Choice between Autotrophy and Heterotrophy in *Pseudomonas oxalaticus*

UTILIZATION OF OXALATE BY CELLS AFTER ADAPTATION FROM GROWTH ON FORMATE TO GROWTH ON OXALATE

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1. The labelling patterns of phosphoglycerate obtained from formate-grown or oxalate-grown *Pseudomonas oxalaticus* after exposure for 15 sec. to [^{14}C]formate or [^{14}C]oxalate respectively were determined. 2. The phosphoglycerate obtained from the formate-grown cells contained 78% of the radioactivity in the carboxyl group. This is in accord with that predicted for operation of the ribulose diphosphate cycle of carbon dioxide fixation. 3. The labelling pattern of the phosphoglycerate obtained from the oxalate-grown cells approached uniformity, as predicted for the heterotrophic pathway of oxalate assimilation. The departure from complete uniformity may have been due to concurrent $^{14}\text{CO}_2$ fixation into C_4 dicarboxylic acids. 4. The labelling pattern of phosphoglycerate obtained from cells that had just started to grow on oxalate after adaptation from formate was determined after incubation of the cells for 15 sec. with [^{14}C]oxalate. This pattern approached uniformity. 5. The pathway of incorporation of $^{14}\text{CO}_2$ into cells that had just started to grow on oxalate after adaptation from formate, in the presence of either formate or oxalate as energy source, was studied by chromatographic and radioautographic analysis. 6. It is concluded from the isotopic data that a mixed heterotrophic-autotrophic metabolism, with the former mode predominating, operates in the initial stages of growth on oxalate after adaptation from growth on formate.

Pseudomonas oxalaticus has been shown (Quayle & Keech, 1959a, 1959b) to grow on formate as sole carbon source by synthesizing its cell constituents autotrophically from carbon dioxide via the ribulose diphosphate cycle, using the energy obtained from oxidation of formate by a nicotinamide nucleotide-linked formate dehydrogenase:

On all other growth substrates tested, including oxalate, the organism grows heterotrophically. Growth on oxalate is of special interest; it has been shown (Quayle, Keech & Taylor, 1961; Quayle & Taylor, 1961; Quayle, 1963a,b) that synthesis of cell constituents occurs in effect from glyoxylate, the glyoxylate being derived from oxalate by the reactions:

The necessary energy is derived from the oxidation of formate produced from the oxalate by the reactions:

Thus the same source of energy, i.e. formate, can drive two different types of assimilatory pathway, depending on whether the formate is supplied as such to the cell from outside, or produced inside the cell from oxalate. The former situation gives rise to autotrophic metabolism, the latter to heterotrophic metabolism. A formate-grown cell when re-suspended in an oxalate-growth medium enters a lag period of no growth, during which time the enzymes necessary to decarboxylate oxalate to formate and to condense oxalate to phosphoglycerate via glyoxylate are synthesized. Under these conditions, at the commencement of growth

on oxalate, the cell also still possesses the enzymes necessary for autotrophic metabolism. The cell thus has open to it the choice of two types of carbon assimilation. When presented with formate it must perforce assimilate carbon autotrophically; however, when presented with oxalate the cell could theoretically use the energy of oxidation of the oxalate not only for heterotrophic assimilation of oxalate, but also for autotrophic fixation of carbon dioxide. This paper describes experiments to test whether this, in fact, happens.

Two experimental approaches have been used. The first approach utilizes the fact that different labelling patterns in phosphoglycerate should result from operation of the two different biosynthetic pathways. Several groups of workers (see Elsdon, 1962) have shown that fixation of $^{14}\text{CO}_2$ by the ribulose diphosphate cycle leads to phosphoglycerate that initially is labelled predominantly in the carboxyl group, and this labelling pattern in phosphoglycerate would therefore be expected after brief autotrophic metabolism of $[^{14}\text{C}]\text{oxalate}$ or $[^{14}\text{C}]\text{formate}$ by *P. oxalaticus*. If, however, oxalate is being assimilated heterotrophically it may be predicted that the consecutive intermediary metabolites of the biosynthetic pathway, namely glyoxylate, tartronic acid semialdehyde, glycerate and phosphoglycerate, would be uniformly labelled even after the briefest incubation with $[^{14}\text{C}]\text{oxalate}$. Thus the labelling pattern in $[^{14}\text{C}]\text{phosphoglycerate}$ should serve as an indicator of the type of assimilatory pathway of $[^{14}\text{C}]\text{oxalate}$ that is being followed.

