in sugar carbon atoms*					
	C-1	C-2	C-3	C-4	C-5	C-6
Isolated urinary ribose	84	121	100	87	68	
Administered glucose-C ¹⁴	111	97	114	100	89	80

* Carbon 3 of ribose and carbon 4 of glucose are given an arbitrary value of 100.

Table III describes the isotope distribution in the urinary ImAA riboside ribose of the rats injected with ribose-C¹⁴. The urinary ribose from the rat (Rat 20) given ribose-1-C¹⁴ had 90 per cent of its isotope in carbon 1. The isotope patterns in ribose excreted by the rat (No. 18) administered randomly-labeled ribose (Table III) and by the animal given randomly-labeled glucose (Table IV) were not markedly different from those in the injected sugars.

DISCUSSION

The advantages of the imidazoleacetic acid technique for studying ribose synthesis were described in a recent report (7). In addition to its relative ease, this method permits the separation of ribose with a much greater incorporation of administered radioactive carbon than is found in simultaneously isolated visceral nucleic acid ribose (7). The quantity of riboside excreted by our normal animals accounted for as much as 30 per cent of the administered ImAA. The one rat given ImAA orally excreted 19 per cent as the riboside. Tabor has shown that with sufficiently small doses of administered ImAA as much as 80 per cent is excreted as the riboside (19).

Although the site of riboside synthesis has not yet been determined, participation of the liver in this process is suggested by the substantial reduction in riboside excretion by the partially hepatectomized rats. The only other experimental condition which led to a very marked reduction in riboside excretion was severe diabetes. The presumed explanation for this observation is the low concentration in diabetic animals of glucose-6-phosphate, the precursor required for ribose synthesis. Whether the moderate reduction in ribose excretion by the hyperthyroid animal is significant will require further observations.

The pattern of isotope distribution in the ribose synthesized from glucose-2-C¹⁴ was remarkably

consistent in all of the normal animals. The C¹⁴ in carbon atoms 1 and 2 of the ribose is considered to reflect ribose production from hexose carbon via the C-1 oxidation pathway, and the nonoxidative mechanism involving the transketolase reaction, respectively. The latter is known to require thiamine pyrophosphate as a coenzyme (20, 21), and an impairment of this reaction has recently been observed in the erythrocytes of thiamine-deficient rats (6). Our Experiments 3, 30 and 32 provide evidence for an interference with transketolase activity *in vivo* in the thiamine-deficient animal. The isotope ratio in ribose carbon atom 1 to that in carbon 2 of 2.6 to 3.1 in these rats, as compared with a ratio of 0.6 in normal animals, is consistent with a profound diminution in the transketolase reaction. Of great interest is the absence of a concomitant reduction in total ribose excretion by the thiamine-deficient rats. Two explanations for this phenomenon are available. One possibility is that sufficient additional ribose was synthesized by way of the C-1 oxidation pathway to compensate for the depression of the transketolase mechanism. A less likely reason stems from the suggestion that isotope normally found in carbon 2 of ribose derived from hexose-2-C¹⁴ may result from an exchange, rather than from a synthetic reaction:

If such were the case, an impairment of the transketolase reaction could result in an alteration of the isotope pattern in ribose without any change in net pentose production. However, the results of the studies of ImAA riboside synthesized from randomly-labeled ribose and from randomly-labeled glucose (see below) indicate that exchange reactions probably have little effect on isotope distribution in ribose synthesized from glucose- C^{14} .

In the rats with regenerating liver the ribose carbon 1 to carbon 2 isotope ratio approached 1.0 and in one study in a tumor-bearing animal it exceeded 1.4. These observations may be interpreted in the light of our present concepts of the physiologic role of the glucose-6-phosphate and 6-phosphogluconic dehydrogenase reactions (4). These reactions, which result in the production of C-1 labeled pentose from C-2 labeled glucose, constitute the principal mechanism for the reduction of triphosphopyridine nucleotide (TPN). Since reduced TPN (TPNH) is required in a large number of reductive synthetic processes, including certain reactions involved in protein synthesis, an increased demand for TPNH might be anticipated in animals with regenerating liver or with rapidly growing tumors. Thus, the increased C-1 to C-2 ratio in these animals may reflect augmented requirements for TPNH. Further efforts to effect an increase in this ratio were made by attempting to stimulate other reactions in which TPNH is an essential cofactor. These include hexose synthesis from lactate (22), fatty acid synthesis from acetate (23), and steroid synthesis (24, 25). The administration of large quantities of lactate, acetate or ACTH, however, did not result in any shift in the ribose C-1 to C-2 ratio. Similarly, no change from the normal was observed in the isotope pattern of ribose synthesized by the thyrotoxic animal, although marked increases in the levels of liver glucose 6-phosphate and 6-phosphogluconic dehydrogenases have been found in thyroid-treated rats (5). The change from the normal in the isotope pattern of ribose obtained 48 and 72 hours, but not in that excreted 24 hours following partial hepatectomy may be related to the observation that regeneration of liver tissue is maximal from 48 to 96 hours following hepatectomy (26).

