and this may be the limiting factor. It is known that respiratory function of the small intestine varies along the intestine, the upper intestine having highest activity (Dickens & Weil-Malherbe, 1941; Fisher & Parsons, 1950*b*; Wilson & Wiseman, 1954).

Peptidases may be concerned with protein synthesis in the intestine as in the root tip of barley (Linderström-Lang, 1952). However, the incorporation of ^{35}S-labelled methionine into intestinal mucosa occurs mainly in the duodenum and the upper ileum (Friedburg, Tarver & Greenberg, 1948). These sites are not coincident with the general region of maximal peptidase activity, which suggests that, in the intestine, the function of peptidases is concerned with digestion and absorption rather than with protein synthesis.

SUMMARY

1. Optimum conditions for the hydrolysis of L-leucylglycine, glycylglycine, glycyl-L-leucine and glycyl-L-alanine, by rat mucosal extracts, have been studied.

2. For all these dipeptides, the highest hydrolytic activity was found to occur in the ileum.

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Biochem. J. (1960) **77**, 356

Metabolism of D- and L-[3-^{14}C]Glycerate by Liver Tissue of the Rat

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It was recently reported (Dickens & Williamson, 1959) that isotopically labelled hydroxypyruvate is well incorporated into liver glycogen by the intact rat and into glucose by rat-liver slices *in vitro*. Possible enzymic routes for this incorporation were suggested, including the possibility that the hydroxypyruvate might first be reduced to glyceric acid. This could either be L-glycerate, known to be produced from hydroxypyruvate by reduced diphosphopyridine nucleotide acting together with the L-lactic dehydrogenase of animal tissues, or D-glycerate produced by an animal enzyme similar to the D-glycerate dehydrogenase which Stafford, Magaldi & Vennesland (1954) discovered in plant

tissues. These possibilities and others are further considered in the Discussion to the present paper.

The most direct approach to this problem was to study the metabolic fate of labelled D- and L-glycerate in liver tissue. This work is reported below.

EXPERIMENTAL

Materials

Radioactive substances. These were obtained from The Radiochemical Centre, Amersham, Bucks, with the following approximate specific activities. Generally labelled [^{14}C]glucose, 10 mc/m-mole; [6-^{14}C]glucose, 2 mc/m-mole; L-[3-^{14}C]serine, 3 mc/m-mole; DL-[3-^{14}C]serine, 1 mc/m-mole. They were diluted as required with non-isotopic material.

Enzymes. D-Glycerate dehydrogenase was prepared from parsley leaves (Stafford *et al.* 1954; cf. Dickens & Williamson, 1958*b*). D-Amino acid oxidase was prepared from pig kidney [Negelein & Brömel (1939) purified to completion of stage 2]. D-Glycerate kinase was prepared from rat liver (Ichihara & Greenberg, 1957). Liver alcohol dehydrogenase was purchased from Boehringer und Söhne, Mannheim.

Methods

These were as described by Batt, Dickens & Williamson (1960*a*) with the following additions.

Glyceric acid was determined either by colorimetric estimation of formaldehyde liberation from C-3 by periodate or by the colour reaction with naphtharesorcinol in nearly conc. H_2SO_4 (Rapoport, 1936; see Dickens & Williamson, 1958*b*). Later the modification of Bartlett (1959) was also used for this purpose. Recrystallized barium DL-glycerate was used as a standard after quantitative conversion into the sodium salt by Na_2SO_4.

Degradation of radioactive substances. The general methods described by Batt, Dickens & Williamson (1960*a*) were followed. Glucose was precipitated from the completely deionized solutions as glucosazone, which was counted for ^{14}C content, recrystallized, re-counted and degraded by the periodate oxidation method. Bacterial degradation of the glucose molecule was also carried out, by fermentation to two molecules of lactate by means of *Lactobacillus casei* (for a full description see Bernstein & Wood, 1957). For this purpose, the deionized medium was concentrated *in vacuo* and the radioactive glucose purified by paper chromatography in ethyl acetate–pyridine–water before fermentation (cf. Batt *et al.* 1960*a*). This method gives the total radioactivity in each of the following pairs of glucose carbon atoms: C-1 + C-6, C-2 + C-5 and C-3 + C-4. On the other hand, periodate degradation of the glucosazone gives the labelling in C-1 + C-2 + C-3; C-4 + C-5; and C-6. Therefore by considering together the degradation by these two methods a more complete picture of the distribution of radioactivity in the glucose molecule is obtainable (cf. Batt, Dickens & Williamson, 1960*b*).