The second approach involves following the path of $^{14}\text{CO}_2$ incorporation into cell constituents by established methods of chromatography and radioautography.

METHODS AND MATERIALS

Maintenance and growth of organism. Cultures of *P. oxalaticus* (OX1) were obtained from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen (culture no. 8642), and maintained on slopes of 1% (w/v) nutrient agar (Oxo Ltd., London, S.E. 1) containing 5 mm-sodium oxalate or -potassium oxalate. Stock cultures were grown at 30°, stored at 2° and subcultured monthly.

The medium used for liquid growth was the same as that used by Jayasuriya (1955) except that 50–100 mm-sodium formate or 50–100 mm-sodium oxalate was used as the carbon source. Generally the organism was grown in either 500–600 ml. of growth medium contained in a 2 l. conical flask or 100 ml. of medium in a 500 ml. flask. The flasks were shaken in a rotary shaker at 30°. Growth was followed by measurement of the increase in extinction at 540 m μ or 700 m μ ; calibration curves relating the respective extinction values to the dry wt. of cells/ml. of culture were used.

Preparation of tracer solutions. Radioactive chemicals were purchased from The Radiochemical Centre, Amersham, Bucks. Solutions were prepared by the methods described by Quayle & Keech (1959a).

Incorporation of $[^{14}\text{C}]\text{formate}$ into formate-grown cells. Cells in the exponential phase of growth on formate were harvested by centrifuging and resuspended at a cell density of 10 mg. dry wt./ml. in 10 ml. of growth medium containing 10 mm-sodium formate as carbon source. The suspension was placed in a cylindrical vessel (10 cm. $\times$ 2.5 cm. diam.) fitted with a sintered-glass bottom. Vigorous aeration in the suspension was maintained by blowing a stream of air through the sintered glass. After aeration for 5 min. at 30°, 1 ml. of sodium $[^{14}\text{C}]\text{formate}$ solution (0.14 M, 200 μC of $^{14}\text{C}/\text{ml.}$) was added, and incubation under conditions of aeration was continued for 15 sec. The entire suspension was tipped into 40 ml. of hot ethanol, and the resulting precipitate removed by centrifuging the mixture when cool. The supernatant was concentrated to a small volume under vacuum, chromatographed and split into ten samples, each of which was separately chromatographed in two dimensions as described below. The salt content of the concentrated solution necessitated its chromatography in small portions.

Incorporation of $[^{14}\text{C}]\text{oxalate}$ into oxalate-grown cells. The experiment was performed with oxalate-grown cells in a fashion similar to that described above for formate-grown cells. The cells were resuspended at a cell density of 9 mg. dry wt./ml. in 8 ml. of growth medium containing 10 mm-potassium oxalate as carbon source. After preliminary aeration for 5 min. at 30°, 2.5 ml. of potassium $[^{14}\text{C}]\text{oxalate}$ solution (0.15 M, 130 μC of $^{14}\text{C}/\text{ml.}$) was added, and incubation under conditions of aeration was continued for 15 sec. The entire suspension was tipped into 40 ml. of hot ethanol, and the resulting precipitate removed by centrifugation. The resulting supernatant was concentrated and split into ten samples, each of which was chromatographed as described below.

Incorporation of $[^{14}\text{C}]\text{oxalate}$ into cells just adapted to growth on oxalate from growth on formate. Cells in the exponential phase of growth on formate were harvested at a cell density of 0.6 mg. dry wt./ml. by centrifuging, and resuspended in oxalate growth medium to a cell density of 0.3 mg. dry wt./ml. After being shaken at 30° for 22 hr. the suspension had passed through the lag phase of no growth and had started to grow on oxalate. The organisms were harvested at a cell density of 0.44 mg. dry wt./ml. and resuspended at a density of 7 mg. dry wt./ml. in 20 ml. of growth medium containing 10 mm-potassium oxalate as carbon source. A portion (8 ml.) of the suspension was aerated for 5 min. at 30° under the same conditions as in the previous two experiments and 2.5 ml. of potassium $[^{14}\text{C}]\text{oxalate}$ solution (0.15 M, 130 μC of $^{14}\text{C}/\text{ml.}$) was added. After incubation for 15 sec. with the tracer, the entire suspension was tipped into 40 ml. of hot ethanol and centrifuged. The resulting supernatant was concentrated and split into ten samples, each of which was chromatographed as described below.

