that the rate of formation of FADH' by dihydro-lipoic acids is the rate-determining step in the catalytic reduction of diphosphopyridine nucleotide by dihydrolipoic acids. Similarly, the rate of reoxidation of FADH' by oxidized lipoic acids is the rate-determining step in the catalytic oxidation of reduced diphosphopyridine nucleotide by oxidized lipoic acids. The results of the rate experiments and titration experiments show that in the overall two-electron oxidation-reduction reaction catalysed by the enzyme the flavinadenine dinucleotide is reduced to FADH' and reoxidized only once per catalytic cycle. The relation of this finding to the reaction mechanism of the enzyme is discussed.

We are indebted to Dr I. C. Gunsalus and Dr L. J. Reed for generous gifts of DL-lipoic acid and lipoyl derivatives and to Miss M. Hamshaw for the preparation of large amounts of enzyme. One of us (C.V.) is an O.E.E.S. Senior Visiting Fellow, working under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.), with financial aid from the Netherlands Organization for the Advancement of Pure Research. The equipment used was largely provided by the Medical Research Council and the Rockefeller Foundation.

REFERENCES

- Alberty, R. A. (1953). *J. Amer. chem. Soc.* **75**, 1928.
 Beinert, H. (1956). *J. Amer. chem. Soc.* **78**, 5323.
 Beinert, H. (1957). *J. biol. Chem.* **225**, 465.
 Dolin, M. (1956). *Arch. Biochem. Biophys.* **60**, 499.
 Dolin, M. (1957). *J. biol. Chem.* **225**, 557.
 Friedmann, H. C. & Vennesland, B. (1958). *J. biol. Chem.* **233**, 1398.
 Gibson, Q. H. (1954). *Disc. Faraday Soc.* **17**, 137.
 Gibson, Q. H. & Antonini, E. (1960). *Biochem. J.* **77** 328.
 Gunsalus, I. C. (1954). *Fed. Proc.* **13**, 715.
 Haas, E. (1937). *Biochem. Z.* **290**, 291.
 Kornberg, A. & Horecker, B. L. (1953). *Biochemical Preparations*, vol. 3, p. 23. New York: John Wiley and Sons Inc.
 Kuhn, R. & Wagner-Jauregg, T. (1934). *Ber. dtsch. chem. Ges.* **67**, 361.
 Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
 Massey, V. (1958). *Biochim. biophys. Acta*, **30**, 205.
 Massey, V. (1960a). *Biochim. biophys. Acta*, **37**, 314.
 Massey, V. (1960b). *Biochim. biophys. Acta*, **38**, 447.
 Massey, V. & Palmer, G. (1960). *Biochem. J.* **74**, 40P.
 Massey, V. & Veeger, C. (1960). *Biochim. biophys. Acta* (in the Press).
 Michaelis, L., Schubert, M. P. & Smythe, C. V. (1936). *J. biol. Chem.* **116**, 587.
 Reed, L. J. (1957). *Advanc. Enzymol.* **18**, 319.
 Sanadi, D. R., Langley, M. & Searls, R. L. (1959). *J. biol. Chem.* **234**, 178.
 Sanadi, D. R., Langley, M. & White, F. (1959). *J. biol. Chem.* **234**, 183.
 Savage, N. (1957). *Biochem. J.* **67**, 146.
 Searls, R. L. & Sanadi, D. R. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 697.
 Straub, F. B. (1939). *Biochem. J.* **33**, 787.
 Veeger, C. & Massey, V. (1960). *Biochim. biophys. Acta*, **37**, 181.
 Whitby, L. G. (1953). *Biochem. J.* **54**, 437.
 Ziegler, D. M., Green, D. E. & Doeg, K. A. (1959). *J. biol. Chem.* **234**, 1916.