[3-^{14}C]Glyceric acid degradation was by means of periodate oxidation, distillation of the [^{14}C]formaldehyde liberated from C-3 of the acid into dimedone, and measurement of radioactivity of the crystalline formaldehyde–dimedone (cf. Batt *et al.* 1960*a*). Unlabelled carrier DL-glycerate was added where necessary.

The methods of measuring the radioactivity of glucosazone, of $^{14}CO_2$ as $Ba^{14}CO_3$, and of [^{14}C]formic acid as $Ba^{14}CO_3$, were also as described by Batt *et al.* (1960*a*).

Conditions of animal experiments. These were similar to those of Dickens & Williamson (1959) and Batt *et al.* (1960*b*). Batches (1 g.) of slices from the livers of fed rats were incubated aerobically in bicarbonate–saline for 90 min. as these authors described, but the substrate consisted of D- or L-[3-^{14}C]glycerate, usually about 5–10 μmoles containing 0·5–1·0 μc, prepared as described below. After the incubation the total CO_2 was liberated by excess (2 ml. 20%, w/v) of trichloroacetic acid and collected and weighed as $BaCO_3$, of which the radioactivity was determined as usual. The deproteinized extract and washings from the slices and incubation medium were made up to 20 ml. (extract *A*). To 2 ml. of this extract, 100 μmoles of DL-glycerate were added for periodate oxidation, with collection and counting of the liberated formaldehyde as the dimedone derivative. The original solution was similarly assayed and the percentage recovery of the [3-^{14}C] of the added glyceric acid was calculated on this basis.

In order to cover the possible conversion of part of the glyceric acid into [3-^{14}C]serine, which also liberates the terminal carbon atom as formaldehyde on treatment with periodate, the remainder (18 ml.) of the deproteinized extract was passed through a column of Amberlite IR-120 (H^+ form) and washed through with water (total eluate *B*, 25 ml.). Aq. 2N-NH_3 soln. (25 ml.) was then run through and the combined eluate and water washing (*C*) concentrated *in vacuo* to 2·0 ml. To measured samples of extracts *A*, *B* and *C*, 100 μmoles of unlabelled carrier DL-serine was added before periodate oxidation. In some experiments, 50 mg. of DL-serine was first added to *A* and the recovered serine fraction (*C* above) was then recrystallized from 60% ethanol–water before periodate degradation. With this procedure recoveries of radioactivity, from standard solutions of glyceric acid and serine in fractions *B* and *C* respectively, were satisfactory.

Eluate *B* also contains radioactive glucose, in experiments with liver slices. Since periodate would also liberate formaldehyde from this glucose, the sugar was removed, before the periodate analysis for the glyceric acid recovery, by the following method. Eluate *B* was shaken for 2 hr. with Bio-Deminrolit G (see Batt *et al.* 1960*a*) when the originally strongly acidic pH had become 5. The volume of filtrate and washings was noted, and 1·0 ml. was removed for estimation of glucose by the Nelson (1944) method; to the remainder a measured amount of glucose was added, and the solution was concentrated *in vacuo* to small volume. This was either run on paper for purification of glucose for fermentation with *L. casei* (see above) or, if the osazone was to be prepared, it was made up to 10 ml. with water and heated with phenylhydrazine etc. as described by Batt *et al.* (1960*a*). The resulting glucosazone was counted at infinite thickness on a planchet, recrystallized and recounted, and finally degraded by periodate oxidation. Recoveries of radioactivity in the glucose fraction were based on these counts and also on persulphate (total) oxidation of a measured portion of eluate, with subsequent counting as $BaCO_3$. The general precautions detailed by Topper & Hastings (1949), including correction for backscatter by $BaCO_3$, were followed in all experiments.