Kinetic analysis of the entry of $[^{14}\text{C}]\text{bicarbonate}$ into cells just adapted to growth on oxalate from growth on formate. Cells in the exponential phase of growth on formate were harvested by centrifuging and resuspended in oxalate growth medium at a cell density of 0.19 mg. dry wt./ml. After shaking for 6 hr. at 30° the cells had passed through the lag phase of no growth and had commenced to grow on oxalate. They were harvested at a cell density of 0.21 mg. dry wt./ml. and divided into two portions. One portion was resuspended at a cell density of 3.1 mg. dry wt./ml. in 9 ml. of a solution containing 50 mm-sodium formate, 10 mm-

sodium phosphate buffer, pH 7.0, and 10mm-ammonium sulphate; the other portion was resuspended at a cell density of 3.9 mg. dry wt./ml. in 9 ml. of a similar solution to that described above except that 50mm-sodium oxalate was substituted for sodium formate. The two cell suspensions were each vigorously aerated at 30° in sintered-glass bubbler vessels (as described above) for 5 min. and 1 ml. of $\text{NaH}^{14}\text{CO}_3$ (0.028 M; 200 μC of $^{14}\text{C}/\text{ml.}$) was added to each. Samples (1 ml.) were rapidly withdrawn by hypodermic syringe and transferred to 4 ml. of ethanol. The resulting precipitates were each extracted with 0.25 ml. of aq. 20% (v/v) ethanol and the extracts combined with their respective supernatants. Each supernatant was concentrated under vacuum to dryness and taken up in 0.25 ml. of aq. 20% ethanol, and 0.1 ml. of each sample was analysed by chromatography and radioautography as referred to below.

Chromatographic analysis, radioautography and identification of labelled compounds. The individual samples from the experiments described above were applied to the origin positions of sheets of Whatman no. 4 chromatographic paper (18½ in. × 22 in.). The methods of two-dimensional chromatography, radioautography, radioassay and identification of labelled compounds have been described previously (Quayle & Keech, 1959a; Large, Peel & Quayle, 1961). For those experiments in which phosphoglycerate was degraded, the radioactive areas on the chromatograms that contained radioactive phosphoglycerate were eluted with water and the solutions treated with Polidase-S (Schwarz Laboratories Inc., New York, N.Y., U.S.A.) according to the procedure of Benson, Bassham & Calvin (1951). The resulting solution of dephosphorylated compounds was then rechromatographed in the same two-dimensional system as before. The labelled glyceric acid on these chromatograms was eluted, and the samples corresponding to each individual experiment were combined.

Preparation of samples for degradation. The samples of purified radioactive glycerate were each mixed with 400 mg. of calcium D-glycerate as carrier and crystallized from aq. ethanol to constant specific radioactivity.

Degradation of labelled compounds. The specific radioactivity of the labelled compounds was determined by total oxidation to carbon dioxide with Van Slyke-Folch reagent

as described by Sakami (1955). Glyceric acid was degraded by the procedure described by Sakami (1955); this involved periodate oxidation, which yielded carbon dioxide, formic acid and formaldehyde from C-1, C-2 and C-3 atoms respectively. The formic acid was oxidized by mercuric acetate to carbon dioxide; the formaldehyde remaining in the solution was oxidized in two steps to carbon dioxide, with sodium hypiodite and then mercuric acetate.

Assay of $^{14}\text{CO}_2$. $^{14}\text{CO}_2$ was converted into $\text{Ba}^{14}\text{CO}_3$ by precipitation with BaCl_2 (5%, w/v) and was then plated in triplicate on paper disks (2.69 cm.²) as described by Sakami (1955). The samples were assayed for radioactivity in a gas-flow counter (model D-47, Nuclear Instrument and Chemical Corp., Chicago, Ill., U.S.A.) and corrected for self-absorption.

RESULTS

Labelling patterns of phosphoglycerate. The labelling pattern of phosphoglycerate obtained from formate-grown *P. oxalaticus* exposed for 15 sec. to ^{14}C formate is shown in Table 1. The carboxyl group contains almost 80% of the radioactivity contained in the molecule. The general features of the pattern are those expected from operation of the ribulose diphosphate cycle of carbon dioxide fixation, the $^{14}\text{CO}_2$ being derived from oxidation of the administered ^{14}C formate. Operation of the cycle should result in uniformity of labelling in C-2 and C-3 atoms; the departure observed may not be significant, as the specific radioactivity of the barium carbonate originating from these carbon atoms was low (for C-3 the radioactivity measured was of the same order as the background count).

