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Tetrose Metabolism

2. THE UTILIZATION OF TETROSES AND TETRITOLS BY RAT TISSUES

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Very little definite information is available about the metabolism of either tetroses or tetrutols in the whole animal or in tissue preparations. This is surprising since there is now a considerable body of evidence that tetroses or their phosphates are capable of a variety of enzymic transformations. Thus the aldotetrose D-erythrose was first implicated in the pentose phosphate pathway of carbohydrate metabolism as a probable product, in the form of D-erythrose 4-phosphate, of the action of transaldolase on sedoheptulose 7-phosphate (Horecker & Smyrniotis, 1954; Horecker, Gibbs, Klenow & Smyrniotis, 1954; Horecker & Smyrniotis, 1955; Horecker, Smyrniotis, Hiatt & Marks, 1955):

Sedoheptulose 7-phosphate + D-glyceraldehyde 3-phosphate $\rightleftharpoons$ D-fructose 6-phosphate + D-erythrose 4-phosphate.

The reversibility of this reaction was considered probable; Racker, de la Haba & Leder (1954) were able to show that fructose 6-phosphate was itself split by transketolase yielding 'active glycolaldehyde' and again, presumably, D-erythrose 4-phosphate. The same product, together with dihydroxyacetone phosphate, was formed by the reversible cleavage by aldolase of sedoheptulose 1:7-diphosphate (Horecker *et al.* 1955; Smyrniotis & Horecker, 1956). Finally, Ballou, Fischer & MacDonald (1955) synthesized chemically D-erythrose 4-phosphate and showed that, with aldolase and dihydroxyacetone phosphate, sedoheptulose 1:7-diphosphate was formed. Kornberg & Racker (1955) showed that the synthetic tetrose ester behaved in the same

manner as the substance obtained as a barium salt (Srere, Kornberg & Racker, 1955) from the action of transketolase on fructose 6-phosphate with glyceraldehyde as 'acceptor aldehyde'.

L-Erythrulose is also a substrate for transketolase (Horecker & Smyrniotis, 1953; Horecker, Smyrniotis & Klenow, 1953); the latter paper, however, suggested that free L-erythrulose cannot be an intermediate in the synthesis by transketolase of heptulose phosphate from pentose phosphate, since added L-erythrulose did not become appreciably radioactive when $[1-^{14}\text{C}]$ ribose 5-phosphate was the substrate, nor did it much dilute the isotope appearing in the sedoheptulose formed. The reverse reaction, the synthesis of L-erythrulose from transketolase acting on hydroxypyruvate with glyceraldehyde as acceptor, was shown by Dickens & Williamson (1956) [cf. Dickens (1958)] by isolation and determination of optical rotation of the recrystallized *o*-nitrophenylhydrazone of the product. L-Erythrulose is also reversibly reduced by the 'diphosphopyridine nucleotide-xylitol (D-xylulose) dehydrogenase' of guinea-pig liver in the presence of reduced diphosphopyridine nucleotide, as is fully described in the preceding paper (Batt, Dickens & Williamson, 1960*b*): the product is L-threitol.

Quite a different route for synthesis of ketotetrose 1-phosphate in liver was discovered by Charalampous & Mueller (1953) and Charalampous (1954), who found that an aldolase-like enzyme from liver causes condensation of dihydroxyacetone phosphate with formaldehyde, yielding an erythrulose 1-phosphate; we have presented evidence (Batt *et al.* 1960*b*) that this is also the L-erythrulose derivative. It was shown much earlier (Meyerhof, Lohmann & Schuster, 1936; cf. Lohmann, 1941) that a ketotetrose 1-phosphate resulted from this reaction when catalysed by crude muscle aldolase, and recently (Peanasky & Lardy, 1958) this has been confirmed with the once-crystallized rabbit-muscle enzyme and also with crystalline ox-liver aldolase. The claim of Charalampous (1954) that a specific liver enzyme, 'phosphoketotetrose aldolase', is involved needs reinvestigation in view of the results obtained by Peanasky & Lardy, whose crystalline liver aldolase had 2.5 times the specific activity of the best preparations of Charalampous, and appeared to be a typical aldolase in its action on fructose 1:6-diphosphate.

The interesting possibility that a diphosphate of a tetrose might be a regulator of the activity of glyceraldehyde 3-phosphate dehydrogenase, which it powerfully and specifically inhibits, has been raised by recent work by Racker, Klybas & Schramm (1959): the substance appears to be D-threose 2:4-diphosphate (Fluharty & Ballou, 1959). D-Erythrose 4-phosphate strongly inhibits phosphoglucose isomerase (Grozi, De Flora & Pontremoti, 1960).

In contrast with these enzymic studies showing varied activities of tetroses and tetrutols, metabolic studies on these substances are largely lacking. Erythritol, as the most available substance in this series, has received some attention (reviewed by Carr & Krantz, 1945). Beck, Carr & Krantz (1938) showed that erythritol and its anhydride, erythritan, were absorbed readily from the gastrointestinal tract of rats, were excreted in the urine in large amounts and were not precursors of liver glycogen in the fasting hen or rat. Meyer, McTiernan & Slater (1934) observed negligible anaerobic acid formation from erythritol by slices of mouse sarcoma 180. The urinary excretion in a normal man of erythritol and its oxidation by crude liver extracts has been briefly reported (Touster, Hecht & Todd, 1959). Metabolic studies on D- and L-threitol, the two optical isomers of erythritol, have not been made, according to Carr & Krantz (1945, p. 180) who state that in their opinion 'these substances will be found refractory to metabolism by the animal body'. This view, which appears to be generally held, is shown in the present work not to be entirely correct.

No studies on the phosphorylation in the animal body of these compounds have been found, but both the chemical and enzymic syntheses of D-erythritol 4-phosphate have been described by MacDonald, Fischer & Ballou (1956) and Shetter (1956) respectively. The latter used extracts of dried erythritol-adapted cells of *Propionibacterium pentosaceum* (Barker & Lipmann, 1949) for the preparation of the kinase, and established the optical specificity, but it should be noted that this compound could also be called L-erythritol 1-phosphate, thus emphasizing the analogy with the L- α -glycerophosphate which is formed by mammalian glycerokinase and adenosine triphosphate (ATP) acting upon glycerol (Bublitz & Kennedy, 1954; Karnovsky, Hauser & Elwyn, 1957):

The 'isotopic asymmetry' of the two [α - ^{14}C]erythritols, and the identity of each of the two D- and L-[α - ^{14}C]threitol has already been discussed (Batt *et al.* 1960b). In the present work only the metabolism of the [1 - ^{14}C]erythritol produced by catalytic hydrogenation of L-[4 - ^{14}C]erythrulose (D-[1 - ^{14}C]erythritol, in the above-mentioned nomenclature) has been studied; it appeared not to be metabolized to radioactive carbon dioxide or glucose by rat-liver slices.

By the methods of preparation, identification and degradation described by Batt *et al.* (1960b) we have also investigated the metabolism in the whole rat and in rat-liver slices of the following specifically labelled tetroses and tetrutols: in the whole rat, L-[1 - ^{14}C]- and L-[4 - ^{14}C]-erythrulose, and in rat-liver slices both of these substrates and also D-[4 - ^{14}C]erythrose, as well as a number of specifically ^{14}C -labelled tetrutols. The percentage of radioactivity appearing in respiratory carbon dioxide, in glucose (or liver glycogen, in the intact rat), and in some cases in the urinary excretion of isotope, have been determined. In addition, the glycogen formed from L-[1 - ^{14}C]- and L-[4 - ^{14}C]-erythrulose has been hydrolysed and the localization of ^{14}C in the individual glucose carbon atoms has been determined. Similar degradations have been made on the glucose formed on incubation of these substances, and also of D-[^{14}C]erythrose, with liver slices. A brief account of this work has been presented (Batt, Dickens & Williamson, 1960a).