Preparation of barium D-*glycerate*

D-Glyceraldehyde, $[\alpha]_D + 16°$, was purchased from Fluka A.G., Buchs, Switzerland, and was oxidized by Br_2 in aqueous solution to D-glyceric acid (Baer, Grosheintz & Fischer, 1939). From the resulting solution the barium salt was prepared, recrystallized three times from aqueous ethanol, and dried over P_2O_5 in a vacuum desiccator until anhydrous. The optical rotation of glyceric acid is unreliable as an indication of optical purity (cf. Rodd, 1952), but $[\alpha]_D$ for the above barium glycerate was $+9·9°$ [*c*, 5·78%, 2 dm. in water; Meyerhof & Schulz (1938) give $+11·5°$]. The addition of ammonium molybdate (final concn. 8·3%) to the neutral sodium salt, prepared quantitatively from the above barium salt by Na_2SO_4 addition, gave $[\alpha]_D + 120°$ calc. for sodium glycerate ($+88·5°$ calc. as barium glycerate). Meyerhof & Schulz (1938) give $[\alpha]_D + 104°$ (basis of calculation not stated); Fager & Rosenberg (1950) found for the sodium salt, molybdate-enhanced, $[\alpha]_D + 83°$, and Stafford *et al.* (1954) give $+75°$ and $+82°$.

Preparation of L-[3-^{14}C]*glyceric acid*

L-[3-^{14}C]Serine (10 mg. containing 20 μc, diluted with 40 mg. of unlabelled L-serine; total 475 μmoles) was deaminated by nitrous acid (Fischer & Jacobs, 1907) to give the corresponding glyceric acid. A parallel experiment was performed with unlabelled L-serine (50 mg.). Both products were passed through Amberlite IR-120 (H^+) columns and washed through with water to 20 ml., concentrated *in vacuo* to syrups, redissolved in 10 ml. of water and reconcentrated to syrups.

The resulting material was passed through Solka Floc cellulose columns, 1 cm. × 15 cm., which had previously been equilibrated with the solvent used [ethanol–aq. NH_3 soln. (sp.gr. 0·88)–water (8:1:1, by vol.)]. Fractions (32) of 3 ml. each were collected, most of the glyceric acid (naphtharesorcinol test) being located in fractions 4–8. These fractions were combined, treated with a little activated charcoal and concentrated to dryness *in vacuo*; the residue (188 μmoles of glyceric acid) was made up to 1·00 ml. for polarimetry in the case of the unlabelled material. Found $[\alpha]_D - 116°$, calc. as the sodium salt, molybdate-enhanced rotation.

The labelled sample, treated exactly similarly, yielded 170 μmoles of L-glyceric acid, but with this material ascending chromatography on paper in the above solvent mixture showed the presence of a trace of a minor radioactive component which moved slightly ahead of the glycerate. No attempt was made to remove this in view of its small contribution to the total radioactivity. Determination of ^{14}C in the formaldehyde liberated from C-3 of the glyceric acid by periodate gave a total recovery of 5·6 μc/170 μmoles of L-glyceric acid.

Preparation of D-[3-^{14}C]*glyceric acid*

DL-[3-^{14}C]Serine was treated with D-amino acid oxidase, the resulting L-[3-^{14}C]serine and hydroxy[3-^{14}C]pyruvate were purified as described in detail by Batt *et al.* (1960*b*). The labelled L-serine was used as described above for the preparation of labelled L-glyceric acid.

The hydroxy[3-^{14}C]pyruvate was reduced to D-glyceric acid by reduced diphosphopyridine nucleotide (DPNH) and the specific dehydrogenase was obtained from parsley leaves as described by Stafford *et al.* (1954) [cf. Holzer & Holldorf (1957*b*)]. Hydroxy[3-^{14}C]pyruvate (156 μmoles) was incubated at 20° in a total volume of 50 ml. containing 1 ml. of D-glyceric acid dehydrogenase, 0·5 ml. of alcohol dehydrogenase, 10 mg. of diphosphopyridine nucleotide (DPN), 0·1 ml. of 2N-NH_3 soln. (to give pH 8·0), and 1 ml. of ethanol. After 30 min., all hydroxypyruvate was reduced as shown by the naphtharesorcinol test (Dickens & Williamson, 1958*b*). The mixture was heated for 10 min. in a boiling-water bath, cooled, filtered, and acidified with 2 ml. of 2N-H_2SO_4. After continuous extraction with ether for 4 days, 10 ml. of water was added to the extract, the ether was evaporated, and the residual aqueous layer contained D-[3-^{14}C]glyceric acid (115 μmoles, 5·0 μc). Chromatography on paper in ethanol–NH_3 soln.–water (8:1:1, by vol.) showed a single peak of radioactivity coinciding with glyceric acid similarly chromatographed (the unlabelled material was detected by spraying the dried paper with ethanolic chlorophenol red, 0·04%).

