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Oxalic Acid Synthesis in Shoots of *Oxalis pes-caprae* (L.)

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Oxalis pes-caprae, a winter-growing weed of economic significance in parts of South Australia and elsewhere, contains up to 16% of the dry weight as oxalic acid, which is present in both photosynthetic and non-photosynthetic tissues.

Present information about the origin of oxalic acid is largely from studies of micro-organisms and animal tissues. In *Aspergillus niger* (strain 315), oxalic acid is formed from glycollic acid via glyoxylic acid (Franke & de Boer, 1959), and in rats glyoxylic acid is converted into oxalic acid (Weinhouse & Friedman, 1951). In contrast with some reports (Zelitch & Ochoa, 1953; Frigerio & Harbury, 1958), it has been shown by Kenton & Mann (1952) and by Richardson & Tolbert (1961*a*) that glycollate oxidase of plants (Clagett, Tolbert & Burris, 1949; Zelitch & Ochoa, 1953) may catalyse the oxidation of glyoxylic acid to oxalic acid.

In plants, a likely precursor of glyoxylic acid is isocitric acid, via the reaction catalysed by isocitratase (Kornberg & Beevers, 1957), but from the studies of Carpenter & Beevers (1959) it appears that this enzyme occurs only in those plant tissues that actively convert fat into carbohydrate, a condition which does not apply to tissues of *Oxalis pes-caprae*. From studies of micro-organisms and animals, other possible precursors are glycine (Weinhouse & Friedman, 1951; Nakada & Weinhouse, 1953), tartaric acid (Kun & Hernandez, 1956) and γ-hydroxyglutamic acid (Maitre & Dekker, 1961). Glyoxylic acid may also be derived from glycollic acid, which can be formed in plants by fixation of carbon dioxide, either in the light (Schou, Benson, Bassham & Calvin, 1950; Kearney & Tolbert, 1961) or in the dark (Kursanov, Kryakova & Vyskrebentsera, 1953). Other possible sources of glycollic acid are fructose 6-phosphate (Bradbeer & Racker, 1961), ribose (Griffith & Byerrum, 1959), hydroxypyruvic acid (Milhaud, Benson & Calvin, 1956), phosphoglycollic acid (Richardson & Tolbert, 1961*b*) and glycolaldehyde (Kun, Dechary & Pitot, 1954; Davies, 1960).

Oxidative cleavage of oxaloacetic acid, as found in *A. niger* at pH values less than 5 (Cleland & Johnson, 1956), is a likely alternative pathway for formation of oxalic acid in *Oxalis*.

This paper describes studies to determine the precursors of oxalic acid in the emergent shoots from the starchy bulbs of *Oxalis pes-caprae* (L.).

MATERIALS

Plants. Bulbs of *Oxalis pes-caprae* were harvested from the field and stored in a cold room at 2° to prevent germination until required. The bulbs were germinated in the dark at room temp. (15–20°) in trays containing moistened vermiculite. Bulbs harvested in February and March were used from March to October, but those stored longer than this period usually failed to germinate. The white shoot that emerged was kept moist and was allowed to grow in the dark for 7–10 days until approx. 3 cm. long. The oxalic acid content of such shoots was usually about 16% of the dry weight.

Chemicals. All ^{14}C-labelled compounds were obtained from The Radiochemical Centre, Amersham, Bucks.: D-[$^{14}C_6$]glucose, specific activity 29·7 μc/mg.; [$^{14}C_2$]glyoxylic acid monohydrate, specific activity 32·6 μc/mg.; sodium [1-^{14}C]glycollate, specific activity 19·4 μc/mg.; [$^{14}C_2$]oxalic acid, specific activity 45·5 μc/mg.; sodium hydrogen [^{14}C]-carbonate, specific activity 270·3 μc/mg.; [$^{14}C_2$]glycine, specific activity 7·6 μc/mg.; L-[$^{14}C_3$]serine, specific activity 27·8 μc/mg.