EXPERIMENTAL

Materials and analytical methods. These were as already described (Batt *et al.* 1960b). In addition, methods of animal experiments and general outlines of procedure, including measurement of radioactivity, were the same as those detailed by Dickens & Williamson (1959) for studies on hydroxypyruvate metabolism in the whole rat and in tissue slices. The following points are additional to those given in that paper.

Degradation of glucose for determination of location of ^{14}C . In the previous work (Dickens & Williamson, 1959), two methods were used for this purpose: fermentation by *Leuconostoc mesenteroides*, which yields isotope values for each of the six glucose carbon atoms, and yeast fermentation, which is quantitative only for determination of C-3 and C-4 of glucose.

In the present work difficulties were encountered in the use of *L. mesenteroides*. The amounts of radioactivity in the glucose were not sufficient to permit its recrystallization,

and it is thought that purification by removal of ionized substances by Bio-Deminrolit G followed by paper chromatography was insufficient to remove some persistent impurity. Consequently, although satisfactory recoveries of radioactivity continued to be obtained with pure test samples of specifically labelled glucose, in experiments where glucose formation from tetroses (particularly L-erythrulose) was being studied in tissue slices, the glucose fraction analysed for radioactivity by *L. mesenteroides* showed rather inconsistent results. In particular, the values obtained for labelling at C-4 of glucose were occasionally suspiciously high. One such example is cited in the preliminary publication, in the experiment with L-[4 - ^{14}C]erythrulose (Batt *et al.* 1960a).

In order to avoid this source of uncertainty, two alternative methods of degradation of glucose have been successfully applied. The former (Topper & Hastings, 1949) was purely chemical, beginning with the preparation of phenylglucosazone from the sample, a step which, together with the recrystallization of this compound, effects sufficient purification for reliable results to be obtained. Periodate oxidation of the recrystallized glucosazone yields C-1, C-2 and C-3 of glucose in the form of the phenyl-osazone of mesoxalic semialdehyde (Topper & Hastings, 1949), the radioactivity of which is measured and compared with the total activity of the recrystallized glucosazone. Also formed by the periodate oxidation are two molecules of formic acid from C-4 and C-5, and one molecule of formaldehyde from C-6. The former is converted into BaCO_3 and the latter into the dimedone compound for counts of specific activity. Details of procedure are given by Batt *et al.* (1960b).

For control of this procedure, D-[1 - ^{14}C]- and D-[6 - ^{14}C]-glucose (obtained from The Radiochemical Centre, Amersham, Bucks.) were appropriately diluted with unlabelled glucose. The subsequent analyses are summarized in Table 1.

Degradation methods and results for the other substrates used have already been described in detail (Batt *et al.* 1960b).

The above-mentioned methods of degradation were those mainly used, but a few experiments made use of *Lactobacillus casei* fermentation (Bernstein & Wood, 1957). Here the glucose molecule is symmetrically cleft into two molecules of lactate, which are then chemically degraded. As a result the methyl groups appear as the sum of C-1 and C-6, the secondary alcohol groups as the sum of C-2 and C-5 and the carboxyl groups as the sum of C-3 and C-4, all being counted as BaCO_3 . Here also satisfactory control analyses on pure samples of labelled glucose were obtained. Because of the complications caused by tetrose metabolites in purification of glucose for the other methods, the precipitation as the osazone and subsequent degradation by periodate oxidation were generally preferred.

Procedure with intact rats. Male albino rats of the Wistar strain (wt. approx. 200 g.) were fasted for 18 hr., injected

Table 1. Degradation of specifically labelled [^{14}C]glucose by periodate oxidation of glucosazone

Substance	C-1, C-2, C-3	C-4, C-5	C-6	Overall recovery (%)
D-[1 - ^{14}C]Glucose	97.8	0.4	1.8	95.0
D-[6 - ^{14}C]Glucose	3.2	5.0	91.8	88.0

intraperitoneally with a trace dose (e.g. $10\ \mu\text{moles}$, $1.5\ \mu\text{C}$) of the labelled tetrose or tetritol, followed by administration by stomach tube of 1 g. of glucose in 3 ml. of water. The rat was placed in a metabolism chamber consisting of a large desiccator with a top tubulure through which a stream of air was continuously drawn, after being freed from CO_2 by passage through large sintered-glass 'bubblers' containing 2N-NaOH solution. The rate of passage of gas was followed on a flow meter, and the effluent gases were passed through two more sintered-glass scrubbers to collect the respiratory CO_2 in NaOH solution free from carbonate. This apparatus was developed in conjunction with Dr G. E. Glock and Dr P. McLean, and it also permitted the separate collection of the rat's urine excreted in the 3 hr. period. The CO_2 was usually collected after each hour for a total period of 3 hr., when the animal was removed and killed, the liver being at once dissected for analysis. For this purpose the weighed liver tissue was first macerated for 1 min. with 15 vol. of 7% trichloroacetic acid and 2 g. of Celite 535, and stirred at 0° for 15 min. The glycogen precipitated from the filtrate by the addition of 1.1 vol. of ethanol was collected after 16 hr. at 0° , washed, dried, weighed and plated on planchets for determination of radioactivity. The glycogen was then hydrolysed by $2\text{N-H}_2\text{SO}_4$ (3 hr. at 100°), the solution deionized with Bio-Deminrolit G and the resulting glucose solution treated with phenylhydrazine for the preparation and recrystallization of the osazone, as described in the references quoted above. In some experiments the glucose solution was first concentrated, streaked upon filter paper for chromatographic purification and eluted with water from the band cut from the paper (see Batt *et al.* 1960b).

The urine collected from the rat-metabolism chambers was measured and the total ^{14}C determined on a small portion by oxidation with the persulphate- AgNO_3 reagent to $^{14}\text{CO}_2$, followed by its conversion into $\text{Ba}^{14}\text{CO}_3$ for counting (Katz, Abraham & Baker, 1954). After deionization with Bio-Deminrolit G, the solution and washings were concentrated *in vacuo* and chromatographed as a band on paper with ethyl acetate-pyridine-water (12:5:4) for 16 hr. Suitable markers were run simultaneously, and the band corresponding to an erythritol marker was eluted, the eluate made to 10 ml. with water and the total $^{14}\text{CO}_2$ liberated from a 5 ml. portion was determined as before by persulphate oxidation. This is a measure of the excreted tetrityls. A large part (average 75%) of the excreted ^{14}C -labelled compounds was removed by the treatment with the mixed-bed resin: the nature of this fraction remains to be investigated.

For the identification of the urinary tetritol, apart from the chromatography which identifies it as either erythritol or threitol (not separated in the solvents used; Batt *et al.* 1960b), electrophoresis on paper in borate at pH 6 (cf. Batt *et al.* 1960b) was also employed. The results (see below) were consistent with the expected nature of this product as threitol, presumably L-threitol produced by diphosphopyridine nucleotide (DPN)-xylitol dehydrogenase (Batt *et al.* 1960b).