The marked randomization of radioactivity in the ribose excreted by the massive ascites tumor-

bearing Rat 17 makes difficult conclusions from the C-1 to C-2 isotope ratio. The randomization may be ascribed to the fact that this animal received glucose-2- C^{14} intraperitoneally. It has previously been shown that small quantities of hexose injected intraperitoneally are almost completely cleaved to triose by ascites tumor cells (16). Hexose synthesized from triose- C^{14} which reaches the livers of ascites tumor-bearing animals shows extensive isotope randomization (16). Marked isotope distribution was also present in the ribose excreted by the normal rat given glucose-2- C^{14} and histamine (Experiment 19). It is likely that the profound depression observed in the histamine-treated animals was accompanied by alterations in many metabolic processes.

The animals given ImAA or histamine and ribose- C^{14} incorporated 4 to 5 per cent of the administered C^{14} in the urinary ribose as compared with an incorporation of less than 1 per cent by rats given glucose- C^{14} . These observations do not permit quantitative conclusions concerning the relative efficiency of the two sugars as riboside ribose precursors because of differences in the ribose and glucose pool sizes. However, the data do exclude the possibility that ribose-carbon must be converted to hexose prior to its incorporation into riboside. Further evidence against such a sequence of events is provided by the isotope distribution in the urinary ribose of Rat 20, the animal given ribose-1- C^{14} . The pattern of radioactivity in the urinary ribose in this experiment was very similar to that in the administered ribose. Had a prior conversion to hexose taken place, the isotope pattern in the urinary ribose would have been vastly different [ribose-1- C^{14} is converted to hexose-1,3- C^{14} both *in vitro* (27) and *in vivo* (28)]. Similar evidence was presented for direct incorporation of ribose-1- C^{14} into nucleic acid ribose of a human carcinoma cell grown in tissue culture (3).

Ribose randomly labeled with C^{14} was administered to Rat 18 in an effort to obtain information concerning the possible role of exchange reactions in determining the isotope pattern of urinary ribose. An interchange of 2 carbon fragments ("active glycolaldehyde") between nonradioactive hexose and randomly- C^{14} -labeled pentose phosphates would be expected to effect a dilution in the radioactivity in carbon 2 relative to carbon 3 of

the urinary ribose, as compared with the pattern in the starting material:

It is apparent that little change in the C-2 to C-3 ratio took place in our study. It is, of course, true that such an exchange reaction could occur only if ribose-5-phosphate were converted to xylulose-5-phosphate, the pentose which acts as the "active glycolaldehyde" donor in the transketolase reaction (29, 30), before all of the ribose-5-phosphate was consumed in riboside synthesis. However, the interconversion of the pentose phosphates is known to be rapid (31), and hence the phosphorylation of ribose would be immediately followed by the presence of an equilibrium mixture of the pentose phosphates. Further evidence against the possibility that exchange reactions have altered the isotope distribution is provided by the study of ribose synthesized from randomly-labeled glucose. Here again, the pattern of radioactivity in the urinary ribose is not markedly different from that in the starting material.

SUMMARY

Ribose synthesis *in vivo* has been studied by isolating imidazoleacetic acid riboside from rats given imidazoleacetic acid and a C¹⁴-labeled sugar. Evidence is presented which indicates an impairment of riboside excretion in partially hepatectomized and in diabetic animals. The isotope distribution in ribose synthesized from glucose-2-C¹⁴ by normal animals is consistent with synthesis via both the oxidative and the nonoxidative reactions of the pentose phosphate pathway. Thiamine deficiency resulted in a marked decrease of ribose synthesis from hexose via the nonoxidative mechanism. An apparent increase in ribose production by way of the oxidative reactions was observed in

rats with regenerating livers and in tumor-bearing animals. These observations are discussed in the light of our present concepts of the role of the oxidative reactions in TPNH production for synthetic processes. Evidence is presented for the direct incorporation of administered ribose into the urinary riboside.

ACKNOWLEDGMENT

The valuable technical assistance of Misses Jacqueline Lareau and Madeline Goldstein is gratefully acknowledged.

