detected in tissues of the chicken, river turtle or frog, all of which contain serine ethanolamine phosphate.

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Properties of a New Glyoxylate Reductase from Leaves

BY I. ZELITCH AND A. M. GOTTO*
Department of Biochemistry, The Connecticut Agricultural Experiment Station, New Haven, Conn., U.S.A., and Medical Research Council Cell Metabolism Research Unit, Department of Biochemistry, University of Oxford

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Observations of the effect of glycollate and glyoxylate added in catalytic quantities in stimulating the rate of oxygen uptake by homogenates of spinach leaves first led to the discovery of a specific $NADH_2$-linked glyoxylate reductase (Zelitch, 1953, 1955). This enzyme (glycollate–NAD oxidoreductase, EC 1.1.1.26) catalyses the reduction of glyoxylate to glycollate (reaction 1) and of hydroxypyruvate to D-glycerate (Stafford, Magaldi & Vennesland, 1954).

$$CHO \cdot CO_2^- + NADH_2 \rightarrow HO \cdot CH_2 \cdot CO_2^- + NAD \quad (1)$$

On the addition of the flavoprotein glycollate oxidase, glyoxylate reductase can transfer hydrogen from $NADH_2$ to oxygen through a series of reactions which constitute a glycollate-oxidation cycle (Zelitch & Ochoa, 1953; Zelitch, 1953).

Although crude preparations of glyoxylate reductase from both spinach and tobacco leaves react with both $NADH_2$ and $NADPH_2$ in the presence of glyoxylate, the highly purified crystalline enzyme from tobacco leaves (Zelitch, 1955) and an enzyme of similar specific activity from spinach leaves (Holzer & Holldorf, 1957) do not function with $NADPH_2$.

* Present address: Department of Biochemistry, Vanderbilt School of Medicine, Nashville, Tenn., U.S.A.

Evidence has been presented that $NADH_2$-linked glyoxylate-reductase activity is associated with chloroplasts in spinach leaves (Zelitch & Barber, 1960), and that glycollic acid plays an important role in the carbon metabolism and respiration of leaves in sunlight (Zelitch, 1958, 1959). These considerations, together with the demonstration of the role of these acids in synthetic reactions of micro-organisms (Kornberg & Gotto, 1961; Kornberg & Sadler, 1961), have stimulated our further interest in the glyoxylate-reductase reaction. We have now found an enzyme in both tobacco and spinach leaves which catalyses the reduction of glyoxylate to glycollate in the presence of $NADPH_2$. This new enzyme has been partially purified and shown to have properties different from the $NADH_2$-linked glyoxylate reductase previously isolated from the same tissues. A preliminary account of this investigation has been presented (Zelitch & Gotto, 1961).

MATERIALS AND METHODS

Leaf extracts. Tobacco (*Nicotiana tabacum*) leaves weighing about 10 g. each from plants grown outdoors, and spinach (*Spinacea oleracea*) leaves purchased locally, were used. The leaves were washed in tap water and stored at 5° in a polythene bag for several hours before extraction.

Tobacco leaves were ground in a chilled mortar with sand and an equal weight of water at 5°, and a total of 0·46 g. of potassium hydrogen carbonate and 0·56 g. of cysteine hydrochloride for each 100 g. of leaves were also added during the process. The thoroughly macerated leaf tissue was squeezed through a double layer of cheese-cloth, and the extract was clarified by centrifuging for 20 min. at 10 000*g*.

Spinach leaves were disrupted in a mechanical plate mill with the addition of 88 mg. of cysteine hydrochloride and 100 mg. of potassium hydrogen carbonate for every 100 g. of leaves. The extract was squeezed through a double layer of cheese-cloth and mixed with filter-paper clippings, and the semi-solid mass was wrapped in sail-cloth and pressed in a hydraulic press. The slightly turbid extract was clarified by filtration through a pad of shredded filter paper on a Büchner funnel.