Experiments with liver slices. Freshly excised liver from fed Wistar rats was sliced and divided into 1 g. batches of slices, which were incubated at 37° with shaking in Krebs-Ringer solution in $\text{O}_2 + \text{CO}_2$ (95:5) exactly as described by Dickens & Williamson (1959), except for the substrate, which consisted of the labelled tetrose or tetritol as shown

in the tables. The subsequent liberation and collection as BaCO_3 of the CO_2 , deproteinization and purification of the glucose contained in the solution were as described by Dickens & Williamson (1959). In addition to the treatment with Bio-Deminrolit G, the filtrate and water washing from the resin were concentrated *in vacuo*, usually to 0.5 or 1.0 ml., and the concentrate was chromatographed on paper [ethyl acetate-pyridine-water (12:5:4)] with suitable markers. The glucose area was cut and eluted with water, the eluate made to 10 ml., and unlabelled carrier glucose (50 mg.) was added after estimation of the glucose content by the method of Nelson (1944). The bands corresponding to the marker erythritol (and in some experiments also marker erythrulose) were also eluted similarly. These fractions are referred to as 'tetritol' and 'ketotetrose' in the Tables. The total ^{14}C in each of these fractions was determined after addition of carrier erythritol or erythrulose, by oxidation to CO_2 of measured portions by means of the persulphate- AgNO_3 reagent (Katz *et al.* 1954), conversion into BaCO_3 , and plating on planchets for determination of radioactivity.

After the total radioactivity in a portion of the purified glucose fraction had been similarly determined, the osazone was prepared from this fraction (Batt *et al.* 1960b) and its radioactivity was determined before and after crystallization. The osazone was then degraded by periodate oxidation as already described.

RESULTS

Metabolism of L-[1- ^{14}C]- and L-[4- ^{14}C]-erythrulose in the intact rat

The data for rats injected with L-[1- ^{14}C]erythrulose (dose 9–10 μmoles ; $1.38\ \mu\text{C}$) and L-[4- ^{14}C]erythrulose (8–9 μmoles ; $1.75\ \mu\text{C}$) are collected in Table 2. Whereas an average of 25.4% of the ^{14}C appeared in the respiratory CO_2 during the 3 hr. of the experiments, only 15.5% of the ^{14}C was converted into CO_2 in closely similar conditions. Incorporation of radioactivity into liver glycogen was, however, slightly higher (4.8% of the dose) with the 4- ^{14}C compound than with the 1- ^{14}C one (3.8%). Amounts between 11 and 26% of the dose appeared in the urine with the two substances, these figures being more variable than the rather constant values for glycogen and CO_2 incorporation. With both the [1- ^{14}C]- and [4- ^{14}C]erythrulose, only about one-fifth of the total urinary excretion is due to tetrityls. The fact that there was very little radioactivity at any point on the paper other than that at which the tetrityls were found, suggests strongly that unknown ionized components of the urine, removed by the treatment with ion-exchange resin, comprise the other main urinary metabolites.

The total percentage of the dose accounted for is, however, considerable (45% for L-[1- ^{14}C]erythrulose, 40% for L-[4- ^{14}C]erythrulose; see Table 2).

Table 3 includes the results obtained on the localization of labelling in glucose carbon atoms

of glycogen by means of periodate degradation of the purified glucosazones. An average of 83 % of the total radioactivity was recovered in the carbon atoms evaluated. Results of separate experiments with different rats were highly consistent and showed a significantly different pattern of labelling of glucose with the two differently labelled erythroses. That with the [4-¹⁴C]-erythrose was more symmetrical, activity in C-1, C-2 and C-3 of glucose being about equal to that in C-6. Most of the activity in the first three carbon atoms is thought, by analogy with results reported later with liver slices, to be contained in C-1, which would thus become approximately equally labelled to C-6.

With the 1-¹⁴C-labelled substrate, about twice as much radioactivity appears in C-1, C-2 and C-3 (again probably mainly in C-1) as is present in C-6 of the glucose isolated from the liver glycogen.

With either isotope labelled erythrose, very little activity (total 5-13 %) appears in C-4 and C-5 of the glucose formed.

The respiratory CO₂ (3 hr. totals only are shown in Table 2) was sampled at 1 hr. intervals; on the average, with [1-¹⁴C]erythrose, 45 % of the ¹⁴CO₂ expired in the 3 hr. period appeared in the first hour, and 35 and 20 % in the second and third hours, showing that the oxidative metabolism continued throughout this period at a steadily diminishing rate. With [4-¹⁴C]erythrose a different picture resulted, 25 % being expired in the first hour and a peak of 45 % being reached in the second hour, falling to 30 % in the third hour of the experiment. The total elimination of ¹⁴CO₂ was also much higher with the 1-¹⁴C-labelled compound, being 25.4 % of the total dose of radioactive carbon, as compared with 15.5 % for the 4-¹⁴C-labelled compound.

Table 2. Utilization of specifically labelled L-erythrose in the intact rat

Rat no.	Wt. of liver glycogen (mg.)	Percentage of dose of ¹⁴ C				Total accounted for (%)
		In ¹⁴ CO ₂	In glycogen	In urine	In urinary tetritol	
L-[1- ¹⁴ C]Erythrulose (dose 10 μmoles, 1.38 μc)						
1	124	25.0	3.0	18.8	4.0	46.8
2	87	23.4	2.4	17.4	—	43.2
3	131	23.8	4.6	14.5	7.9	42.9
4	190	29.4	5.1	10.9	2.6	45.4
Average	133	25.4	3.8	15.4	4.8	44.6
L-[4- ¹⁴ C]Erythrulose (dose 8-9 μmoles, 1.75 μc)						
5	88	18.4	4.2	25.7	—	48.6
6	112	13.2	5.4	24.0	3.1	42.6
7	100	15.6	4.2	16.6	3.7	36.8
8	63	14.6	5.4	13.1	3.5	33.0
Average	91	15.5	4.8	19.9	3.4	40.3

Table 3. Distribution of ¹⁴C in liver glycogen from rats injected with labelled L-erythroses

Rat numbers are the same as those of Table 2. Liver glycogen was isolated, hydrolysed, and glucosazone degraded (see text).

	Percentage of total activity in glucose C atoms			Total ¹⁴ C recovery on degradation (%)
Rat no.	C-1 + C-2 + C-3	C-4 + C-5	C-6	
L-[1- ¹⁴ C]Erythrulose				
1	61	13	26	83
2	64	13	23	83
3	59	11	30	94
4	64	6	29	80
Average	62	11	27	85
L-[4- ¹⁴ C]Erythrulose				
5	49	10	41	84
6	53	5	42	84
7	43	10	47	83
Average	48	8	45	84

Nature of urinary tetrilotols

In order to obtain sufficient material to ascertain the nature of urinary tetrilotols, half-portions of each of the chromatographically separated tetrilotol fractions from the urine of four rats which had received injections of L-[1-¹⁴C]erythrulose (see Table 1) were pooled, evaporated to 0.5 ml. *in vacuo* and streaked on Whatman no. 1 paper (24 cm. × 20 cm.). Markers of erythritol and threitol were applied at each side, and the paper was subjected to electrophoresis in saturated aqueous boric acid adjusted to pH 6.0 with 10N-NaOH; 400v, 10 mA; 17 hr. at 4°. Erythritol moved 8 mm. towards the cathode, threitol and marginal spots of the urinary tetrilotol moved 32 mm. towards the anode (periodate-benzidine

dip was used on marginal strips for localization). The initial total radioactivity of the pooled fractions (determined before concentration on one-tenth of a portion) was 0.12 μ C. The total radioactivity eluted from the cut-out band corresponding with the threitol marker gave a recovery of 42% as [¹⁴C]threitol. No other spots were detectable on the paper after the electrophoresis and it is concluded that the urinary tetrilotol was exclusively threitol.