The labelling pattern of phosphoglycerate obtained from oxalate-grown *P. oxalaticus* exposed for 15 sec. to ^{14}C oxalate is also shown in Table 1. Uniform labelling of the molecule would result in 33% of the radioactivity being present in each carbon atom. The pattern obtained approximates

Table 1. *Distribution of radioactivity in glyceric acid isolated from P. oxalaticus growing on ^{14}C formate or ^{14}C oxalate*

Growth conditions	Tracer used	Carbon atom no.	Sp. radioactivity of $\text{Ba}^{14}\text{CO}_3$ derived from each C atom (counts/min./mg. of BaCO_3)	Sum of sp. radioactivities of individual C atoms	Sp. radioactivity of $\text{Ba}^{14}\text{CO}_3$ obtained from combustion × no. of C atoms in molecule	Percentage of total radioactivity in molecule contributed by each C atom
Grown on formate	^{14}C Formate	1	21.1	27.2	27.15	77.6
		2	4.5			16.5
		3	1.6			5.9
Grown on oxalate	^{14}C Oxalate	1	8.0	19.8	21.4	40.5
		2	7.3			36.9
		3	4.5			22.6
Grown on formate and then adapted to grow on oxalate	^{14}C Oxalate	1	13.5	34.0	38.1	39.7
		2	11.3			33.2
		3	9.2			27.1

to this but C-1 does contain more, and C-3 less, than uniformity requires. Fixation of labelled carbon dioxide by phosphopyruvate might result in an enhanced amount of radioactivity in C-1. It has been shown that whole cells growing on oxalate fix small amounts of $^{14}\text{CO}_2$ into malate (Quayle & Keech, 1960), and cell-free extracts of the oxalate-grown organism incorporate $^{14}\text{CO}_2$ into malate and aspartate in the presence of phosphopyruvate (Quayle *et al.* 1961). Such fixation of $^{14}\text{CO}_2$, followed by randomization of the label between C-1 and C-4 of fumarate and subsequent decarboxylation of derived oxaloacetate, would result in phosphopyruvate, and hence derived phosphoglycerate, with predominant labelling in the carboxyl group. Combination of this species of labelled phosphoglycerate with that coming from condensation of oxalate by the biosynthetic pathway might then result in a labelling pattern with enhanced radioactivity in C-1. This would not explain the lower radioactivity of C-3.

The labelling patterns observed in phosphoglycerate as a result of heterotrophic growth on oxalate or autotrophic growth on formate are clearly distinct enough for a determination to be made of the major metabolic route followed by oxalate carbon atoms in a cell that is competent either to incorporate them as such heterotrophically via the glycerate pathway or to incorporate autotrophically

carbon dioxide derived from the oxalate by decarboxylation and oxidation. Thus a culture of formate-grown *P. oxalaticus* was resuspended in oxalate growth medium until the organism had adapted to the new substrate and had just started to grow on it. It was then harvested and exposed to ^{14}C oxalate for 15 sec. under the same conditions as were used in the above two isotopic experiments. The radioactive phosphoglycerate obtained from this experiment was dephosphorylated and degraded as before. The results of this degradation are shown in Table 1. The labelling pattern shows a close approximation to uniformity; once again C-1 contains more, and C-3 less, radioactivity than 33% of the total.

The conclusion from this result is that the major pathway of oxalate assimilation followed by *P. oxalaticus* when it commences growing on oxalate, subsequent to formate, is heterotrophic rather than autotrophic. The assumptions upon which this conclusion is based are examined in the Discussion section.

Kinetic analysis of $^{14}\text{CO}_2$ uptake. A culture of formate-grown *P. oxalaticus* was resuspended in oxalate growth medium until it had adapted and had just started to grow on the new substrate. The cells were harvested and divided into two: one half was resuspended in formate growth medium and the other half in oxalate growth medium. Each

Table 2. *Distribution of radioactive carbon among the non-volatile labelled components of the ethanol-soluble fractions of a suspension of P. oxalaticus grown on formate and just adapted to growth on oxalate incubated with ^{14}C bicarbonate in the presence of formate or oxalate*