Sodium glyoxylate monohydrate and DL-isocitric acid, trisodium salt, were obtained from the Sigma Chemical Co.; L-malic acid (California Corp. for Biochemical Research), citric acid (Univar.) and tartaric acid (British Drug Houses Ltd.) were A.R. grade. Glycollic acid, L.R. grade (British Drug Houses Ltd.) was recrystallized. Phenol and chloroform were A.R. grade (British Drug Houses Ltd.). Butan-1-ol, L.R. grade (Colonial Sugar Refineries Ltd.), and propionic acid, L.R. grade (British Drug Houses Ltd.), were redistilled before use. *p*-Dioxan (A.R.; British Drug Houses Ltd.) was used in conjunction with naphthalene, 2,5-diphenyloxazole and *p*-bis-2,5-phenyloxazylbenzene, all A.R. scintillation grade [Nuclear Enterprises (G.B.) Ltd., Scotland]. Silicic acid, 100 mesh (A.R., Mallinckrodt Chemical Works) was suspended in water and fine particles were removed by suction before use.

METHODS

Treatment of shoots with radioactive compounds. Solutions used (final vol., 0·2 ml.) contained 100 μc of ^{14}C-labelled compound except that the solution of [$^{14}C_2$]glyoxylic acid contained 30 μc. Glucose was dissolved in M-sodium acetate buffer, pH 5·0; acidic compounds were adjusted to pH 5·0 with 0·05N-NaOH, and sodium hydrogen carbonate was used as supplied. With a microsyringe, portions (0·01 ml.)

of the appropriate solution were injected into each of 20 shoots attached to the *Oxalis* bulbs. The bulbs were maintained at room temp. for 1 hr.; the shoots were then detached and extracted immediately as described below. This procedure was used throughout, except that 10 of the shoots injected with glucose were detached and extracted after 1 hr. and the remaining 10 after 24 hr. Although no conclusive evidence is available, preliminary studies with dyes suggest that the injected compounds are rapidly transported throughout the shoot tissue.

Extraction, separation and identification of compounds containing radioisotope. The compounds used were D-[$^{14}C_6$]glucose, [$^{14}C_2$]glyoxylic acid monohydrate, sodium [1-^{14}C]glycollate, [$^{14}C_2$]oxalic acid, sodium hydrogen [^{14}C]carbonate, [$^{14}C_2$]glycine and L-[$^{14}C_3$]serine. After treatment, the shoots were extracted by dispersing in a Potter–Elvehjem homogenizer in 10 ml. of ethanol at room temp. It was assumed that this treatment prevented any further enzymic changes since the final pH was 1–2. The suspension was centrifuged and the precipitate extracted with successive portions of water (10 ml.) until no radioactivity was detected in the supernatant.

For each experiment, the combined extract (approx. 40 ml.) was applied to a column (1 cm. × 1 cm.) of Dowex 50 (H^+ form), the effluent of which flowed directly on to a column (2 cm. × 1 cm.) of Dowex 2 (HCO_3^- form). The columns were washed with several volumes of water (approx. 20 ml.). The material which passed through both columns contained non-ionic compounds (mainly sugars). The amino acids were then eluted from the column of Dowex 50 with approx. 30 ml. of aq. 0·5N-NH_3 soln. and the organic acids were eluted from the column of Dowex 2 with approx. 50 ml. of 0·5N-HCl. Elution was continued until no further radioactivity could be detected in the eluate. In each of the three fractions so obtained, the compounds containing ^{14}C were then identified by paper chromatography and radioautography, and quantitatively estimated by separation with column chromatography and measurement of ^{14}C activity.

Paper chromatography and radioautography. Whatman no. 1 paper (46 cm. × 57 cm.) was washed with 0·1N-HCl in ethanol and subsequently with glass-distilled water until the pH of the eluate was no longer acidic. Amino acid and organic acid mixtures were chromatographed in two dimensions with phenol saturated with water at 22° and butan-1-ol–propionic acid–water (47:22:31, by vol.) as described by Benson *et al.* (1950). After being dried in a stream of warm air the chromatograms were placed in contact with Kodirex X-ray film [Kodak (Australasia) Ltd.] and developed at suitable intervals. Amino acids were located on paper chromatograms with 5% (w/v) of ninhydrin in butan-1-ol, and organic acids with aq. 10% (w/v) potassium ferrocyanide, 0·5% (w/v) ferric ammonium sulphate in 70% (v/v) ethanol (Martin, 1955).

Extracts containing a low radioactivity were reduced to a volume such that 0·1–0·2 μc could be spotted on chromatograms in a volume of 0·2–0·5 ml. Where possible approx. 1 μc was applied. A mixture of glyoxylic acid, glycollic acid, malic acid, citric acid, isocitric acid and tartaric acid (0·01 ml. of a mixture 0·1N in respect of each acid) was co-chromatographed with organic acid extracts.