Biochem. J. (1960) **77**, 351

The Hydrolysis of Dipeptides by Different Regions of Rat Small Intestine

By G. B. ROBINSON AND B. SHAW*

Department of Medical Biochemistry and Pharmacology, The Medical School, Birmingham 15

(Received 25 March 1960)

Recent studies of the small intestine have shown that its absorptive capacity for different nutrients varies according to the region. In the rat, Fisher & Parsons (1950b), showed that glucose is most rapidly absorbed in the duodenum and this is also reported to be the most active area for the absorption of iron (Brown & Justus, 1958). On the other hand, the distal half of the small intestine in the rat seems to be the site of maximal absorption of vitamin B₁₂ (Coates & Holdsworth, 1958), of ¹³¹I-

labelled fat (Benson, Chandler, Vansteehuyse & Gagnon, 1956) and of water (Barry, Matthews & Smyth, 1959). Kremens, Linner & Nelson (1954) have shown, using dogs, that the distal half of the small intestine is more important than the proximal half in maintaining fat balance.

Information on the site of absorption of protein breakdown products, i.e. peptides and amino acids, is scanty. Neil (1959) has reported that, in the rat, the rates of absorption of L-cystine and L-cysteine are highest in the duodenum, decrease in the jejunum and are lowest in the ileum; Korelitz & Frank (1959) have shown that translocation of

* Present address: Boots Pure Drug Co. Ltd., Biochemistry Department, Research Division, Island Street, Nottingham.

alanine in guinea pig occurs more swiftly in the jejunum than in the ileum. Experiments *in vitro* on the absorption of peptides have shown that small quantities of glycylglycine can penetrate through the intestine (Agar, Hird & Sidhu, 1953; Newey & Smyth, 1959a; Wiggans & Johnston, 1959), as can traces of L-leucylglycine (Agar, Hird & Sidhu, 1953). Further, the evidence of Florey, Wright & Jennings (1941) indicates that the peptidases of the succus entericus are confined to cells desquamated from the mucosa. These findings suggest that peptide hydrolysis may occur primarily inside the mucosal cell rather than in the intestinal lumen, a possibility stated by Newey & Smyth (1959b).

It was of interest, therefore, to investigate the distribution of peptidase activity in small intestine, to further the understanding of the processes involved in protein digestion and absorption.

A preliminary report of the present work has appeared elsewhere (Shaw & Robinson, 1958).

MATERIALS AND METHODS

Dipeptides. These were obtained commercially and were checked for purity by paper chromatography before and after acid hydrolysis. Where necessary these were purified by recrystallization.

Animals. Adult, male, albino rats of the Glaxo strain were used; the average weight was 250 g. They were allowed only water for 16 hr. before use, to ensure an empty intestine.

Enzyme extracts. All operations were carried out at 4°. The animal was killed by a blow on the head, the small intestine rapidly dissected out and divided into duodenum, jejunum and ileum. For the purposes of characterization, the duodenum was taken as the section of intestine from the pylorus to the ligament of Treitz, the jejunum as the proximal third of the remaining small intestine and the ileum as the distal two-thirds.

The central inch of each of these sections was cut out and slit longitudinally to expose the mucosal surface. This surface was then washed quickly with a fine jet of distilled water to remove adhering mucus, and the mucosa was scraped away with a scalpel. Histological examination showed that all the mucosa was removed by this procedure leaving only a thin layer of submucosa attached to the musculature. The mucosal scrapings from each section were then separately homogenized with a known volume of water in a Potter-Elvehjem-type homogenizer. The volume of distilled water used, 5–10 ml., was determined by preliminary experiments, to give a convenient enzyme concentration for assay. The homogenates were left for 2 hr. to allow complete extraction of the enzymes from the cell debris. They were then centrifuged for 20 min. at 26 000 g in a refrigerated centrifuge, and the supernatant was used as the enzyme extract. The pH throughout this process was approximately 6.5.

This method of extraction was compared with extractions with 10% glycerol or dilute buffers at different pH values and was as effective as, but no better than, these. The residue showed no activity with dipeptide substrates, nor was any obtained by further extraction with water.

Hydrolysis of dipeptide. The ninhydrin method of Moore & Stein (1948) was used and the increase in final colour was the basis for estimating the degree of hydrolysis of the dipeptides. However, the colour yield is not linearly related to the α -amino nitrogen when the extinction exceeds 0.5 (Moore & Stein, 1948), and, in addition, the colour yields of some peptides do not correspond with the theoretical ones (Yanari, 1956). Accordingly, for each dipeptide used, a standard curve was constructed by using mixtures of the dipeptide and its constituent amino acids; this related the colour yield (measured as extinction), to the degree of hydrolysis of the peptide.