^{14}C Bicarbonate (1 ml., 28.4 mM, 200 μC of ^{14}C /ml.) was added at zero time to (a) a 9 ml. suspension of bacteria (28 mg. dry wt.) vigorously aerated in 50 mM-sodium formate, 10 mM-sodium phosphate buffer, pH 7.0, and 10 mM- $(\text{NH}_4)_2\text{SO}_4$, and to (b) a 9 ml. suspension of bacteria (35 mg. dry wt.) vigorously aerated in 50 mM-sodium oxalate, 10 mM-sodium phosphate buffer, pH 7.0, and 10 mM- $(\text{NH}_4)_2\text{SO}_4$. Samples (1 ml.) were withdrawn by hypodermic syringe at known times and transferred to 4 ml. of ethanol (95%). The precipitates were each extracted with 0.25 ml. of aq. 20% (v/v) ethanol, and each extract was combined with the supernatant. The samples were analysed by two-dimensional chromatography. The radioactive areas were located by radioautography and directly counted with a mica-end-window Geiger-Müller tube.

Extracellular carbon source	Time (sec.)	Radioactivity (counts/min.)						
		Phosphorylated compounds	Malate	Glycine, serine, glutamate	Aspartate	Citrate	Unknowns	Total
(a) Formate	7	76	7	—	—	—	—	83
	15	30	13	12	10	7	7	79
	23	81	16	13	15	16	13	154
	30	106	34	33	23	14	27	237
	38	106	34	47	28	14	9	238
	45	95	24	55	20	11	14	219
(b) Oxalate	7	36	12	—	10	1	—	59
	16	58	22	17	27	16	7	147
	24	70	33	52	54	27	13	249
	33	72	53	57	57	16	14	269
	43	70	60	72	65	21	16	304
	54	75	54	96	55	24	13	317

Fig. 1. Variation with time of the percentage distribution of ^{14}C from $[^{14}\text{C}]$ bicarbonate incorporated into the constituents of the ethanol-soluble fraction of *P. oxalaticus* grown on formate, just adapted to growth on oxalate, and resuspended in growth media with (a) formate and (b) oxalate as energy source. Conditions were as described in Table 2. ▲, Phosphorylated compounds; ■, malate; ○, aspartate; △, glycine + serine + glutamate; ●, citrate.

culture was injected with $[^{14}\text{C}]$ bicarbonate and samples were rapidly withdrawn for analysis by chromatography and radioautography. The results of the experiment are given in Table 2. The curves obtained by plotting against time the percentage of the total radioactivity fixed that was contributed by each constituent of the samples are shown in Figs. 1(a) and 1(b). In Fig. 1(a) it can be seen that, as would be expected, the pattern of $^{14}\text{CO}_2$ uptake by the cells in the presence of extracellular formate is characteristic of autotrophic metabolism, i.e. the majority of the radioactivity fixed at the earliest time is contained in phosphorylated compounds, and the initial slope of the percentage curve for phosphorylated compounds is negative. The pattern is similar to that obtained by Quayle & Keech (1959a) for $^{14}\text{CO}_2$ uptake by *P. oxalaticus* in the presence of formate after prolonged growth on formate. Fig. 1(b) shows that the pattern of $^{14}\text{CO}_2$ uptake by the cells in the presence of extracellular oxalate is qualitatively similar to that obtained with extracellular formate but differs quantitatively. The proportion of the total fixed radioactivity present in phosphorylated compounds at all times is considerably less in the presence of extracellular oxalate than in the presence of extracellular formate. It may be noted that *P. oxalaticus* after prolonged growth on oxalate fixes very little $^{14}\text{CO}_2$ in the

presence of oxalate, and the main product is malate (Quayle & Keech, 1960).

The conclusion from these results is that some carbon dioxide is fixed autotrophically in the presence of oxalate as energy source by cells just adapted from formate, but this is quantitatively less than in the presence of formate as energy source.

DISCUSSION

Interpretation of the two kinds of experiment reported, involving determination of labelling pattern on the one hand and following the kinetics of $^{14}\text{CO}_2$ uptake on the other, is subject to the kind of uncertainties common to many radioactive-label experiments aimed at assessing the relative contributions of two simultaneously operating pathways. The conclusion drawn from the study of labelling patterns is based on the assumption that, in any mixed heterotrophic-autotrophic metabolism, the gross labelling pattern of phosphoglycerate is made up of two differently labelled species, the relative amount of label in each being proportional to the flow of carbon atoms through the two respective pathways. It is not difficult to envisage circumstances in which this assumption could not be valid, e.g. if the two species of phosphoglycerate were contained in separate pools of

different sizes and turnover rates. If this were so the labelling data could overestimate the contribution made by the pathway that went through the pool of phosphoglycerate of larger size.