Other samples of D-[3-^{14}C]glyceric acid and unlabelled D-glyceric acid were similarly prepared enzymically from the corresponding lithium hydroxypyruvates, prepared according to Dickens & Williamson (1958*a*).

Action of D-*glyceric acid kinase on preparations of* D- *and* L-*glycerate*

Ichihara & Greenberg (1957) and Holzer & Holldorf (1957*a*) have described the preparation from liver of a kinase which specifically phosphorylates D-glyceric acid in the presence of adenosine triphosphate (ATP). The rat-liver enzyme has been used to assess the optical purity of the specimens of D- and L-glycerate prepared as above. Preliminary manometric experiments in bicarbonate media having shown that CO_2 evolution with ATP and the enzyme (plus magnesium and fluoride ions) occurred only

Table 1. *Action of* D-*glyceric acid kinase on samples of* D- *and* L-[3-^{14}C]*glycerate*

For details see text.

Addition	Tubes A	B	C	D
ATP (10 μmoles)	−	+	−	+
DL-Glycerate (10 μmoles)	+	+	+	+
D-Glycerate kinase (0·5 ml.)	+	+	+	+
[3-^{14}C]Glycerate (2 μmoles)	D-	D-	L-	L-
After incubation				
Wt. of formaldehyde–dimedone (mg.)	36·3	36·4	37·7	37·9
Radioactivity (counts/min.)	191	8	607	605
[^{14}C]Glycerate utilized (%)	0	96	0	<0·5

with the D-isomer, the binding of the $CH_2 \cdot OH$ group in the [3-^{14}C]glycerate samples was determined by the loss of ability to liberate formaldehyde on treatment with periodate that occurs on phosphorylation of this group to give 3-phosphoglycerate. For this purpose tubes were set up as shown in Table 1. Potassium phosphate buffer, pH 7·4 (0·5 ml., 0·1 M), $MgCl_2$ (0·1 ml., 0·1 M) and water to a total volume of 2 ml. were added to each tube. Incubation for 40 min. at 37° was ended by cooling to 0°, addition of 0·5 ml. of 20% (w/v) trichloroacetic acid and removal of precipitated protein. Samples (1 ml.) were diluted to 25 ml. and portions of 1 ml. were neutralized with 2 N-NaOH to phenol red; 5 ml. of water and 0·5 ml. of 0·2 M-sodium periodate were added, followed after 10 min. at room temperature by 1·0 ml. of 5·4% (w/v) sodium arsenate. The solution was adjusted to approx. pH 3 and after a further 5 min. the volume was made up to 25 ml. After the addition of 1·0 ml. of 0·1 M-unlabelled formaldehyde to 5 ml. portions, the addition of 50 ml. of cold saturated aqueous dimedone precipitated the formaldehyde–dimedone which was filtered off on 1 cm.^{2} paper disks, washed, dried and counted on planchets as usual, with the results shown in Table 1. It is clear that only the D-form of glyceric acid is phosphorylated by the kinase and that the optical purity of the two samples is probably 96% or more, according to this sensitive assay method. Control experiments with DL-glycerate showed no interference by the presence of the L-isomer.

RESULTS

Metabolism of D- *and* L-*glycerates by rat-liver slices*

Table 2 summarizes the results of experiments in which [3-^{14}C]glycerate, D- and L-isomers, was incubated with rat-liver slices aerobically for 90 min. at 37° as described in the Methods section. In the first three experiments of Table 2 duplicate batches of slices from the same rat's liver were directly compared. Consistent differences were not observed, whether the D- or L-glycerate was the substrate. The percentage utilization, that is the difference between the total added periodate-liberated radioactivity and that remaining after incubation, was on the average 30%. Only a little over 3% of the added ^{14}C appeared as respiratory carbon dioxide, whereas about 10% entered the glucose molecule. This average glucose incorporation is a little lower than the mean value (15·7%) observed with hydroxy[3-^{14}C]pyruvate in otherwise similar experiments (Dickens & Williamson, 1959). In the case of rat no. 3, a parallel experiment in which 0·12 μc (15 μmoles) of lithium hydroxy[3-^{14}C]pyruvate was incubated with slices from the same liver, showed 12·5% of the ^{14}C as respiratory carbon dioxide, 18·7% incorporation into glucose, and 5·3% into serine.