Metabolism of tetroses and tetrilotols in liver slices

Table 4 shows the percentage of tracer doses of radioactivity converted into ¹⁴CO₂, [¹⁴C]glucose and [¹⁴C]tetrilotol on incubation with rat-liver slices (see Experimental section) of the following substances: L-[1-¹⁴C]erythrulose (10 μ moles, 0.45 μ C/vessel);

Table 4. *Metabolism of tetroses and tetrilotols in rat-liver slices*

For details of substrates see text. Period: 90 min. at 37°; O₂ + CO₂ (95:5). Respiratory CO₂, glucose, threitol and, in some experiments, erythrulose were isolated from incubation mixture after shaking 1 g. (wet wt.) of slices with substrate as shown.

Rat no.	Percentage of added ¹⁴ C found in			
	Resp. CO ₂	Glucose	Tetrilotol	Erythrulose
Substrate: L-[1- ¹⁴ C]Erythrulose (amount per vessel: 10 μ moles, 0.45 μ C)				
8	13.9	4.8	—	—
9	9.6	6.2	19	18
10	14.9	4.5	13	—
11	12.0	4.3	—	6
12	8.9	3.7	8	—
13	12.9	2.3	6	—
Average	12.0	4.3	11.5	12
Substrate: L-[4- ¹⁴ C]Erythrulose (amount per vessel: 10 μ moles, 1.2 μ C)				
14	4.9	1.4	—	—
9	6.9	2.5	22	18
10	7.1	3.8	12	16
11	4.9	2.8	—	9
12	3.7	1.9	10	—
13	4.1	2.4	5	—
Average	5.3	2.4	12	14
Substrate: D-[4- ¹⁴ C]Erythrulose (amount per vessel: 10 μ moles, 0.48 μ C)				
15	1.3	3.6	—	—
15	0.7	1.7	—	—
Substrate: Tetrilotols from reduction of labelled L-erythruloses				
14 ^a	1.0	0.50	—	—
14 ^b	0.9	0.75	—	—
17 ^c	1.2	0.50	—	—
18 ^d	0.0	0.04	—	—
19 ^e	1.0	0.10	—	—
19 ^f	1.4	0.13	—	—
20 ^e	2.0	0.35	—	—
20 ^f	1.1	0.30	—	—

^{a, b} Substrate, 10 μ moles (0.48 μ C) of catalytic hydrogenation product from L-[1-¹⁴C]erythrulose (example of duplicate batches of slices).

^c 6 μ moles (0.50 μ C) of tetrilotols as described above from hydrogenation of L-[4-¹⁴C]erythrulose.

^d 6 μ moles (0.69 μ C) of electrophoretically separated erythritol fraction from catalytic hydrogenation of L-[4-¹⁴C]-erythrulose.

^e Product of enzymic reduction of L-[1-¹⁴C]erythrulose; 5.1 μ moles (0.68 μ C) = L-[1-¹⁴C]threitol.

^f Product of enzymic reduction of L-[4-¹⁴C]erythrulose; 5.0 μ moles (0.71 μ C) = L-[1-¹⁴C]threitol.

L-[4- ^{14}C]erythrulose (10 μmoles , 1.2 μC); the mixed tetrutols formed by catalytic hydrogenation of each of the above ketotetroses (see Batt *et al.* 1960b) containing 0.48–0.69 μC in 6–10 μmoles of total tetrutol; and electrophoretically purified erythritol isolated from the catalytic hydrogenation, as described above, of L-[4- ^{14}C]erythrulose. Finally, L-[1- ^{14}C]threitol, produced by reduction of labelled L-erythrulose with reduced diphosphopyridine nucleotide (DPNH) and the DPN-xylitol (D-xylulose) dehydrogenase, was tested as substrate; it was shown in the preceding paper that this product is formed either from L-[1- ^{14}C]erythrulose or L-[4- ^{14}C]erythrulose, both of which give the same ^{14}C -labelled compound on enzymic reduction by this enzyme (Batt *et al.* 1960b).

Preliminary experiments on slices of liver tissue of two rats, incubated with D-[4- ^{14}C]erythrulose, are also included in Table 4; this substance was prepared chemically from D-[6- ^{14}C]glucose (Batt *et al.* 1960b) and used in amounts of 10 μmoles (0.48 μC)/vessel.

Of the two labelled ketotetroses included in Table 4, the 1- ^{14}C -labelled compound is much the richer source of respiratory $^{14}\text{CO}_2$ (average 12.3% compared with 5.2% for the 4- ^{14}C -labelled compound), though it contributes about the same radioactivity (av. 12%) to the tetrutol (presumably L-threitol), which accumulates in the incubation medium: the amount of the latter is, however, very variable in individual experiments. The incorporation into glucose of ^{14}C also varies considerably from rat to rat, but tends to be higher, sometimes twice as high, with the [1- ^{14}C]ketotetrose. The extent of incorporation (1.3–6% of the dose) was in a similar range to that observed for D- and L-serine (Dickens & Williamson, 1959) and for a number of related compounds cited in that paper.

Table 4 shows also the behaviour with liver slices of D-[4- ^{14}C]erythrulose. The amount of radioactivity appearing in the respiratory CO_2 is much less than with either of the labelled ketotetroses (average 1.1%), whereas the incorporation into glucose is in the two experiments (average 3%) about the same. It would be desirable to extend these experiments with 1- ^{14}C -labelled erythrulose but this compound was not available.

By comparison with the above keto- and aldotetroses, the tetrutols studied appear to be poorly metabolized.

The catalytic hydrogenation (Batt *et al.* 1960b) of L-erythrulose produces a mixture consisting of about 75% of erythritol and 25% of L-threitol. The erythritol fraction isolated from the mixture by high-voltage electrophoresis (Table 4, rat 18^d) did not appear to be utilized appreciably, since ^{14}C from the 4- ^{14}C -labelled compound contributed no detectable radioactivity to the respiratory CO_2 and

a very low amount (0.04% of the total activity) to the glucose isolated after incubation.

The catalytic reduction products from L-[1- ^{14}C]erythrulose (rat 14, duplicates 14^a and 14^b) were, however, incorporated appreciably (1% as $^{14}\text{CO}_2$, 0.6% as [^{14}C]glucose). Closely similar figures were obtained with the mixed tetrutols from L-[4- ^{14}C]erythrulose (rat 17^c; in this and the following examples only means of duplicate batches of slices, which agreed well, are reported). This similarity is understandable since both substances give the same threitol (L-[1- ^{14}C]- or L-[4- ^{14}C]-threitol, which are identical: Batt *et al.* 1960b). Presumably only the threitol component is metabolized, though poorly. This view was confirmed by studies on the product of enzymic reduction of the two labelled L-erythruloses, prepared by means of DPNH and the DPN-xylitol (L-xylulose) dehydrogenase (rats 19 and 20^e and 20^f). These products have been shown by specific degradation methods (Batt *et al.* 1960b) to have 90% or more of the isotopic labelling in the named carbon atoms.