Materials. Sodium glyoxylate (Radin & Metzler, 1955), lithium hydroxypyruvate (Dickens & Williamson, 1958), potassium pyruvate (Korkes, del Campillo, Gunsalus & Ochoa, 1951) and oxaloacetic acid (Heidelberger, 1953) were prepared in the Laboratory. $NADH_2$ and $NADPH_2$, of the highest grade commercially available, were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A.

Assay of glyoxylate reductase. Initial rates were determined spectrophotometrically by measuring the rate of decrease of extinction at 340 mμ on the reduction of glyoxylate to glycollate in the presence of reduced dinucleotide. Cuvettes of 1 cm. light-path contained 50 μmoles of potassium phosphate buffer, pH 6·4, enzyme extract, 0·15–0·25 μmole of $NADH_2$ or $NADPH_2$, and water to give a final volume of 3 ml. At zero time, 3 μmoles of sodium glyoxylate were added, and the rate of decrease of extinction was observed at 15 sec. intervals for 1 min. A blank cuvette contained all of the components of the reaction mixture except reduced dinucleotide. The reactions were followed with a Beckman DU spectrophotometer or with a Cary recording spectrophotometer. From the molecular extinction coefficient, $6{\cdot}22 \times 10^6$ cm.^{2} mole^{-1} (Horecker & Kornberg, 1948), the enzyme activity was calculated in arbitrary units, 1 unit being defined as the amount of enzyme required to oxidize 1 μmole of reduced dinucleotide/min. in the presence of glyoxylate. Under these conditions of assay the activity was proportional to enzyme concentration up to about 0·2 unit. Protein was determined by measurement of the extinction at 260 and 280 mμ (Warburg & Christian, 1941).

The concentration of sodium glyoxylate used in the assay, 1 mM, was sufficient to give maximal rates for the $NADPH_2$-linked glyoxylate reductase, but would provide far less substrate than that required to obtain maximal rates of the $NADH_2$-linked glyoxylate reductase.

RESULTS

Partial separation of dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase from dihydronicotinamide–adenine dinucleotide-linked glyoxylate reductase of tobacco leaves

Some activity had previously been observed with $NADPH_2$ in assays of glyoxylate reductase in extracts of spinach and tobacco leaves, but this activity disappeared during the purification of the $NADH_2$-linked enzyme. During fractionation of extracts from tobacco leaves with ammonium sulphate, the $NADH_2$-linked glyoxylate-reductase activity was found in fractions precipitated by low and high ammonium sulphate concentrations (Table 1), but almost all of the $NADPH_2$-linked activity was found in the protein precipitated by higher concentrations of ammonium sulphate. Such experiments, in which the ratio of the activity of the $NADPH_2$-linked enzyme to that of the $NADH_2$-linked enzyme was varied from 0·18 to 4·2 by the simple procedure of ammonium sulphate fractionation, provided the first direct evidence that these two enzymic activities are associated with different proteins.

Table 2 shows that the new reaction is analogous to the $NADH_2$-linked activity found previously. For each mole of $NADPH_2$ oxidized, 1 mole of glyoxylate was reduced to glycollate; hence the reaction catalysed by the new enzyme is established as:

$$CHO \cdot CO_2^- + NADPH_2 \rightarrow HO \cdot CH_2 \cdot CO_2^- + NADP \quad (2)$$

Partial separation of dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase from spinach leaves

Further purification of the $NADPH_2$-linked enzyme from tobacco leaves in good yields proved difficult. Since a highly purified $NADH_2$-linked glyoxylate reductase had already been isolated by

Table 1. *Partial separation of dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase from tobacco leaves*

An extract of tobacco leaves (55 g. without midribs) was prepared as described in the Materials and Methods section; the volume of clear extract was 52 ml. Three preparations were obtained by successive addition of 12·7 g., 1·8 g. and 14·6 g. of ammonium sulphate respectively to the extract. Each of these fractions, designated 'low', 'middle' and 'high' respectively, was dissolved in 4·5 ml. of 0·01 M-potassium phosphate buffer, pH 7·0, containing 5 mM-ethylenediaminetetra-acetate.