In samples of the reaction mixture used in each assay, small amounts of protein were present from the enzyme extract. Preliminary experiments showed that the colour given by this protein with the ninhydrin reagent was negligible and did not interfere with the assay.

Determination of enzyme activity. As the intestinal mucosa contains many enzymes which hydrolyse peptides, preliminary experiments were performed to ascertain the optimum conditions for measurement of enzyme activity. Although the term 'peptide-splitting activity' has been used, it is possible that, under the conditions of assay, more than one enzyme is involved.

Measurements of peptidase activity were made as follows: equal volumes (0.08 ml.) of 0.2M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, at the optimum pH of the activity being measured, and 0.0625M-dipeptide solution were incubated together at 37° in a small tube sealed with a rubber stopper. After 5 min., 0.04 ml. of enzyme extract was added, the tube was shaken quickly and returned to the water bath. Two samples of 0.01 ml. were withdrawn immediately and pipetted into test tubes containing 1 ml. of ninhydrin reagent which stopped the reaction. Similar samples were withdrawn at known time intervals in a 1 hr. period and the degree of hydrolysis was measured. All volumes were measured with an Agla micro-syringe (Burroughs Wellcome and Co. Ltd.).

Blank determinations carried out with boiled enzyme alone, dipeptide alone, and dipeptide together with boiled enzyme, showed no increase in ninhydrin colour.

When metal ions were used in an attempt to activate the enzyme extract, the metal ion was added to the reaction mixture in the buffer; molarities stated refer to the final molarity in the reaction mixture.

The final molarity of the substrate in the reaction mixture was, in all cases, 0.025M.

The extracts of the three sections from one animal were assayed concurrently.

Units of peptide-splitting activity. One unit represents the hydrolysis of 1 mole of substrate in 10 min. Results are expressed as units of activity/mg. of protein.

Determination of protein. Protein nitrogen was determined by the micro-Kjeldahl method; protein was calculated as mg. of N $\times$ 6.25.

RESULTS

Characterization of peptide-splitting activities

L-Leucylglycine. Enzymes hydrolysing this substrate are widely distributed and have been demonstrated in rat intestinal mucosa by Berger & Johnson (1940). These authors showed that maxi-

mum activity occurred at pH 8.6–8.8 and that, after dialysis, activation by Mg^{2+} and Mn^{2+} ions could be obtained. The crude preparation studied in our experiments had a pH optimum at pH 8.5–8.6 (Fig. 1) and activity was decreased by 20% when $0.01M-Mg^{2+}$ was added to the reaction mixture. Therefore assays were carried out at pH 8.6 without added metals. Activity was quite stable, only decreasing by 40% after three days at 0° ; this was in contrast to the instability of the other enzymes studied.

The reaction followed zero-order kinetics and the rate of reaction was proportional to enzyme concentration (Fig. 2).

Glycylglycine. An enzyme, hydrolysing glycylglycine, was demonstrated in rat intestinal mucosa by Berger & Johnson (1940). A similar enzyme was found in rat muscle by Smith (1948*a*); this

enzyme was activated by Co^{2+} ions, and to a lesser extent by Mn^{2+} ions at pH 8.0.

The extracts studied by us had quite low activity when no metals were added, but activity was increased about threefold by $mm-Co^{2+}$. The activated preparation had two pH optima, at 7.4 and at 8.2 (see Fig. 1). Activity at pH 7.4 was decreased by 10% with $mm-Mn^{2+}$, but enhanced about threefold by $mm-Co^{2+}$. At pH 8.2 activity was similarly increased by $mm-Co^{2+}$ but was also

Fig. 1. pH optima for the hydrolysis of dipeptides by mucosal extracts. ●, Glycylglycine; ○, L-leucylglycine. Reaction mixtures contained 0.08 ml. of 0.2M-tris buffer, 0.08 ml. of 0.0625M-dipeptide, 0.04 ml. of mucosal extract; incubated for 30 min. at 37° . For glycylglycine the reaction mixture contained $0.2\mu M-Co^{2+}$.