Separation and estimation of acidic and neutral amino acids. Columns (150 cm. × 1·5 cm.) were prepared from Dowex 50 resin (X8; H^+ form) that had been previously graded into particles of 35–70 μ diameter as described by Hamilton (1958). The extract containing amino acids was applied to the column and amino acids were separated by chromatography as described by Moore, Spackmann & Stein (1958), 2 ml. fractions being collected. The ninhydrin reagent of Moore & Stein (1954) was used with even-numbered tubes for detection and estimation of each amino acid. As CO_2 is evolved in the ninhydrin reaction and would constitute a possible loss of $^{14}CO_2$, fractions used for amino acid assays were not used in the determination of ^{14}C activity but odd-numbered fractions in each peak were combined and assayed for radioactivity.

Separation and estimation of organic acids. The fraction of the shoot extract containing organic acids was neutralized with 0·5N-NaOH and then evaporated to dryness with a stream of cold air. The residue was dissolved in 1·5 ml. of 0·5N-H_2SO_4 and the organic acids were separated by chromatography on a survey column (15 cm. × 1 cm.) of silicic acid gel by the method of Bulen, Varner & Burrell (1952). Glyoxylic acid (sodium salt), glycollic acid, malic acid, citric acid, isocitric acid and tartaric acid (2 mg. of each compound in 0·25 ml. of 0·5N-H_2SO_4) were co-chromatographed with the acids from the plant extract. The organic acids in the fractions collected from the column eluate were estimated by titration with 0·01N-NaOH and then the appropriately combined fractions were evaporated to dryness on a steam bath; the residue was dissolved and made up to 5·0 ml. in water. Samples (0·1 ml.) were used for estimation of ^{14}C activity.

Glycollic acid and oxalic acid do not separate on the silicic acid column under the conditions used. Oxalic acid was therefore separated from the mixture of these acids as its calcium salt. The precipitate obtained by addition of an excess of $CaCl_2$ was collected by centrifuging and washed three times with dilute acetic acid previously adjusted to pH 5·5 with 0·5N-NaOH. The glycollic acid in the supernatant and washings was then estimated as described for the other organic acids. The precipitate of calcium oxalate was dissolved in 0·5N-HCl (2·0 ml.) and portions (0·02 ml.) were assayed for ^{14}C activity.

Determination of radioactivity. An Ekco scaler and multiplier unit (type N530F, Ekco Electronics Ltd.) was used.

(*a*) Amino acids separated on Dowex 50 (H^+ form). The liquid-scintillation technique of Butler (1961) was used. The scintillator solution consisted of naphthalene (120·0 g.), 2,5-diphenyloxazole (4·0 g.) and *p*-bis-2,5-phenyloxazylbenzene (0·5 g.) dissolved in 1 l. of *p*-dioxan. The radioactivity in a 0·2 ml. portion of the amino acid solution (in 0·2M-sodium citrate buffer) could be determined in 5·0 ml. of scintillator. A counting efficiency of 36% was obtained with a type EM79514S photomultiplier tube (Ekco Electronics Ltd.) operating at 1200v.

(*b*) All other samples containing ^{14}C activity. A known volume (0·02–0·1 ml.) of each solution containing radioactivity was plated in an infinitely thin film on copper planchets. After the film had been dried under an infrared lamp the radioactivity was determined with a Geiger–Müller end-window tube, type E.W. 3H (20th Century Electronics, Ltd.), operating at 600v. Under these conditions a counting efficiency of 4–5% was obtained. A minimum of 1000 counts was recorded for each sample and duplicate samples were counted three times. The plating and counting procedure was repeated when more than 3% variation in duplicates was obtained.

Table 1. *^{14}C-labelled compounds detected in* Oxalis *shoots by paper chromatography and radioautography*

White shoots of *Oxalis* bulbs were injected with various ^{14}C-labelled compounds as indicated; after 1 hr. (and also after 24 hr. with glucose), the shoots were removed and extracted with ethanol and then with water as described in the text. Labelled compounds in the extract were separated by paper chromatography and detected by radioautography; they were classified visually as in group A (considerable incorporation) or in group B (slight incorporation of label).