In a few experiments in which the incorporation into serine of ^{14}C from the labelled glyceric acids was measured, the amount was also lower than for hydroxypyruvate, namely about 0·9–1·6% of the total added radioactivity. However, the observed incorporation into glucose of the labelled glycerate was rather variable and in some experiments reached values (e.g. rat no. 3, 16%) equal to those previously reported for the incorporation of hydroxy[3-^{14}C]pyruvate.

In order to decrease the possibility that the almost equal incorporation of ^{14}C from the samples of D- and L-glycerate might have been due to the highly preferential use of a radioactive impurity (e.g. of a trace of the opposite optical isomer), the experiments summarized in Table 3 were carried out. Graded amounts, down to one-tenth of the quantity of total glycerate normally used, were incubated with liver slices in the usual way.

Table 2. *Metabolism of* D- *and* L-[3-^{14}C]*glycerate in rat-liver slices*

Figures represent percentage of radioactivity present in the named fraction after incubation. For details see text.

	D-[3-^{14}C]Glycerate				L-[3-^{14}C]Glycerate			
			Incorporated into glucose				Incorporated into glucose	
Rat no.	$^{14}CO_2$*	Residual in medium†	% of added*	% of utilized‡	$^{14}CO_2$*	Residual in medium†	% of added*	% of utilized‡
1	2·6	61	7·2	18	3·4	68	7·9	25
	3·1	59	8·3	20	—	—	—	—
2	3·4	61	11·6	30	3·5	70	10·0	33
	4·3	67	11·4	34	—	—	—	—
3	3·0	80	15·4	77	4·4	78	12·9	58
	2·9	75	16·8	67	—	—	—	—
4	—	—	—	—	2·8	72	7·2	26
	—	—	—	—	3·1	76	6·3	26
Mean	3·2	67	11·8	41	3·4	73	8·9	34

* As percentage of total added radioactivity.

† By periodate oxidation and determination of radioactivity of liberated [^{14}C]formaldehyde; as percentage of added radioactivity.

‡ Calculated as percentage of (100-residual in medium).

Even at the concentration of 1 μmole/vessel there was no change in the percentage utilization, yield of radioactive carbon dioxide, or of glucose (Table 3).

The glucose formed in the experiments recorded in Table 2 was purified and degraded in order to determine the localization of ^{14}C in the various glucose carbon atoms (Table 4). Degradation with *L. casei* showed that 85–90% of the total radioactivity of the glucose resided in C-1 and C-6, with very low amounts in C-2 and C-5 and less than 10% in C-3 and C-4 combined. This picture was identical for D- and L-[3-^{14}C]glycerate as substrates. By purification of the glucose through the glucosazone and chemical degradation of the latter by means of periodate oxidation, C-6 was shown to have about 60% of the total glucose activity, whence it follows, by comparison with the figures for C-1 + C-6 in Table 4, that C-1 has about 30% of the total activity. Again, C-4 and C-5 together had less than 10%. Once more, no essential difference was observed whether D- or L-[3-^{14}C]-glycerate was the substrate used.

DISCUSSION

The results presented above leave little doubt that both D- and L-[3-^{14}C]glycerate are metabolized by rat-liver slices in a quantitatively closely similar manner. Both isomers are utilized to almost the same extent by liver slices; both make a similar, rather low, contribution to the respiratory carbon dioxide; both are incorporated into glucose formed by the slices to a similar extent and contribute ^{14}C nearly identically to the individual carbon atoms of the glucose molecule.

Enzymes metabolizing D- *and* L-*glycerate*

Since D-glycerate is the only isomer as yet reported to be present, as phosphoglycerate, in animal tissues, and a kinase for only the D-isomer is present in liver tissue (Ichihara & Greenberg, 1957; Holzer & Holldorf, 1957*a*); this result is a surprising one. However, it is worth noting that L-glycerate is a substrate, as effective as L-lactate, for the crystalline L-lactic dehydrogenase of animal

Table 3. *Effect of diminishing amount of added* D- *and* L-[3-^{14}C]*glycerate on the extent of its metabolism by rat-liver slices*

Expts. were under the usual incubation conditions (for details see text). Values represent percentage of total radioactivity in the fractions named. Specific activities were: D-isomer 0·05 μc/μmole and L-isomer 0·089 μc/μmole. Different rats were used for each of the two isomers.