Fig. 2. Plot of zero-order rate constants (K_0) against protein content of the reaction mixture for the hydrolysis of dipeptides by mucosal extracts. ●, Glycylglycine (measured at pH 8.2); ○, L-leucylglycine (measured at pH 8.6) $K_0 = \mu\text{moles of substrate hydrolysed/min.}$ Reaction mixture contained 0.08 ml. of 0.2M-tris buffer, 0.08 ml. of 0.0625M-dipeptide and 0.04 ml. of mucosal extract. For glycylglycine the reaction mixture contained $0.2\mu M-Co^{2+}$. Temp. 37° .

Fig. 3. pH optima for the hydrolysis of dipeptides by mucosal extracts. ●, Glycyl-L-alanine; ○, glycyl-L-leucine. Conditions as for Fig. 1.

Fig. 4. Plot of first-order rate constant (K_1) against protein content of the reaction mixture for the hydrolysis of dipeptides by mucosal extracts. ●, Glycyl-L-leucine (measured at pH 8.6); ○, glycyl-L-alanine (measured at pH 8.3). Values for K_1 are derived from a graph of $\log S/(S-x)$ against time where S is the initial substrate concn. and x is the concn. after a known time for each reaction. Reaction conditions as for Fig. 2.

increased two and a half times by mM-Mn²⁺, indicating that two enzymes were present.

Measurement of the distribution along the intestine was carried out at pH 8.2 in the presence of mM-Co²⁺.

The reaction was zero order, and the rate of peptide splitting was proportional to enzyme concentration (see Fig. 2).

Glycyl-L-leucine. This was shown to be hydrolysed by an enzyme from rat muscle by Smith (1948b). The enzyme retained its activity well at 0°, and was activated by Zn²⁺ in the presence of phosphate ions.

Activity in intestinal mucosa was maximal at two pH optima, 7.6 and 8.6 (see Fig. 3). At pH 7.6, the crude extract was inhibited by mM-Co²⁺, -Mg²⁺, -Mn²⁺ and -Zn²⁺, to the extent of 70, 20, 50 and 90 % respectively. At pH 8.6, the corresponding inhibitions were 25, 35, 25 and 30 %. Contrary to

the findings of Smith, inhibition with Zn²⁺ occurred, even when 0.2 M-phosphate buffer was used. Activity, at both pH optima, was very labile, 50–60 % being lost after 16 hr. at 0°; some preparations lost all their activity after this treatment.

Assays on distribution were performed at pH 8.6 without added metals.

Hydrolysis followed first-order kinetics, and the rate was proportional to enzyme concentration (Fig. 4).

Glycyl-L-alanine. Price, Meister & Greenstein (1949) showed that this peptide was hydrolysed by rat kidney.

Activity of the mucosal extract had one pH optimum at 8.3 (Fig. 3) and was inhibited by 0.001 M-Co²⁺, -Mg²⁺, -Mn²⁺, and -Zn²⁺. It was extremely labile, all activity being lost when the extract was kept at 0° overnight. The lability

Table 1. *Distribution of peptide-hydrolysing activity in rat small intestine*

1 unit is equivalent to the hydrolysis of 1 μ mole of substrate in 10 min. Levels of activity in: D, duodenum; J, jejunum; I, ileum.

Substrate	Expt. no.	Activity (units/mg. of protein)		
		Duodenum	Jejunum	Ileum
L-Leucylglycine	1	13.2	12.7	19.7
	2	9.8	15.0	13.7
	3	8.4	10.9	12.6
	4	5.6	9.2	15.8
	5	1.8	3.14	14.0
	6	5.8	6.93	9.2
	Mean	7.4	9.6	14.1
<i>P</i> (D - J) > 0.1		<i>P</i> (J - I) < 0.1		<i>P</i> (D - I) = 0.01
Glycylglycine	1	3.0	8.6	9.5
	2	0.9	1.33	7.7
	3	7.5	7.60	9.1
	4	6.1	6.78	12.5
	5	4.9	6.8	11.9
	6	5.9	11.1	11.4
	Mean	4.7	7.0	10.31
<i>P</i> (D - J) > 0.1		<i>P</i> (J - I) < 0.05		<i>P</i> (D - I) = 0.001
Glycyl-L-leucine	1	18.2	21.8	30.7
	2	13.8	38.0	36.0
	3	21.4	27.9	42.8
	4	16.2	28.8	26.6
	5	14.4	24.8	39.9
	6	14.1	20.0	23.2
	7	28.8	52.5	68.2
	Mean	18.1	30.5	38.2
<i>P</i> (D - J) < 0.05		<i>P</i> (J - I) > 0.1		<i>P</i> (D - I) < 0.01
Glycyl-L-alanine	1	12.05	19.6	23.5
	2	18.9	18.4	27.0
	3	14.1	26.0	38.9
	4	11.9	18.5	29.6
	5	20.0	20.0	20.8
	6	18.0	25.2	25.3
	Mean	15.8	21.3	27.5
<i>P</i> (D - J) > 0.1		<i>P</i> (J - I) = 0.05		<i>P</i> (D - I) < 0.01