Compound injected	^{14}C-labelled acids in shoot extract: Group A	Group B
D-[$^{14}C_6$]Glucose*	Malic, citric, isocitric, Asp, Glu	Glycollic, oxalic and unidentified acids, Ser, Ala, Val, Leu, Ileu
D-[$^{14}C_6$]Glucose†	Oxalic, glycollic, glyoxylic, Glu	Asp, Ser, Ala, Val, Leu, Ileu
[$^{14}C_2$]Glyoxylic acid	Glyoxylic, oxalic, glycollic, Gly, Ser	Malic
[1-^{14}C]Glycollic acid	Glycollic, oxalic, glyoxylic, Gly, Ser	Asp, Glu
[$^{14}C_2$]Oxalic acid	Oxalic, glycollic	Glyoxylic, Glu, Asp, Ser, Gly, Ala
Sodium hydrogen [^{14}C]carbonate	Malic, Glu, Asp	Citric, isocitric, Ser, Ala, Val
[$^{14}C_2$]Glycine	Gly, Ser	Glyoxylic, malic, glycollic, oxalic, citric, isocitric, Ala, Asp
L-[$^{14}C_3$]Serine	Ser	Glycollic, glyoxylic, oxalic, malic, citric, Gly

* After 1 hr. † After 24 hr.

Table 2. *Distribution of ^{14}C activity in sugars, amino acids and organic acids of* Oxalis *shoots*

White shoots of *Oxalis* bulbs were injected with various ^{14}C-labelled compounds as indicated; after 1 hr. (and also after 24 hr. with glucose), the shoots were removed and extracted with ethanol and then with water as described in the text. The compounds in the extracts were fractionated into sugars, amino acids and organic acids and the ^{14}C activity of each was determined. The radioactivity in each fraction is expressed as a percentage of the total activity of the three fractions.

Compound injected	Distribution of ^{14}C activity in shoot extract (%): Sugars	Amino acids	Organic acids
D-[$^{14}C_6$]Glucose*	77·1	5·7	17·2
D-[$^{14}C_6$]Glucose†	17·3	2·2	80·5
[$^{14}C_2$]Glyoxylic acid monohydrate	—	13·1	86·9
[1-^{14}C]Glycollic acid	—	5·0	95·0
[$^{14}C_2$]Oxalic acid	—	0·9	99·1
Sodium hydrogen [^{14}C]carbonate	—	40·7	59·3
[$^{14}C_2$]Glycine	—	98·7	1·3
L-[$^{14}C_3$]Serine	—	99·7	0·3

* After 1 hr. † After 24 hr.

RESULTS

The distribution of ^{14}C among organic acids and amino acids after injection of suitably labelled compounds was first determined by paper chromatography and radioautography. The results (Table 1) provided essential information for a quantitative study of the relative distribution of radioactivity among the compounds detected by radioautography. The relative distribution of radioactivity among the sugar, amino acid and organic acid fractions obtained from the injected shoots is shown in Table 2.

To assess the relative importance of various precursors in the production of oxalic acid, an examination was made of the quantitative distribution of ^{14}C activity among specific amino acids (Table 3) and organic acids (Table 4).

DISCUSSION

It is evident that emergent white shoots of *Oxalis pes-caprae* utilize carbohydrate from the starch-filled bulb for formation of oxalic acid that rapidly accumulates in the shoots. The presence of oxalic acid in shoots formed within 2 days of germination has been established during this work, confirming and extending earlier observations by Michael (1959). With the exception of the bulb, it appears likely that oxalic acid is a compulsory end product of the metabolism of growing tissues of this plant; the expressed sap has a pH value of about 2. The oxalic acid is probably stored within numerous small vacuoles which we have observed by electron microscopy in the cells of the emergent shoots.

Within 1 hr., 17% of the activity of injected

Table 3. *Distribution of* ^{14}C *activity among amino acids from extracts of* Oxalis *shoots*

Compounds were injected and extracts prepared as described in the text and Table 2. The amino acids in shoot extracts were fractionated on a column of Dowex 50 (X 8; H^+ form) and the amount of radioactivity in each fraction was estimated as described in the text. The results are expressed as a percentage of the total ^{14}C activity recovered from *Oxalis* shoots.