Isotopic identity of [1- ^{14}C]- and [3- ^{14}C]-dihydroxyacetone

Preparation of [α - ^{14}C]dihydroxyacetone. L-Erythrulose (100 μmoles) was incubated at 37° for 2 hr. with [^{14}C]formaldehyde (50 μmoles , 4.0 μC) and yeast transketolase (0.4 ml.) in 2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer (100 ml., 0.2M, pH 7.6), MgCl_2 (50 μmoles) and thiamine pyrophosphate (0.5 mg.; cf. Dickens & Williamson, 1958; for details see Batt *et al.* 1960b). In 120 min. 33 μmoles of formaldehyde had been utilized (acetylacetone analysis on 0.2 ml. portions). Ice-cold 20% perchloric acid (0.5 ml.) was added, the small precipitate of protein was removed and the supernatant was deionized with Bio-Deminrolit G. The filtrate and washings were evaporated to dryness *in vacuo*, the residue was redissolved in 1.0 ml. of water and chromatographed on paper as a band [solvent: butan-2-one–acetic acid–aqueous 4% (w/v) boric acid (9:1:1)]. The position of the dihydroxyacetone band was detected by marginal markers, the band was cut from the paper and eluted with water, the eluate was evaporated *in vacuo* to dryness and the evaporation repeated several times with additions of methanol to remove boric acid. Finally the aqueous solution of [α - ^{14}C]dihydroxyacetone (10 μmoles) was made to 1.0 ml.

Enzymic phosphorylation of [α - ^{14}C]dihydroxyacetone and reduction to phosphoglycerol. Glycero-kinase was prepared from rat liver (Bublitz & Kennedy, 1954). The solution of labelled dihydroxyacetone (10 μmoles in 1.0 ml.) was transferred to a Warburg vessel with glycerokinase (2.0 ml.), MgCl_2 (20 μmoles), NaHCO_3 (100 μmoles), sodium

adenosine triphosphate (Sigma Chemical Co., Mo., U.S.A.; 60 μ moles) and water (to 6.0 ml.). Incubation at 37° under $N_2 + CO_2$ (95:5, v/v) yielded CO_2 equivalent to 9.0 μ moles of dihydroxyacetone phosphate formation. After deproteinization at 0° with 20% perchloric acid (0.2 ml.), centrifuging, cautious neutralization of the supernatant with 20% KOH and removal of perchlorate by centrifuging, the final solution contained dihydroxyacetone phosphate. This was reduced by addition of α -glycerophosphate dehydrogenase (C. F. Boehringer und Söhne, Mannheim, Germany; 0.08 ml.), DPNH (0.9 ml., 0.5%) in tris buffer (1.3 ml., 0.2M, pH 7.6). The photometrically determined DPNH oxidation corresponded to the formation of 4.8 μ moles of α -glycerophosphate. Unlabelled α -glycerophosphate (560 μ moles) was then added, and the mixture was heated at 100° for 3 min., when 2.6 ml. of 50% (w/v) barium acetate was added to the cooled solution. The precipitate was removed, the supernatant and washings were adjusted to pH 9.0 by means of saturated $Ba(OH)_2$ and 4 vol. of ethanol were added. After being kept at 0° for 24 hr. the precipitate was collected, washed and dried (136 mg.; ester P content equiv.: 300 μ moles of barium α -glycerophosphate). This was fractionated by absorption on a column of Dowex-1 (formate form; 1 cm. $\times$ 18 cm.), washed with water (20 ml.) and eluted with 0.1M-ammonium formate, with collection of 10 ml. fractions. The fractions between 300 and 400 ml. of total eluate contained the single peak of α -glycerophosphate, which was again precipitated as barium salt from the concentrated eluate.

Degradation of the [^{14}C]glycerophosphate. Barium α -glycerophosphate (13.0 mg., prepared as described above) was dissolved in water (2.5 ml.) with a few drops of N-HCl. The barium was removed by sodium sulphate (0.12 ml., 0.5M) and unlabelled sodium α -glycerophosphate (to a total of 120 μ moles) was added: total volume was 10.0 ml. After ^{14}C determination by persulphate oxidation of a small sample, sodium periodate (0.6 ml., 0.5M) was added and then $2N-H_2SO_4$ to pH 3. After keeping the solution for 1 hr. at room temperature, sodium sulphite (1 ml., 2%) was added. After a further 10 min. the solution was passed through an Amberlite IRA-400 (OH^- form) column (1 cm. $\times$ 10 cm.) and washed through with water to 50 ml. total eluate, a small portion of which was analysed for formaldehyde (acetylacetone method: 120 μ moles) and the formaldehyde in the whole was converted into the dimedone derivative for determination of radioactivity (43% of total radioactivity of original solution). The glycolaldehyde 2-phosphate, the other product formed from α -glycerophosphate by periodate oxidation, was eluted from the well-washed column by 20 ml. of

$2N-H_2SO_4$ (117 μ moles of total phosphate). After neutralization to pH 7.5, alkaline phosphatase (Sigma Chemical Co.; 20 mg.) was added, followed by incubation at 37° for 2 hr. Repetition of the periodate treatment on the hydrolysate gave 92 μ moles of formaldehyde, and the formaldehyde dimedone was prepared from the distillate of the neutralized solution. The second steam-distillate after acidification gave formic acid, which was converted into CO_2 by $HgCl_2$ -acetate oxidation and converted into $BaCO_3$ for determination of radioactivity (cf. Batt *et al.* 1960b).

The results showed the following percentage of total radioactivity in the named carbon atoms of glycerol in α -glycerophosphate: C-1, 52%; C-2, 5%; C-3, 43%. It is clear from this result that both primary alcohol groups of dihydroxyacetone are approximately equally labelled, indicating that there is no appreciable enzymic discrimination between these two groups either by the transketolase reaction itself or by the kinase which phosphorylates the dihydroxyacetone formed by the transketolase. In accordance with theoretical expectations, therefore, dihydroxyacetone, unlike glycerol, behaves as a symmetrical molecule in these enzymic reactions.

DISCUSSION

Metabolism of L-erythrulose in the intact rat

The results summarized in Table 2 show clearly that ^{14}C from both C-1 and C-4 of the L-erythrulose molecule is fairly extensively utilized in the rat. Elimination in the urine is considerable, about one-third to one-sixth of the urinary excretion being due to a reduction of the ketotetrose to the corresponding tetritol, a threitol being formed which has been distinguished electrophoretically from erythritol, and is presumed to be L-threitol produced by the action of the DPN-xylitol (D-xylulose) dehydrogenase on L-erythrulose (Hollmann & Touster, 1957; Hickman & Ashwell, 1959; Hollmann, 1959).

Apart from the urinary excretion, extensive oxidation to respiratory carbon dioxide occurs, though to a different extent for individual carbon atoms, being more than 50% higher for C-1 of erythrulose than for C-4. This finding indicates that oxidation does not follow the complete conversion into a symmetrical molecule, such as reduction to threitol, before oxidation. Moreover, the time course of production of carbon dioxide is different for the two labelled erythruloses. Again, incorporation into liver glycogen is quite high for both labelled erythruloses, but higher for the ^{14}C -4- than for the ^{14}C -1-labelled compound, i.e. in the opposite sense to the evolution of carbon dioxide from these two carbon atoms.

Table 5. Distribution of ^{14}C in glucose isolated from liver slices incubated with L-[1- ^{14}C]- or L-[4- ^{14}C]-erythrulose or D-[4- ^{14}C]erythrulose

The percentage activity in the specified carbon atoms of the glucose molecule are given.