Step	Total $NADPH_2$-linked reductase units	Total $NADH_2$-linked reductase units	Ratio: $\frac{NADPH_2\text{-linked activity}}{NADH_2\text{-linked activity}}$
Extract	52	33	1·6
'Low' ammonium sulphate	2·0	11	0·18
'Middle' ammonium sulphate	2·9	1·4	2·1
'High' ammonium sulphate	46	11	4·2

Holzer & Holldorf (1957) from spinach leaves, this tissue was investigated as a possible source of the $NADPH_2$-linked enzyme. Spinach leaves were found to provide extracts suitable for the partial separation of the $NADPH_2$-linked glyoxylate reductase, and by following procedures similar to those described by Holzer & Holldorf (1957) it was possible to obtain preparations in which the ratio of glyoxylate-reductase activities linked with $NADPH_2$ and with $NADH_2$ varied from 0·10 to 70. One such experiment is summarized in Table 3 and was carried out as follows.

Initial ammonium sulphate fractionation. An extract was prepared from 2250 g. of spinach leaves as described in the Materials and Methods section. All procedures were carried out at 0–5° unless otherwise stated. Solid ammonium sulphate, 21 g. for each 100 ml. of extract, was added with stirring to give a 30% saturated solution. After being stirred continuously for 30 min., the turbid solution was filtered through coarse fluted filter paper and the residue was discarded. More ammonium sulphate (10·5 g. for each 100 ml. of the original extract) was added to the filtrate to give 45% saturation. The turbid solution was filtered twice through fluted filter paper to obtain a clear filtrate. The residue was dissolved in 0·01 M-potassium phosphate buffer (containing 1 mM-ethylenediaminetetra-acetate), pH 7·6, the volume of which was one-twentieth of that of the original extract. This fraction (Table 3, step 2), which comprises protein precipitated between 30 and 45% saturation, was stored frozen and, after further purification, served as a source of the $NADH_2$-linked enzyme.

More ammonium sulphate (10·5 g. for each 100 ml. of the original extract) was added to the clear filtrate to give 60% saturation. The mixture was filtered twice through the same fluted filter paper, and the residue was dissolved in buffer as above. This fraction (Table 3, step 3) contained most of the $NADPH_2$-linked glyoxylate-reductase activity.

Ethanol fractionation to purify the dihydronicotinamide–adenine dinucleotide phosphate-linked enzyme. The preparation obtained by fractionation between 45 and 60% saturation of ammonium sulphate was dialysed against 20 vol. of 0·01 M-potassium citrate buffer, pH 5·4, with stirring, for 90 min. The final pH of the enzyme solution was about 6·0. If turbidity appeared at this stage, the solution was clarified by centrifuging. Ethanol at 0° was added in small portions with constant stirring while the enzyme preparation was kept at 0° until the mixture contained 22% (v/v) of ethanol (i.e. 22 ml. of ethanol was added for each 78 ml. of solution). Stirring was continued for 1 hr., and the solution was then centrifuged at 0° and the precipitate discarded. Ethanol was added to the supernatant fluid at 0° to raise its concentration to 30% (v/v) (i.e. 8 ml. of ethanol was added for each 92 ml. of the original 45–60% saturated ammonium sulphate fraction after dialysis). The temperature was then lowered to −2° and the stirring continued for 1 hr. The fraction that had been precipitated was collected by centrifuging at −2° and dissolved in ice-

Table 2. *Stoicheiometry of the dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate-reductase reaction*