REFERENCES

- Bernstein, I. A. Synthesis of ribose by the rat. *Biochim. biophys. Acta* 1956, **19**, 179.
- Marks, P. A., and Feigelson, P. The biosynthesis of nucleic acid ribose and of glycogen glucose in the rat. *J. biol. Chem.* 1957, **226**, 1001.
- 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.
- Horecker, B. L., and Hiatt, H. H. Pathways of carbohydrate metabolism in normal and neoplastic cells. *New Engl. J. Med.* 1958, **258**, 177 and 225.
- Glock, G. E., and McLean, P. A preliminary investigation of the hormonal control of the hexose monophosphate oxidative pathway. *Biochem. J.* 1955, **61**, 390.
- Brin, M., Shohet, S. S., and Davidson, C. S. Effect of thiamine deficiency on mammalian erythrocyte metabolism. *Fed. Proc.* 1956, **15**, 224.
- Hiatt, H. H. Studies of ribose metabolism. II. A method for the study of ribose synthesis *in vivo*. *J. biol. Chem.* 1957, **229**, 725.
- Tabor, H., and Hayaishi, O. The excretion of imidazoleacetic acid riboside following the administration of imidazoleacetic acid or histamine to rats. *J. Amer. chem. Soc.* 1955, **77**, 505.
- Karjala, S. A. The partial characterization of a histamine metabolite from rat and mouse urine. *J. Amer. chem. Soc.* 1955, **77**, 504.
- Hiatt, H. H., and Lareau, J. Studies of ribose metabolism. VII. A quantitative assessment of ribose biosynthesis from hexose via the C-6 oxidation pathway. *J. biol. Chem.* Oct., 1958.
- Takagi, Y., and Horecker, B. L. Purification and properties of a bacterial riboside hydrolase. *J. biol. Chem.* 1957, **225**, 77.
- Somogyi, M. Determination of blood sugar. *J. biol. Chem.* 1945, **160**, 69.
- Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem.* 1944, **153**, 375.

14. Albaum, H. G., and Umbreit, W. W. Differentiation between ribose-3-phosphate and ribose-5-phosphate by means of the orcinol-pentose reaction. *J. biol. Chem.* 1947, **167**, 369.
15. Drury, A. N., Harris, L. J., and Maudsley, C. Vitamin B deficiency in the rat. Bradycardia as a distinctive feature. *Biochem. J.* 1930, **24**, 1632.
16. Hiatt, H. H. Glycolytic activity *in vivo* of the mouse Ehrlich ascites tumor. *Cancer Res.* 1957, **17**, 240.
17. Bauer, H., and Tabor, H. The synthesis of imidazoleacetic acid. *Biochem. Prep.* 1957, **5**, 97.
18. Hiatt, H. H., Goldstein, M., Lareau, J., and Horecker, B. L. The pathway of hexose synthesis from pyruvate in muscle. *J. biol. Chem.* 1958, **231**, 303.
19. Tabor, H. The fate of histamine in the body *in Ciba Foundation Symposium on Histamine*. Boston, Little, Brown & Co., 1956, p. 318.
20. Horecker, B. L., and Smyrniotis, P. Z. The coenzyme function of thiamine pyrophosphate in pentose phosphate metabolism. *J. Amer. chem. Soc.* 1953, **75**, 1009.
21. Racker, E., de la Haba, G., and Leder, I. G. Thiamine pyrophosphate, a coenzyme of transketolase. *J. Amer. chem. Soc.* 1953, **75**, 1010.
22. Ochoa, S., Mehler, A. H., and Kornberg, A. Biosynthesis of dicarboxylic acids by carbon dioxide fixation. I. Isolation and properties of an enzyme from pigeon liver catalyzing the reversible oxidative decarboxylation of l-malic acid. *J. biol. Chem.* 1948, **174**, 979.
23. Langdon, R. G. The biosynthesis of fatty acids in rat liver. *J. biol. Chem.* 1957, **226**, 615.
24. Hayano, M., and Dorfman, R. I. On the mechanism of C-11 β -hydroxylation of steroids. *J. biol. Chem.* 1954, **211**, 227.
25. Olson, J. A., Jr., Lindberg, M., and Bloch, K. On the demethylation of lanosterol to cholesterol. *J. biol. Chem.* 1957, **226**, 941.
26. Higgins, G. M., and Anderson, R. M. Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch. Path.* 1931, **12**, 186.
27. 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.
28. Hiatt, H. H. Glycogen formation via the pentose phosphate pathway in mice *in vivo*. *J. biol. Chem.* 1957, **224**, 851.
29. Srere, P. A., Cooper, J. R., Klybas, V., and Racker, E. Xylulose-5-phosphate, a new intermediate in the pentose phosphate cycle. *Arch. Biochem.* 1955, **59**, 535.
30. Horecker, B. L., Hurwitz, J., and Smyrniotis, P. Z. Xylulose 5-phosphate and the formation of sedoheptulose 7-phosphate with liver transketolase. *J. Amer. chem. Soc.* 1956, **78**, 692.
31. Dickens, F., and Williamson, D. H. Pentose phosphate isomerase and epimerase from animal tissues. *Biochem. J.* 1956, **64**, 567.