(a) Degradation by <i>Leuconostoc mesenteroides</i>							
Rat no.	Substrate	C-1	C-2	C-3	C-4	C-5	C-6
8	L-[1- ¹⁴ C]Erythrulose (duplicate runs)	{ 42 47	0	0	8 12	2 4	48 37
14	L-[4- ¹⁴ C]Erythrulose	23	3	9	29	4	37
(b) Degradation by <i>Lactobacillus casei</i>							
		C-1 + C-6	C-2 + C-5	C-3 + C-4			
21	L-[1- ¹⁴ C]Erythrulose	88	1	11			
(c) Periodate oxidation of glucosazone							
		C-1 + C-2 + C-3	C-4 + C-5	C-6	Total ¹⁴ C recovered (%)		
10 } 11 } 12 } 13 }	L-[1- ¹⁴ C]Erythrulose	{ 62 69 72 62	6 6 7 5	32 25 21 33	97 80 84 88		
Average		66	6	28	88		
10 } 11 } 12 } 13 }	L-[4- ¹⁴ C]Erythrulose	{ 25 34 42 40	18 16 17 3	57 50 41 57	88 100 98 85		
Average		35	13	52	93		
16	D-[4- ¹⁴ C]Erythrulose	22	5	73	84		

Incorporation of isotope into the glucose carbon atoms of liver glycogen (Table 3) shows that with the [4- ^{14}C]erythrulose labelling is probably symmetrical and mainly in C-1 and C-6 of glucose, but with [1- ^{14}C]erythrulose it appears mainly in C-1 and much less in C-6. Labelling in C-4 plus C-5 of glucose is at a low level, suggesting that incorporation of respiratory $^{14}\text{CO}_2$, which is well known to enter in positions C-3 and C-4, is not an important feature of erythrulose metabolism. These results on glucose formation are in excellent general agreement with those found for liver slices, as far as the different experimental conditions permit.

Metabolism of L-erythrulose and D-erythrulose in rat-liver slices. Comparison of the results given in Tables 2 and 4 and 3 and 5 shows such a close similarity for the metabolism of L-erythrulose in the intact rat and in rat-liver slices respectively that the liver may well be the main site of ketotetrose metabolism in the body. In the shorter (90 min.) experiments with liver slices, less percentage oxidation of both C-1 and C-4 of erythrulose to carbon dioxide occurred than in the 180 min. experiments in the whole rat, but nevertheless the proportions of oxidation at C-1 relative to C-4 show the same excess of oxidation at C-1 over C-4. The incorporation into glucose by the liver tissue of fed rats (used for the tissue-slice experiments) was in extent about the same as the incorporation (in fasting rats) into liver glycogen observed in the

intact animals. However, while the average C-4 incorporation into glucose was greater than C-1 incorporation in the whole rat, the reverse was true in the rat-liver slices.

As in the intact rat, reduction of a part (average 12%) of the labelled erythrulose to tetritol also occurred, even under the aerobic conditions of the slice experiments, and unchanged erythrulose was recovered from the medium in amount about equal to that so reduced.

Localization of isotope from the two labelled erythruloses in the glucose formed by the tissue slices was almost the same as the localization in liver glycogen in the intact rat (Tables 3 and 5), the main difference being the slightly lower C-1 to C-3 glucose labelling with [4- ^{14}C]erythrulose in rat-liver slices compared with that in the whole animal. Again, this might be connected with the use of fed rats for slices and fasted rats for experiments on the whole animal.

Degradation of glucose formed by the slices was more detailed (Table 5) than that of liver glycogen and included differentiation of labelling among C-1, C-2 and C-3 (by *Leuconostoc mesenteroides*), showing that most of the labelling was in C-1 with much lower amounts, or none, in C-2 and C-3. The peculiar and possibly erroneous results for C-4 of glucose by this method have already been discussed. *Lactobacillus casei* fermentation (Table 5) gave results in good agreement with those for

L. mesenteroides, showing 88% of total ^{14}C in C-1 plus C-6. Taking the results as a whole, this figure was fairly consistently obtained for combined radioactivity of the two terminal glucose carbons, whether $[1-^{14}\text{C}]$ - or $[4-^{14}\text{C}]$ -erythrulose was the ketotetrose administered: the main difference being due to the preponderance of $1-^{14}\text{C}$ of erythrulose C-1 of glucose, whereas $4-^{14}\text{C}$ of erythrulose enters mainly C-6 of the glucose molecule. Possible explanations of this finding are discussed below.

Tables 4 and 5 show some preliminary results obtained when $\text{D}-[4-^{14}\text{C}]$ erythrulose was incubated with rat-liver slices. The preponderance of labelling introduced into C-6 of glucose is even higher than with $[4-^{14}\text{C}]$ erythrulose as substrate, being more than three times as high as in C-1 + C-2 + C-3 of glucose combined.

The tetritols are poorly metabolized by liver slices according to these experiments (Table 4), and erythritol appears not to be utilized. However, L-threitol does appear to enter the metabolic pathways leading to glucose as well as those giving carbon dioxide. One route for this would be the preliminary dehydrogenation of L-threitol to L-erythrulose by means of the DPN-xylitol (D -xylulose) dehydrogenase and DPNH. This reaction we have shown (Batt *et al.* 1960b) to give erythrulose almost equally labelled in C-1 and C-4 when terminally labelled threitol (i.e. $1-^{14}\text{C}$ or $4-^{14}\text{C}$, both substances being therefore enzymically identical) is the substrate. Consequently L- $[1-^{14}\text{C}]$ threitol should be metabolized after this dehydrogenation in exactly the same way as an equimolar mixture of $[1-^{14}\text{C}]$ - and $[4-^{14}\text{C}]$ -erythrulose. According to the results of Table 5, already discussed, this would be expected to give almost equal labelling of C-1 and C-6 of glucose. This possibility remains to be determined, however, owing to the much smaller incorporation and lesser availability of labelled threitol, the metabolism of which is on this hypothesis controlled by the rate of its enzymic dehydrogenation to erythrulose.

Some preliminary observations were made on the effect on the labelling of L- $[1-^{14}\text{C}]$ - and L- $[4-^{14}\text{C}]$ -erythrulose when these were incubated with rat-liver slices, with subsequent isolation and degradation of the erythrulose remaining, the general conditions being the same as in Table 4. Little exchange of isotope between C-1 and C-4 of erythrulose had occurred, showing that little of the residual erythrulose could have been reversibly equilibrated under these conditions with L- $[1-^{14}\text{C}(4-^{14}\text{C})]$ threitol. However, if, as is clear from our observations, the aerobic utilization of L-erythrulose is much more rapid than that of L-threitol, it is not necessarily to be expected that erythrulose entering the slices should exchange in this way, but it might be

drained off more rapidly into other metabolic channels. Further work is needed on this point, e.g. under anaerobic conditions where oxidative utilization of erythrulose would not compete with the above-described exchange reaction.

Some possible routes of tetrose and tetritol metabolism. Fig. 1 summarizes the reactions already described at the beginning of this paper, or, in some cases (indicated by broken arrows) hypothetical reactions, which may be supposed to be concerned in the metabolism of L-erythrulose and L-threitol.