suggested that the enzyme responsible for the hydrolysis of glycyl-L-alanine was identical with that for glycyl-L-leucine, but after 14 hr. at 0°, activity against glycyl-L-leucine decreased by 50 % whereas that against glycyl-L-alanine decreased by 80 %, indicating different enzymes.

The activity was sufficiently labile at 37° to decrease during the reaction period. This was reflected in the plot of $\log [S/(S-x)]$ against time (where S is the initial concentration and x is the concentration after a known time), giving a curve instead of the expected straight line. Rate measurements were taken from the tangent to the curve at the origin, and were proportional to enzyme concentration (see Fig. 4). If high concentrations of extract were used, a straight line could be obtained but a high concentration could not always be obtained in distribution experiments because of variations in the mucosa; the tangent method was therefore used.

Assays were carried out at pH 8.3 without any activation.

Distribution of peptide-splitting activity

There was an increase in peptide-splitting activity along the intestine; it was lowest in the duodenum and became progressively higher toward the ileum (Table 1). The difference between the levels of activity in duodenum and ileum is significant, $P \leq 0.01$. With glycyl-L-alanine and glycylglycine, the differences between ileum and jejunum are significant, $P = 0.05$ and $P < 0.05$ respectively, but the differences are not as clear as for the other two peptides.

The differences between duodenum and jejunum are only significant in the case of glycyl-leucine.

Experiments performed on consecutive sections of ileum showed that activity in any one region was not abruptly different from that of the neighbouring regions; extracts of four consecutive inch samples showed activities of 13.0, 14.0, 14.5, 13.0 units/mg.

of protein. This indicates that the change in peptidase activity is a gradual one and does not vary at random.

Peptidase activities were related to protein content and not to serosal length as the latter is difficult to measure accurately. There remained the possibility that there is a variation in protein content along the intestine, which would cause ambiguous results. It has been demonstrated by Wood (1944) and Fisher & Parsons (1950*a*) that there is a greater mucosal area per unit of intestinal length in the jejunum of rat than in the ileum. Accordingly we estimated the amount of protein obtained from samples of intestinal tissue used for preparation of enzyme extracts and compared them (Table 2). These results showed no significant difference of protein content per unit of serosal length in the different regions of the intestine. There is a high variance due to individuality and difficulty in measuring the length of the section.

DISCUSSION

Although optimum conditions for each peptide were used, this does not ensure that only one enzyme is being measured in the crude mucosal extract and indeed it is likely that activity is a result of the simultaneous action of a number of enzymes. The results obtained here are, therefore, a measure of the distribution of the ability of the intestine to hydrolyse peptides presented to it, rather than the distribution of particular enzymes. The results obtained support other experimental data suggesting that the intestine does not have a constant function along its length, but that there are variations of function according to region.

Fisher (1954) has suggested that protein may be partly absorbed as peptides. Recent investigations have shown that when the mucosa, *in vitro*, is presented with peptides, the constituent amino acids appear on the serosal side of the intestine, although small amounts of glycylglycine (Agar, Hird & Sidhu, 1953; Newey & Smyth, 1959*a*; Wiggans & Johnston, 1959), and L-leucylglycine (Agar, Hird & Sidhu, 1953) are translocated. Newey & Smyth (1959*b*) have further demonstrated that peptides, as such, enter the mucosa. It seems likely that the peptidases of the intestine are associated with this process. The high level of activity in the ileum may indicate a site of maximal absorption.

The amino acids, alanine, cystine and cysteine, are most rapidly absorbed in the upper intestine where peptidase activity is low. Peptidases may be involved in this but may not limit absorption. Absorption of amino acids is dependent upon the respiratory function (Gibson & Wiseman, 1951; Wiseman, 1953; Friedhandler & Quastel, 1955),

Table 2. Protein content of different regions of the intestine

Total protein was extracted from 1-in. segments of rat small intestine.