The complete reaction mixture contained, in a total volume of 3·0 ml., 50 μmoles of potassium phosphate buffer, pH 6·4, 1·8 μmoles of $NADPH_2$, 2·6 μmoles of sodium glyoxylate, and about 0·1 unit of $NADPH_2$-linked glyoxylate reductase obtained from the 'high' ammonium sulphate fraction (Table 1). The reactions were carried out at 22° for 15 min., and terminated by the addition of 3 ml. of 95% (v/v) ethanol. Utilization of $NADPH_2$ was determined by the decrease in extinction at 340 mμ in the Beckman spectrophotometer (Horecker & Kornberg, 1948). Glyoxylate was assayed by the method of Friedemann & Haugen (1943). Glycollate was determined after separation on a column of Dowex 1 (Zelitch, 1958) by the colorimetric method of Calkins (1943).

System	Glyoxylate utilized (μmoles)	$NADPH_2$ utilized (μmoles)	Glycollate produced (μmoles)
Complete	1·6	1·7	1·7
Enzyme omitted	0	0	0
$NADPH_2$ omitted	0	0	0
Glyoxylate omitted	0	0	0

Table 3. *Partial separation of dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase and dihydronicotinamide–adenine dinucleotide-linked glyoxylate reductase from spinach leaves*

Experimental details are given in the text.

Step	Vol. (ml.)	$NADPH_2$-linked reductase: Total units	$NADPH_2$-linked reductase: Specific activity (unit/mg. of protein)	$NADH_2$-linked reductase: Total units	$NADH_2$-linked reductase: Specific activity (unit/mg. of protein)	Ratio: $\frac{NADPH_2\text{-linked activity}}{NADH_2\text{-linked activity}}$
1. Extract	1830	282	0·015	211	0·011	1·3
2. Ammonium sulphate, 30–45% saturation	105	32·2	0·007	36·5	0·008	0·89
(*a*) Acid precipitation	87	3·4	0·005	26·9	0·038	0·13
(*b*) Ammonium sulphate, 17–29% saturation	23	1·8	0·008	17·8	0·077	0·10
3. Ammonium sulphate, 45–60% saturation	122	176	0·10	13·9	0·008	13
(*a*) Ethanol, 22–30% (v/v)	40	8·6	0·092	0·2	0·002	43
(*b*) Ethanol, 30–40% (v/v)	40	12·5	0·36	0·2	0·006	64

cold buffer (volume one-third of that of the original 45–60% saturated ammonium sulphate fraction after dialysis) as above to provide the first active enzyme preparation.

The temperature of the supernatant fluid was lowered to −6° and ethanol was added to bring the concentration to 40% (v/v) (i.e. 10 ml. of ethanol was added for each 90 ml. of the original 45–60% saturated ammonium sulphate fraction after dialysis). The mixture was stirred for 1 hr., and the precipitated protein was then collected by centrifuging at −6°. The 30–40% fraction was suspended in chilled buffer (volume one-third of that of the original 45–60% saturated ammonium sulphate fraction after dialysis) and the turbid solution was ground in a glass homogenizer and clarified by centrifuging. This fraction (Table 3, step 3*b*) and the preceding one were made 50% saturated with ammonium sulphate (by adding 35 g. of the solid for each 100 ml. of solution), and the resulting suspension was stored under refrigeration. Preparations at this stage of purification were used to study the properties of the $NADPH_2$-linked glyoxylate reductase. In the overall procedure the specific activity of the $NADPH_2$-linked enzyme was increased 20-fold and the activity with respect to the $NADH_2$-linked enzyme was increased 50-fold.