Of the known enzymic reactions, the action of transketolase on L-erythrulose might perhaps be expected to be of primary importance. This would yield 'active glycolaldehyde' together with an equimolar amount of glycolaldehyde (route A, Fig. 1). The metabolism of free glycolaldehyde in rats has been studied by Friedmann, Levin & Weinhouse (1956), who found that 2.25% of the dose of $[2-^{14}\text{C}]$ glycolaldehyde was incorporated into liver glycogen, the distribution in glucose carbon atoms 1-6 being respectively 21, 26, 6, 7, 22 and 19%. Entry of glycolaldehyde (B, Fig. 1) was considered to be indirect via its transformation into glycine and serine. In our experiments $[2-^{14}\text{C}]$ glycolaldehyde would arise from transketolase action upon $[4-^{14}\text{C}]$ erythrulose. However, the low incorporation into C-2, C-3, C-4 and C-5 observed by us with this compound suggests that this route can play only a minor part in the metabolism of L-erythrulose in rat liver. The same conclusion can be drawn from the behaviour of the 'active glycolaldehyde' moiety (C, Fig. 1). Hiatt (1957) found that $\text{D}-[1-^{14}\text{C}]$ ribose was converted into liver glycogen in the mouse by a transketolase pathway about as effectively as D -glucose, the labelling being mainly at C-1 (40%) and C-3 (45%) of the glucose molecule. Transketolase action on L- $[1-^{14}\text{C}]$ erythrulose would be expected to give a similar distribution. However, in our experiments, in addition to labelling at C-1 (44%), low or zero labelling at C-3 and fairly heavy labelling (28%) at C-6 were observed, and this again appears to exclude a transketolase route of metabolism of L-erythrulose. It may be added that transketolase also appeared to play only a minor part in the metabolism of hydroxypyruvate in rat-liver tissue (Dickens & Williamson, 1959).

The other known metabolic reaction of free erythrulose is its reversible reduction to L-threitol (D, Fig. 1). The possible role of this in metabolism of L-threitol via erythrulose has already been discussed, and also the possibility that this enzymic exchange might cause double-ended labelling of erythrulose, as indicated on Fig. 1 by the double-labelling of the (symmetrical) L-threitol so produced (cf. Batt *et al.* 1960b). The resulting

L-[1:4- ^{14}C]erythrulose would be expected to contribute isotope to all those carbon atoms denoted in Fig. 1 by * and °, the symbols used for 1- ^{14}C and 4- ^{14}C respectively. Incubation of liver slices with [1- ^{14}C]- and [4- ^{14}C]-erythrulose did not, however, cause much randomization between C-1 and C-4 of the residual erythrulose, as has already been mentioned.

We are therefore compelled to look for other and as yet unknown pathways of erythrulose metabolism. Of several possible routes the most probable include (E, Fig. 1) phosphorylation of L-

erythrulose to give the 1-phosphate; a reaction closely resembling the action of fructokinase and ATP on fructose, but not in fact brought about by that enzyme with L-erythrulose as substrate (Batt *et al.* 1960b). L-Erythrulose 1-phosphate is split by an aldolase reaction (H, Fig. 1) to give reversibly dihydroxyacetone phosphate and formaldehyde; this reaction has already been discussed (see also Batt *et al.* 1960b), but whereas we have shown above that dihydroxyacetone behaves isotopically as expected, i.e. symmetrically, C-1 and C-3 being identical groups, this does not apply to dihydroxy-

Fig. 1. Some possible metabolic pathways for specifically labelled tetroses and L-threitol. The following enzymic pathways of L-erythrulose are shown: A. Transketolase (with thiamine pyrophosphate as coenzyme) cleavage. B. [2- ^{14}C]glycolaldehyde incorporation into liver glycogen (Friedmann, Levin & Weinhouse, 1956) by an unknown route. C. Transketolase and transaldolase incorporation of CH₂·OH-labelled 'active glycolaldehyde' into liver glycogen (Hiatt, 1957). D. DPNH reduction of L-erythrulose by the DPNH-xylitol (D-xylulose) dehydrogenase of liver. Reversibility causes randomization of erythrulose C-1 and C-4 labelling. E. Hypothetical phosphorylation with ATP plus a kinase. F. Hypothetical C-1 oxidation and decarboxylation to (non-natural) L-glyceric acid. G. Incorporation of L-[3- ^{14}C]glycerate into liver glycogen (F. Dickens & D. H. Williamson, unpublished results). H. Phosphoketotetrose aldolase (Charalampous & Mueller, 1953; Charalampous, 1954) or aldolase from liver or muscle (Peanasky & Lardy, 1958). I. Triose phosphate isomerase. J. Aldolase and reversal of glycolysis. K. Formaldehyde dehydrogenase (Strittmatter & Ball, 1955). L. Serine aldolase (serine transhydroxymethylase; Alexander & Greenberg, 1955). * and °, 1- ^{14}C - and 4- ^{14}C -labelled atoms respectively (see text).

acetone phosphate, in which the labelling at C-1 as shown in Fig. 1 is therefore preserved from that originally present in [1-¹⁴C]erythrulose 1-phosphate. If free dihydroxyacetone were an intermediate this would not apply, and glucose formed by way of the phosphorylation of the free substance should therefore present approximately equal labelling in C-1, C-3, C-4 and C-6, which was not observed.

Fig. 1 shows in outline the isomerization (*I*) of the triose phosphates and their condensation by aldolase to give fructose 1:6-diphosphate, which would yield (*J*) glucose equally labelled in C-1 and C-6 if equilibration by the triose phosphate isomerase (*I*) were complete; otherwise a preponderance of isotope at C-1 of glucose over that at C-6 would be expected. However, in fed animals particularly, dilution of the labelled glyceraldehyde phosphate produced by reaction *I* with tissue triose may lower the extent of labelling in C-6 of glucose by this route.

The formaldehyde (derived from C-4 of erythrulose) produced in reaction *H* can be oxidized (*K*) via formate to carbon dioxide or by combination with tetrahydrofolic acid can give hydroxymethyl-tetrahydrofolic acid, the well-known source of 'active one-carbon transfer' (*L*) for formation of C-3 of serine and incorporation into purines etc. Formate carbon enters the glucose molecule by this route predominantly at C-1 and C-6 of glucose (Bernstein, 1953). By this aldolase route therefore C-4 of erythrulose would only enter the glucose molecule indirectly, C-1 would do so predominantly at positions 1 and 6 of glucose, and only C-4 of erythrulose would be converted into carbon dioxide, via formaldehyde oxidation.

The experimental findings both in the intact rat and in liver slices show that C-1 of erythrulose is the main carbon atom metabolized to carbon dioxide, which is not in accord with the above-mentioned pathway being the principal one. Moreover, the expected approximately equal labelling at C-1 and C-6 of the glucose formed from L-[1-¹⁴C]erythrulose, and much lower expected glucose labelling from the 4-¹⁴C-labelled compound, are not consistent with the observed percentage incorporation or with the distribution of radioactivity in the glucose carbon atoms (Tables 2-5).

The remaining possible route included in Fig. 1 (*F*) for the incorporation of L-erythrulose follows the postulated removal of C-1 of erythrulose by oxidation and decarboxylation of the resulting keto acid. This would yield ¹⁴CO₂ only from 1-¹⁴C of erythrulose, which, in fact, gave much higher yields than [4-¹⁴C]erythrulose both in the whole animal and in liver slices (Tables 2 and 4). However, if this were the only route, no labelling, other than minor amounts at C-3 and C-4 of glucose due

to carbon dioxide fixation, should have entered the glucose when [1-¹⁴C]erythrulose was the substrate, and this was not so. The other product besides carbon dioxide from C-1 oxidation of erythrulose is expected to be L-glyceric acid (the 3-¹⁴C-labelled acid from [4-¹⁴C]erythrulose as substrate). F. Dickens & D. H. Williamson (unpublished work) have found that both D- and L-[3-¹⁴C]glycerate are incorporated into rat-liver glycogen (or glucose when slices are used) to about the same extent and with similar labelling of glucose (at C-1 and C-6). Consequently, L-glycerate could well be an intermediate as shown (*G*) in Fig. 1. By this route [4-¹⁴C]erythrulose would give glucose labelled at C-1 and C-6, though the expected equal labelling at each of these carbon atoms was not fully realized in the present experiments, there being considerable variations with the different experimental conditions.