Substrate ...	D-[3-^{14}C]Glycerate			L-[3-^{14}C]Glycerate		
Amount per vessel (μmoles)	CO_2	Residual in medium	Glucose	CO_2	Residual in medium	Glucose
10	3·7	51	8·6	2·5	61	3·5
4	—	—	—	2·5	62	3·8
2	5·8	49	9·7	2·2	56	3·3
1	4·8	48	7·3	—	—	—

Table 4. *Degradation of* [^{14}C]*glucose formed by liver slices incubated with* D- *or* L-[3-^{14}C]*glycerate*

(*a*) Degradation with *Lactobacillus casei*

Substrate	Radioactivity in glucose carbon atoms (% of total) C-1 + C-6	C-2 + C-5	C-3 + C-4
D-[3-^{14}C]Glycerate	90	1·5	9
L-[3-^{14}C]Glycerate	85	6	9·5

(*b*) Degradation of the glucosazones with periodate

	C-1 + C-2 + C-3	C-4 + C-5	C-6
D-[3-^{14}C]Glycerate*	39	3	58
	31	5	64
L-[3-^{14}C]Glycerate*	37	9	54
	28	5	67

* The upper and lower rows of figures in each bracketed set each refer to duplicate batches of liver slices from the same rat.

skeletal and heart muscle (Meister, 1952; Stafford *et al.* 1954) and is also a substrate for the flavocytochrome b_2 enzyme known as yeast lactic dehydrogenase (Dickens & Williamson, 1956), the product in the direction of dehydrogenation being hydroxypyruvate with both enzymes. We later found (Dickens & Williamson, 1958*a*, legend to Fig. 2) that a highly purified preparation of cytochrome b_2 kindly supplied by Professor E. Boeri is also active in this respect, like the cruder preparations used by Dickens & Williamson (1956). It remains to be seen whether the crystalline, yeast lactic-dehydrogenase preparation (Appleby & Morton, 1959*a*, *b*) also oxidizes L-glycerate. The identity of the product formed by dehydrogenation of L-glycerate with the yeast enzyme was originally shown to be hydroxypyruvate by colorimetric tests (Dickens & Williamson, 1956, 1958*b*) and later photometrically (F. Dickens & D. H. Williamson, unpublished work) by its reduction with DPNH and the dehydrogenase from parsley leaves (Stafford *et al.* 1954); this latter enzyme is specific for hydroxypyruvate and glyoxylate (cf. Holzer, 1959, p. 184). Thus yeast and animal tissues possess L-lactic-dehydrogenase systems which, though entirely different otherwise in their prosthetic group and coenzyme requirements, can both oxidize L-glycerate to hydroxypyruvate.

Many plant tissues contain, in addition to the above dehydrogenase which produces D-glycerate specifically from hydroxypyruvate and DPNH but does not reduce pyruvate to lactate, a second dehydrogenase system of the more familiar lactic-dehydrogenase type which reduces both pyruvate and hydroxypyruvate in presence of DPNH (Stafford *et al.* 1954). Presumably the product of the latter reaction would be L-glycerate, corresponding with the known presence of L-lactic dehydrogenase in plant tissues. Bacterial systems oxidizing both D- and L-lactate are also known (cf. De Ley & Schel, 1959).

Thus enzyme systems which dehydrogenate D- or L-lactate may or may not also dehydrogenate D- or L-glycerate, though more commonly the substrate specificity of systems, oxidizing α-hydroxy acids, is not marked. This applies also to L-malic dehydrogenase, which seems better described as an α-hydroxydicarboxylic acid dehydrogenase, according to Davies & Kun (1957).

In addition to the well-known DPN-linked L-lactic-dehydrogenase system of animal tissues, less well characterized enzymic systems which specifically oxidize D-lactate are present. In view of the low specificity of this type of enzyme for individual hydroxy acids (though specificity for the D- or L-series is usually complete) it seems desirable briefly to consider some examples here, although most of these have not yet been tested with D- and L-glycerate as substrates. Meyerhof & Lohmann (1926) found that rat-kidney slices and yeast cells could oxidize both D- and L-lactate. Baker (1952) found that washed particles from rat kidney and liver oxidized both D- and L-hydroxy acids, the activity towards the D-isomers being predominantly in the particles of the kidney preparation used; glycerate was not tested. With rabbit kidney and liver particles, D-lactate oxidation did not require added DPN and pyruvate was the product (Huennekens, Mahler & Nordmann, 1951; Mahler, Tomisek & Huennekens, 1953). Oxidation of D-lactate is not coupled with reductive carbohydrate synthesis in liver (Hoberman, 1958, 1960). Recently Tubbs & Greville (1959) have obtained a D-lactate dehydrogenase in soluble form from rabbit-kidney mitochondria; L-lactate was not attacked and the extracted enzyme required activation by aging (5 days at 0°) or by dilute cyanide.