Separation of dihydronicotinamide–adenine dinucleotide-linked glyoxylate reductase. Further partial purification of the $NADH_2$-linked enzyme from the same initial preparation was accomplished by methods based on the work of Holzer & Holldorf (1957). The thawed fraction obtained from the 30–45% saturated ammonium sulphate fraction (Table 3, step 2) was brought to pH 4·7 by the dropwise addition of N-acetic acid. The resulting turbid suspension was centrifuged at 10 000*g* for 15 min., and the supernatant fluid was stored in the cold overnight. The fluid was then centrifuged again to yield a clear fraction in which the $NADH_2$-linked enzyme was purified about fivefold over the preceding step with good recovery of the activity (Table 3, step 2*a*). The solution was made 17% saturated with respect to ammonium sulphate (by adding 11·9 g. of the solid for each 100 ml. of solution) and centrifuged. The precipitate was discarded, and the supernatant was made 29% saturated with respect to ammonium sulphate (by adding 8·4 g. of the solid for each 100 ml. of the original solution obtained by the acetic acid treatment above). The mixture was centrifuged, and the precipitate was dissolved in water (volume one-fifth of that of the original solution obtained by the acetic acid treatment) and clarified by centrifuging. This fraction (Table 3, step 2*b*), rich in $NADH_2$-linked glyoxylate-reductase activity, was stored in the cold as a suspension in 30% saturated ammonium sulphate, and preparations at this stage of purification were later used in experiments where the $NADH_2$-linked activity was required. The specific activity of such preparations was increased sevenfold, and the ratio of this activity with respect to the $NADPH_2$-linked enzyme was increased 13-fold.

Comparison of the properties of dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase and dihydronicotinamide–adenine dinucleotide-linked glyoxylate reductase

The highly purified $NADH_2$-linked enzymes described by Zelitch (1955) and Holzer & Holldorf (1957) were characterized by their high Michaelis constants, K_m, at optimum pH values with glyoxylate as substrate, these being approximately 10 mM. A high K_m, about 10 mM, was also observed with our preparation of partially purified $NADH_2$-linked glyoxylate reductase from spinach leaves (Table 3). A much lower K_m for the $NADPH_2$-linked enzyme, however, was obtained with preparations from both tobacco (0·32 mM) and spinach (0·13 mM) leaves. Thus the affinity for glyoxylate of the $NADPH_2$-linked enzyme in both of the tissues examined is about 100 times as great as that previously found for the $NADH_2$-linked enzyme.

D-Glycerate-dehydrogenase activity (Stafford *et al.* 1954) was associated with both of the highly purified $NADH_2$-glyoxylate reductases reported by Zelitch (1955) and Holzer & Holldorf (1957). For the enzyme from spinach leaves, the ratio of the maximal rate with glyoxylate as compared with hydroxypyruvate as substrate was reported to be about 0·25 for the $NADH_2$-linked enzyme. At optimum substrate concentrations, the rate with the $NADPH_2$-linked reductase with glyoxylate was as much as 17 times as great as that with hydroxypyruvate with the partially purified $NADPH_2$-linked reductase from spinach leaves, and ratios of about 5 were observed with ammonium sulphate fractions from tobacco leaves. Thus the new reductase appears to have considerably less D-glycerate-dehydrogenase activity associated with it than the $NADH_2$-linked reductase studied previously.

When enzyme activity sufficient to reduce 0·1 μmole of glyoxylate/min. in the presence of $NADPH_2$ in the standard assay was tested with other possible substrates, no activity was observed with pyruvate (3 μmoles) or oxaloacetate (3 μmoles).

The pH optimum in phosphate buffer appeared to be about the same for the glyoxylate reductases from both spinach and tobacco leaves. The highest rate of activity is always observed between pH 6·0 and 6·5, with half-maximal velocities occurring at about pH 8·0.

Determination of glyoxylate with dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase

The properties of the activity of the new enzyme, namely high specificity together with a low K_m, and a favourable equilibrium, should make this glyoxylate reductase a convenient means for the rapid, sensitive and specific estimation of glyoxylate. One need only observe the extent of oxidation of an excess of $NADPH_2$ at 340 mμ in the presence of the partially purified enzyme to determine glyoxylate. Such determinations on pure samples of sodium glyoxylate are shown in Fig. 1, and demonstrate that satisfactory results can be obtained by this procedure.