Thus it is probable that several different pathways of metabolism for L-erythrulose exist simultaneously in the rat liver. One of these is the reversible reduction (*D*, Fig. 1) to L-threitol, which, as already described, could lead to partial or even complete randomization of ¹⁴C between C-1 and C-4 of the erythrulose, whichever labelled form of erythrulose was the starting material. The probability that L-threitol itself is metabolized in liver by its primary conversion by this dehydrogenase into L-erythrulose has already been discussed. Evidently the extent of this randomization would greatly tend to complicate the interpretation and, if complete, should even render the metabolism of C-1- and C-4-labelled erythrulose the same, which was not so.

Finally, the metabolism of D-[4-¹⁴C]erythrulose (not included in Fig. 1) would be expected to begin with its transketolase conversion to give D-[6-¹⁴C]-glucose 6-phosphate, which should yield glucose and glycogen labelled exclusively in the C-6 position. Although 73% of the labelling was in fact at C-6, the mode of entry of nearly all the remaining radioactivity into positions C-1-C-3 of glucose remains to be studied, by means of D-erythrulose labelled on carbon atoms other than C-4.

SUMMARY

1. The metabolism of a number of specifically ¹⁴C-labelled tetroses and tetrulose has been studied in the intact rat and in rat-liver slices aerobically incubated.

2. In the intact fasted rat, intraperitoneally administered specifically labelled L-erythrulose is fairly extensively metabolized. Average recoveries of ¹⁴C during 3 hr. after the injection were, from the 1-¹⁴C-labelled compound, 25% in respiratory carbon dioxide, 3.8% in liver glycogen and

15% in urinary excretion. From the 4-¹⁴C-labelled compound the corresponding values were 15.5, 4.8 and 20% respectively.

3. The time course of ¹⁴CO₂ elimination in respiration during the 3 hr. experiments differs for the two labelled erythroses.

4. The glucose of the liver glycogen is differently labelled according to the labelled erythrose given. With [1-¹⁴C]erythrose 62% of total labelling appeared in C-1 + C-2 + C-3 of glucose and 27% in C-6. With the 4-¹⁴C-labelled compound a more symmetrical labelling (48 and 45% respectively) was observed in these groups of glucose carbon atoms. Minor amounts (about 10%) were found in C-4 + C-5 of glucose.

5. Rat-liver slices also metabolize L-erythrose, an average of only 13% of the substrate being recovered after incubation for 90 min. Of the remainder, ¹⁴CO₂ formation accounted for 12% of 1-¹⁴C and 5% of 4-¹⁴C of the original erythrose activity, glucose contained 4.3 and 2.7% respectively, and reduction of part of the erythrose to tetritol accounted for 12% with either labelled erythrose.

6. Degradation of the glucose produced by rat-liver slices incubated with labelled L-erythrose showed that the pattern of labelling was essentially similar to that in the whole animal, except for a slight preponderance in C-6 over C-1 + C-2 + C-3 when [4-¹⁴C]erythrose was the substrate. More elaborate degradations were done on the glucose produced by liver slices, and showed that with both labelled ketotetroses nearly all the labelling reported for C-1 + C-2 + C-3 of glucose is in fact in C-1.

7. The urinary metabolites included about 4% of the dose of ¹⁴C as tetritols, threitol being the only detectable substance in this fraction of the excretion products; no erythritol was formed. The nature of the tetritols produced in the slice experiments was not studied, but it is probable that in both instances reduction of L-erythrose to L-threitol had occurred by means of the diphosphopyridine nucleotide-xylitol (D-xylulose) dehydrogenase of mammalian liver.

8. D-[4-¹⁴C]Erythrose is metabolized by rat-liver slices, yielding in 90 min. 1% of the labelling as ¹⁴CO₂ and about 2.5% as glucose. Here the labelling of the glucose carbon atoms is predominantly (73%) in C-6 and 22% in C-1 + C-2 + C-3 with only 5% in C-4 + C-5.

9. The metabolism of some labelled tetritols in rat-liver slices has been studied. [1-¹⁴C]Erythritol, electrophoretically separated from the product of catalytic hydrogenation of L-[4-¹⁴C]erythrose, was not appreciably converted into ¹⁴CO₂ or [¹⁴C]-glucose. Various preparations of L-threitol, produced from L-[1-¹⁴C]- and L-[4-¹⁴C]-erythrose,

were metabolized, though to a less extent than L-erythrose itself. It is suggested that preliminary enzymic oxidation to L-erythrose would be consistent with the extent of L-threitol metabolism observed.

10. Possible pathways of tetrose metabolism are discussed. It was shown in connexion with the incorporation into glucose that the two CH₂·OH groups of free dihydroxyacetone behave, as expected, identically in enzymic reactions, unlike the corresponding groups in glycerol.

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Endogenous Sulphate Acceptors in Rat Liver

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There is now abundant evidence to show that adenosine 3'-phosphate 5'-sulphatophosphate (Robbins & Lipmann, 1957) is an active form of sulphate from which the sulphate group can be transferred to various acceptors under the influence of specific sulphokinases to form sulphate esters or sulphamino compounds (Lipmann, 1958; Gregory & Robbins, 1960). In the presence of added adenosine triphosphate, Mg^{2+} and SO_4^{2-} ions, yeast preparations synthesize adenosine 3'-phosphate 5'-sulphatophosphate by a two-stage reaction. The first stage involves the reversible action of an adenosine triphosphate-sulphurylase to give adenosine 5'-sulphatophosphate and pyrophosphate and this is followed by non-reversible phosphorylation of adenosine 5'-sulphatophosphate by adenosine triphosphate in the presence of a specific adenosine 5'-sulphatophosphate-kinase to give adenosine 3'-phosphate 5'-sulphatophosphate (Bandurski, Wilson & Squires, 1956). The synthesis of adenosine 3'-phosphate 5'-sulphatophosphate by the soluble material of the cytoplasm of mammalian liver is also known to be a two-stage process, and although an unequivocal proof is not yet available it seems reasonably certain that the steps are the

same as those in the yeast preparations (Robbins & Lipmann, 1958).

On incubation of particle-free high-speed supernatants of iso-osmotic-potassium chloride homogenates of rat liver with adenosine triphosphate, SO_4^{2-} and Mg^{2+} ions, it was observed that the rate of formation of adenosine 3'-phosphate 5'-sulphatophosphate diminished with increasing length of incubation and, eventually, after reaching a maximum concentration, the overall amount of adenosine 3'-phosphate 5'-sulphatophosphate decreased. Some of the diminution in the rate of formation of the compound might be attributable to the accumulation of pyrophosphate, which would tend to reverse the first stage of synthesis of adenosine 3'-phosphate 5'-sulphatophosphate (Robbins & Lipmann, 1958) and actual loss of the compound would be expected owing to the action of a specific 3'-nucleotidase (Brunngraber, 1958). With $^{35}SO_4^{2-}$ ions, followed by chromatography and radioautography of the incubates after various times, it has been shown that a further contributing factor to disappearance of adenosine 3'-phosphate 5'-sulphatophosphate is the transfer of sulphate from the compound to endogenous acceptors to