The enzyme is also present in rat-liver mitochondria and is quite strongly active when tested with DL-glycerate as substrate, the product reacting as hydroxypyruvate in the Dickens & Williamson (1958*b*) naphtharesorcinol test (P. K. Tubbs & G. D. Greville, personal communication). The peculiar activation phenomenon with this enzyme may well have masked its presence in earlier attempts to isolate D-hydroxy acid dehydrogenases from animal tissues. The nature of the terminal acceptor and the physiological role of this enzyme remain to be investigated, but it appears possible that it may be a route for the reversible dehydrogenation of D-glycerate to hydroxypyruvate in animal tissues. The known DPN-linked L-lactate dehydrogenases of animal tissues constitute an electrophoretically heterogeneous group of proteins even from the same organ (Wieland & Pfleiderer, 1957; Wieland & Jeckel, 1957, see Holzer, 1959), but as a group would appear to be, like the crystalline L-lactic dehydrogenase of muscle, a probable source of reversible hydroxypyruvate production from L-glycerate in animal tissues. We have, in fact, found that incubation of hydroxypyruvate with mouse-ascites-tumour cells leads to the appearance of L-glycerate ($[\alpha]_D$, molybdate-enhanced, $-90°$ as sodium salt) in the medium (F. Dickens & D. H. Williamson, unpublished work).

Metabolism of D- *and* L-*glycerate in liver tissue*

The results described in the present paper show not only the approximately equal utilization of D- and L-glycerate by liver tissue of the rat, but also that the pattern of incorporation of [3-^{14}C] of both isomers into the glucose molecule by liver tissue is closely similar. In isotopic experiments of this kind it is important to eliminate the possibility

that the presence of a small percentage of the labelled opposite isomer might be responsible for the observed incorporation of isotope. It is believed that this explanation of the results may be excluded on the following grounds. The optical rotation of non-isotopic glycerate prepared by the same method as that used for the labelled material was satisfactory. The specific D-glycerate kinase, prepared from rat liver, phosphorylated the $^{14}CH_2 \cdot OH$ group of the D-[3-^{14}C]glycerate preparation (95 % of calculated amount) but not that of the L-[3-^{14}C]glycerate (< 0·5 %). The incubation of liver slices with progressively lower amounts of each labelled isomer did not affect the per cent of substrate utilized, the $^{14}CO_2$ liberated, or the incorporation of isotope into glucose by the liver slices (Table 3). Since the incorporation into glucose reached 16 % of that added in some experiments (Table 2), a large contamination with the opposite isomer would have been necessary to explain the results on this ground.

The actual distribution of radioactivity among the glucose carbon atoms (Table 4) showed approximately 60 % in C-6 and 30 % in C-1 of the glucose molecule, with much lower amounts in the other carbon atoms. A symmetrical entry of two molecules of [3-^{14}C]glycerate would, of course, be expected to give 50 % of the total activity of the glucose in each of the carbon atoms C-1 and C-6. The results therefore suggest that although the three-carbon chain of both D- and L-glycerate entered intact into glucose, with the unlabelled carboxyl groups as expected in C-3 and C-4, some dilution of the dihydroxyacetone phosphate which forms C-1 to C-3 of the glucose had occurred. Minor secondary reactions (probably $^{14}CO_2$ fixation into C-3 + C-4, accounting for about 9 % of the total incorporation) also occurred, as would be expected from the observed production of respiratory $^{14}CO_2$ (Tables 2 and 4).