The conclusion drawn from the experiment that followed the kinetics of $^{14}\text{CO}_2$ uptake is subject to the same limitations as above if separate pools of phosphoglycerate exist. Also, in comparing the results of the two experiments, there is a technically unavoidable difference between the two experimental conditions. In the assessment of pathways by difference of labelling pattern, the $^{14}\text{CO}_2$ whose autotrophic fixation is being determined is arising intracellularly from oxalate breakdown, whereas in the kinetic experiment the $^{14}\text{CO}_2$ is being presented from outside of the cell. If the resulting intracellular carbon dioxide concentration were widely different in the two cases it might affect the relative extent to which autotrophic metabolism operated, and hence the two experimental approaches might give different answers. The kinetic experiments, as conducted, clearly demonstrate that some autotrophic fixation of carbon dioxide is taking place at the expense of the energy of oxalate oxidation in a cell of the appropriate enzymic constitution. It is not possible, however, from this experiment to determine what proportion of the total metabolism is autotrophic. The uniformity of the labelling pattern found in phosphoglycerate indicates that if there is any contribution of autotrophic metabolism it could only at the most be a minor one. We thus conclude, bearing in mind the possible sources of error in the two experimental approaches, that a mixed heterotrophic-autotrophic metabolism, with the former mode predominating, operates in the initial stages of growth on oxalate after adaptation from growth on formate.

The energy requirements for the biosynthesis of one molecule of triose phosphate by the two alternative modes of metabolism are very different. Triose phosphate synthesis by the ribulose diphosphate cycle of carbon dioxide fixation may be expressed by:

Triose phosphate synthesis by the glycerate pathway of oxalate utilization may be expressed by:

Thus the heterotrophic pathway is more economical of energy to the extent of $2\text{NADH} + 5\text{ATP/mol.}$ of triose phosphate formed. It should be remembered that the energy source is the same in both cases, i.e. reaction (1) and (6). Hence it would be expected on energetic grounds that, in a cell that is enzymically competent to assimilate oxalate carbon atoms heterotrophically or carbon dioxide autotrophically, the former metabolism would be favoured. The conclusion that the organism carries out a mixed heterotrophic-autotrophic metabolism, with the former predominating under the special circumstances obtaining on commencement of growth on oxalate after adaptation from formate, is thus in accord with expectation. This particular mixed metabolism is essentially a temporary process for, on continued growth on oxalate, cessation of synthesis of the key enzymes of the autotrophic pathway switches the metabolism virtually completely over to heterotrophy.

Further aspects of the choice of metabolic pathway by *P. oxalaticus*, when growing on mixtures of formate and oxalate as well as on other substrates, are discussed in the succeeding paper (Blackmore & Quayle, 1968).

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REFERENCES

- Benson, A. A., Bassham, J. A. & Calvin, M. (1951). *J. Amer. chem. Soc.* **73**, 2970.
- Blackmore, M. A. & Quayle, J. R. (1968). *Biochem. J.* **107**, 705.
- Elsden, S. R. (1962). In *The Bacteria*, vol. 3, p. 1. Ed. by Gunsalus, I. C. & Stanier, R. Y. New York and London: Academic Press Inc.
- Jayasuriya, G. C. N. (1955). *J. gen. Microbiol.* **12**, 419.
- Large, P. J., Peel, D. & Quayle, J. R. (1961). *Biochem. J.* **81**, 470.
- Quayle, J. R. & Keech, D. B. (1959a). *Biochem. J.* **72**, 623.
- Quayle, J. R. & Keech, D. B. (1959b). *Biochem. J.* **72**, 631.
- Quayle, J. R. & Keech, D. B. (1960). *Biochem. J.* **75**, 515.
- Quayle, J. R., Keech, D. B. & Taylor, G. A. (1961). *Biochem. J.* **78**, 225.
- Quayle, J. R. & Taylor, G. A. (1961). *Biochem. J.* **78**, 611.
- Quayle, J. R. (1963a). *Biochem. J.* **87**, 368.
- Quayle, J. R. (1963b). *Biochem. J.* **89**, 492.
- Sakami, W. (1955). *Handbook of Isotope Tracer Methods*. Cleveland, Ohio: Department of Biochemistry, Western Reserve University.