Expt. no.	Protein (mg.)		
	Duodenum	Jejunum	Ileum
1	8.2	8.7	5.7
2	9.5	10.0	7.9
3	7.9	9.0	8.1
4	7.5	7.2	8.2
5	7.5	8.9	8.8
6	7.1	7.7	6.0
7	7.1	6.5	6.5
8	9.0	9.1	9.1
Mean	7.97	8.3	7.5

$P > 0.1$ for all cases.

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 ³⁵S-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.

We are indebted to Professor A. C. Frazer for his continued interest and encouragement throughout this work. One of us (G.B.R.) wishes to thank the University of Birmingham for a University Research Scholarship which made this work possible.

REFERENCES

- Agar, W. J., Hird, F. J. R. & Sidhu, G. S. (1953). *J. Physiol.* **121**, 255.
 Barry, B. A., Matthews, J. & Smyth, D. H. (1959). *J. Physiol.* **149**, 78*p*.

- Benson, J. A., Chandler, G. N., Vansteehuyse, F. E. & Gagnon, J. O. (1956). *Gastroenterology*, **30**, 53.
 Berger, J. & Johnson, M. J. (1940). *J. biol. Chem.* **133**, 157.
 Brown, E. R. & Justus, B. W. (1958). *Amer. J. Physiol.* **194**, 319.
 Coates, M. E. & Holdsworth, E. S. (1958). *Biochem. J.* **69**, 20*p*.
 Dickens, F. & Weil-Malherbe, H. (1941). *Biochem. J.* **35**, 7.
 Fisher, R. B. (1954). *Protein Metabolism*, p. 12. London: Methuen and Co. Ltd.
 Fisher, R. B. & Parsons, D. S. (1950*a*). *J. Anat., Lond.*, **84**, 272.
 Fisher, R. B. & Parsons, D. S. (1950*b*). *J. Physiol.* **110**, 281.
 Florey, H. W., Wright, R. D. & Jennings, M. A. (1941). *Physiol. Rev.* **21**, 36.
 Friedburg, F., Tarver, H. & Greenberg, D. M. (1948). *J. biol. Chem.* **173**, 355.
 Friedhandler, L. & Quastel, J. H. (1955). *Arch. Biochem. Biophys.* **56**, 424.
 Gibson, Q. H. & Wiseman, G. (1951). *Biochem. J.* **48**, 426.
 Korelitz, B. I. & Frank, E. D. (1959). *Gastroenterology*, **36**, 94.
 Kremens, A. J., Linner, J. H. & Nelson, C. H. (1954). *Ann. Surg.* **140**, 439.
 Linderström-Lang, K. U. (1952). *Lane Medical Lectures*, vol. 6, p. 51. Stanford: Stanford University Press.
 Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
 Neil, M. W. (1959). *Biochem. J.* **71**, 118.
 Newey, H. & Smyth, D. H. (1959*a*). *J. Physiol.* **145**, 48.
 Newey, H. & Smyth, D. H. (1959*b*). *J. Physiol.* **146**, 11*p*.
 Price, V. E., Meister, A. & Greenstein, J. P. (1949). *Fed. Proc.* **8**, 238.
 Shaw, B. & Robinson, G. B. (1958). *Abstr. Comm. 4th int. Congr. Biochem., Vienna*, no. 12-49, p. 155.
 Smith, E. L. (1948*a*). *J. biol. Chem.* **173**, 571.
 Smith, E. L. (1948*b*). *J. biol. Chem.* **176**, 9.
 Wiggans, D. S. & Johnston, J. M. (1959). *Biochim. biophys. Acta*, **32**, 69.
 Wilson, T. H. & Wiseman, G. (1954). *J. Physiol.* **123**, 126.
 Wiseman, G. (1953). *J. Physiol.* **120**, 63.
 Wood, H. O. (1944). *J. Anat., Lond.*, **78**, 103.
 Yanari, S. (1956). *J. biol. Chem.* **220**, 683.

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

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

BY F. DICKENS AND D. H. WILLIAMSON

Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W. 1

(Received 8 April 1960)

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 gly-

ceric 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