At present, an explanation of these results for D-glycerate can be advanced but that for L-glycerate is more speculative. D-[3-^{14}C]Glycerate would be expected to be phosphorylated by the known D-glycerate kinase of liver to give 3-phospho-D-glycerate, which by the well-recognized reversal of D-glyceraldehyde 3-phosphate dehydrogenase would yield D-[3-^{14}C]glyceraldehyde 3-phosphate (cf. Holzer, 1959, p. 192). In the livers of well-fed rats, such as were used in our experiments, a pool of unlabelled dihydroxyacetone phosphate would be available which, through the action of aldolase, would form D-[6-^{14}C]fructose 1:6-diphosphate with the D-[3-^{14}C]glyceraldehyde 3-phosphate thus provided. At the same time, the highly active triose phosphate isomerase of rat liver would convert some of the labelled glyceraldehyde 3-phosphate into [1-^{14}C]dihydroxyacetone 1-phosphate. The aldolase condensation of the latter with D-[3-^{14}C]glyceraldehyde 3-phosphate would yield, as expected, D-[1:6-$^{14}C_2$]fructose 1:6-diphosphate. On this view the dilution of labelling at C-1 of the glucose formed via the fructose diphosphate is readily understandable, and this should be greater in fed animals. There is considerable evidence (see Wood, 1955) that equilibration by triose phosphate isomerase may be incomplete in such instances.

Although this explanation of D-glycerate metabolism is acceptable, that for L-glycerate requires consideration; and since both isomers are so similarly utilized, the fact that L-glycerate is not known to be capable of phosphorylation in liver, and in any event belongs to the wrong optical species for D-glucose formation, may even shed some doubt on the validity of the above explanation for D-glycerate. Naturally, the assumption of the presence in liver of an active racemase for glycerate would be an easy way out of this dilemma, but this assumption has no supporting evidence. On the other hand, both D- and L-glycerate, as we have seen, can be oxidized by liver enzyme systems to give hydroxypyruvate. [3-^{14}C]Hydroxypyruvate is very readily built into glucose by the liver (Dickens & Williamson, 1959), slice experiments closely comparable with those in the present work showing 15·7 ± 2·1 % incorporation, a value almost equal to the highest reported in this paper for [3-^{14}C]glycerate (Table 2). This raises the possibility that L-glycerate, and perhaps even D-glycerate also, may be metabolized via hydroxypyruvate, which as we have seen, could be formed enzymically from either isomer in liver. Until the route by which hydroxypyruvate is incorporated into the glucose molecule is known [for a discussion, see Dickens & Williamson (1959)], this possibility must remain speculative. At the present time, however, no suitable primary enzymic reaction of L-glycerate, other than its dehydrogenation to hydroxypyruvate, is known.

SUMMARY

1. The preparation and tests of optical purity of samples of D- and L-[3-^{14}C]glycerate are described.

2. The metabolism of these substances by respiring slices of rat liver has been studied.

3. Both isomers are consumed to an equal extent and contribute nearly identically to the ^{14}C appearing in the respiratory carbon dioxide and in the glucose formed. The latter may incorporate in 90 min. up to 16 % of the added isotope.

4. Distribution of ^{14}C from both isomers among the glucose carbon atoms is the same: approximately 60 % of the total labelling being in C-6,

30% in C-1, and 10% in C-3+C-4, of the glucose molecule.

5. Possible metabolic routes which might explain this unexpected metabolic similarity of the two isomers are discussed.

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The Reduction of Oxidized Glutathione in Erythrocyte Haemolysates in Pernicious Anaemia

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The erythrocytes and cells from certain tissues of vitamin B_{12}-deficient rats (Ling & Chow, 1953, 1954; Register, 1954; Kasbekar, Lavate, Rege & Sreenivasan, 1959) and chicks (Hsu, Chow & Okuda, 1959) but not mice (Jaffé, 1958) have unusually low concentrations of reduced glutathione, which rise to within the normal ranges soon after adequate administration of the vitamin. The reason for these low concentrations, however, has not been determined. Stekol (1954) found, using labelled glycine or cystine, that the synthesis of glutathione, *in vivo*, from its three amino acid components was not significantly different from the normal in the liver of vitamin B_{12}-deficient rats, Kasbekar & Sreenivasan (1959); who found a small decrease in the rate of this synthesis in erythrocytes taken from vitamin B_{12}-deficient rats, could not rule out that this might have been due to an observed slight haemolysis of the incubated cells.

Vitamin B_{12} has been implicated in the reduction of disulphides in rat-liver homogenates (Dubnoff, 1950), and it is therefore possible that the reduction of oxidized glutathione (a disulphide) is impaired