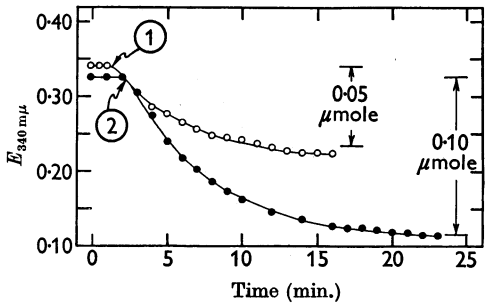

Fig. 1. Spectrophotometric determination of glyoxylate with dihydronicotinamide–adenine dinucleotide phosphate-linked glyoxylate reductase. Each cuvette contained, in 3·0 ml., 50 μmoles of potassium phosphate, pH 6·4, $NADPH_2$, and 0·48 mg. of a partially purified $NADPH_2$-linked glyoxylate-reductase preparation from spinach leaves. Blank cuvettes contained all of the components except $NADPH_2$. ○, At arrow 1, 0·050 μmole of sodium glyoxylate was added to each cuvette; the determined value was 0·055 μmole (110%). ●, At arrow 2, 0·10 μmole of sodium glyoxylate was added to each cuvette; the determined value was 0·102 μmole (102%).

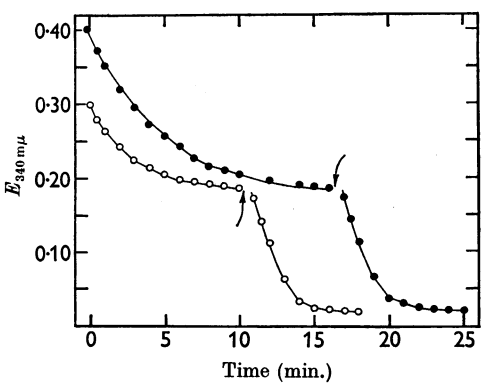

Fig. 2. Spectrophotometric determination of dihydronicotinamide–adenine dinucleotide phosphate and dihydronicotinamide–adenine dinucleotide in mixtures with the use of glyoxylate reductases. Each cuvette contained, in 3 ml., 50 μmoles of potassium phosphate, pH 6·4, 3 μmoles of sodium glyoxylate, and $NADPH_2$ and $NADH_2$ in concentrations estimated solely by their extinctions at 340 mμ. Blank cuvettes contained all components except reduced dinucleotides. At zero time, partially purified $NADPH_2$-linked glyoxylate reductase was added, and at the time indicated by the arrow a preparation of $NADH_2$-linked glyoxylate reductase was added. ●, The mixture contained 0·111 μmole of $NADPH_2$ and 0·082 μmole of $NADH_2$; the determined values were 0·104 μmole of $NADPH_2$ (94%) and 0·078 μmole of $NADH_2$ (95%). ○, The mixture contained 0·058 μmole of $NADPH_2$ and 0·086 μmole of $NADH_2$; the determined values were 0·054 μmole of $NADPH_2$ (93%) and 0·080 μmole of $NADH_2$ (93%).

Determination of dihydronicotinamide–adenine dinucleotide and dihydronicotinamide–adenine dinucleotide phosphate in mixtures with glyoxylate reductases

In the presence of an excess of glyoxylate, the two glyoxylate reductases can also be utilized to determine $NADH_2$ and $NADPH_2$ in mixtures of the dinucleotides in a simple and convenient manner. Although such analyses should be more accurate with the use of highly purified enzymes, determinations can be carried out satisfactorily with the cruder preparations isolated from spinach leaves (Table 3). One can determine $NADPH_2$ first by adding a preparation of $NADPH_2$-linked glyoxylate reductase, and, when all of the $NADPH_2$ is oxidized and the reaction has stopped, the $NADH_2$ concentration is determined similarly by the addition of $NADH_2$-linked glyoxylate reductase. By this method, with the concentration of each reduced dinucleotide added to the mixture estimated from the extinction of each component individually determined at 340 mμ, values of at least 93% of the known added amounts for both $NADPH_2$ and $NADH_2$ (Fig. 2) were obtained. Thus the kinetic and thermodynamic properties of these reductases may make them useful analytical reagents for the estimation of $NADH_2$, $NADPH_2$ and glyoxylate.

DISCUSSION

Although the new $NADPH_2$-linked glyoxylate reductase of leaves has not as yet been obtained completely free from the $NADH_2$-linked glyoxylate reductase, the substantial separation of these two activities that has been achieved leaves no doubt that a second protein catalyses the $NADPH_2$-linked activity. In addition, the significantly different K_m values and the substrate specificity support this view.

Racker (1961) suggested that, in the metabolism of leaves, the glycollate–glyoxylate cycle (Zelitch, 1953; Zelitch & Ochoa, 1953) could play a role similar to that of other systems in which the activity of a dehydrogenase outside the mitochondria is linked with oxidative enzymes inside. Schäfer & Lamprecht (1961) have also postulated such a role for the glycollate–glyoxylate system in transporting hydrogen from $NADH_2$ in the cytoplasm through the mitochondrial membrane of liver tissue. If the glycollate–glyoxylate cycle operates in the living leaf, it may interact with NADP-linked dehydrogenases as well as NAD-linked dehydrogenases. Moreover, in the leaf, the oxidative functions of the chloroplast (Zelitch & Barber, 1960) as well as the mitochondria must be considered.

The kinetic properties of the pairs of glyoxylate reductases of spinach and tobacco leaves do not

help to decide which may have greater importance in the tissue. The much lower K_m for the $NADPH_2$-linked reductase would suggest that it may be favoured, but at optimum substrate concentrations the maximal rate of glyoxylate reductase is about twice as great with $NADH_2$ as with $NADPH_2$. Thus more experimental work will be needed before the physiological role of the two reductases in leaves will be better understood.

SUMMARY

1. A new glyoxylate reductase, which catalyses the reduction of glyoxylate to glycollate in the presence of $NADPH_2$, has been demonstrated and separated to a large extent from the $NADH_2$-linked glyoxylate reductase in both tobacco and spinach leaves. Unlike the $NADH_2$-linked glyoxylate reductase of leaves, the new enzyme has a low Michaelis constant for glyoxylate, 0·13 mM, and reacts only slowly with hydroxypyruvate. Pyruvate and oxaloacetate are not substrates.

2. The kinetic properties and favourable equilibrium should make the $NADPH_2$-linked glyoxylate reductase a useful reagent for the analytical estimation of glyoxylate. Together with $NADH_2$-linked glyoxylate reductase, both enzymes may be used to provide a convenient method for determining the concentration of $NADPH_2$ and $NADH_2$ in mixtures of the two dinucleotides.

3. Although there is increasing evidence of the importance of glycollate and glyoxylate in the carbon metabolism and respiration of leaves, especially in sunlight, the physiological role of the glyoxylate reductases remains uncertain.

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Further Studies on the Entry Rates of Acetate and Glucose in Sheep, with Special Reference to Endogenous Production of Acetate

BY E. F. ANNISON AND R. R. WHITE
School of Rural Science, University of New England, Armidale, New South Wales, Australia

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The measurement of acetate and glucose turnover in sheep in separate experiments was described by Annison & Lindsay (1961) and Annison & White (1961). Isotope-dilution techniques were used to measure the rates of entry of substrates into body pools and a considerable range of turnover rates was observed in animals maintained on similar diets. These differences between animals, which were presumably due to differences in endocrine balance, emphasized that the interrelations of acetate and glucose turnover could be more conveniently studied by methods allowing simultaneous measurement of the rates of entry of both acetate and glucose. In the present studies, this has been achieved by the infusion of both labelled acetate and labelled glucose. Measurements of the