Compound injected	Distribution of total ^{14}C activity recovered from shoots (%)											Percentage of ^{14}C loaded on column and recovered as specific amino acids
	Cysteic acid	Taurine*	Asp	Ser	Glu	Gly	Ala	Val	Ileu	Leu	Phe	Unidentified	
D-[$^{14}C_6$]Glucose†	0·22	—	2·05	0·47	1·52	—	0·34	0·22	0·25	0·13	—	0·17	94·1
D-[$^{14}C_6$]Glucose‡	0·39	—	0·21	0·12	0·61	0·04	0·18	0·21	0·12	0·11	0·07	—	92·6
[$^{14}C_2$]Glyoxylic acid monohydrate	0·12	0·03	0·21	7·39	0·05	3·85	0·09	—	—	—	—	—	89·6
Sodium [1-^{14}C]glycollate	0·50	0·34	0·53	2·41	0·27	0·68	0·03	0·08	—	—	—	—	96·7
[$^{14}C_2$]Oxalic acid	—	—	0·20	0·11	0·22	(0·22)§		0·07	—	—	—	—	90·5
Sodium hydrogen [^{14}C]carbonate	0·57	1·38	15·10	1·38	17·42	0·16	1·55	0·86	—	—	—	—	94·4
[$^{14}C_2$]Glycine	0·40	0·20	4·24	20·73	0·10	59·71	1·58	0·10	—	—	—	0·30	88·7
L-[$^{14}C_3$]Serine	—	0·10	0·10	81·95	0·10	2·40	0·40	0·40	—	—	—	0·30	86·0

* Tentatively identified.
‡ After 24 hr.
† After 1 hr.
§ These two amino acids did not separate satisfactorily.

D-[$^{14}C_6$]glucose was recovered in the organic acid fraction and within 24 hr. about 80% was recovered (Table 2). As shown qualitatively by Table 1, and quantitatively by Table 4, there was greater incorporation of label into each of the isolated acids of the tricarboxylic acid cycle (about 2% in each) than into glycollic acid, glyoxylic acid and oxalic acid (about 1% in each). In 24 hr., however, the acids of the tricarboxylic acid cycle contained 4–5% of the radioactivity, whereas glycollic acid, glyoxylic acid and oxalic acid contained 13·5, 14·3 and 21·4% respectively, in agreement with the qualitative observations (Table 1).

It is clear that oxalic acid is formed from both glycollic acid and glyoxylic acid (Tables 1 and 4). With [$^{14}C_2$]glyoxylic acid, in 1 hr. about 30% of the recovered activity remained in the glyoxylic acid, and the glycollic acid and oxalic acid each contained about 21%. These results are consistent with a dismutation reaction as reported for *A. niger* by Franke & de Boer (1959). With [1-^{14}C]-glycollic acid, 55% of the activity remained in the glycollic acid, and 10·2 and 12·9% in the glyoxylic acid and oxalic acid respectively (Table 4). Although most of the recovered activity was in the oxalic acid fraction, the incorporation of ^{14}C label from injected oxalic acid into glycollic acid (5·9%), glyoxylic acid (1·3%) and lesser amounts into each of the isolated acids of the tricarboxylic acid cycle (Table 4) indicates that oxalic acid itself is not metabolically inert in *Oxalis*.

In confirmation of the qualitative findings (Table 1), Table 3 shows that ^{14}C is incorporated into both serine and glycine from either glyoxylic acid or glycollic acid. However, with [$^{14}C_2$]glyoxylic acid, and with [1-^{14}C]glycollic acid, the amount of incorporation into glycine (3·9 and 0·7%) is considerably less than into serine (7·4 and 2·4% respectively). A large proportion (20·7%) of the total activity recovered from the plant is incorporated into serine from glycine (Table 3), whereas both Table 1 and Table 3 show that serine is comparatively inert; this probably accounts for the relative distribution of the label from glyoxylic acid and glycollic acid into glycine and serine. The relatively low incorporation of label from [$^{14}C_2$]glyoxylic acid into glycine, which is unusual in plants (cf. Tolbert & Cohen, 1953), is probably also a consequence of the relatively rapid synthesis of oxalic acid from glyoxylic acid. A similar channelling of glyoxylic acid into oxalic acid rather than into glycine was observed by Weinhouse & Friedman (1951) in the rat.

With sodium hydrogen [^{14}C]carbonate, the total radioactivity recovered from the shoots was about 1% of that obtained with most other compounds used; the activity was distributed between the

Table 4. *Distribution of* ^{14}C *activity among organic acids from extracts of* Oxalis *shoots*

Compounds were injected and extracts prepared as described in the text and Table 2. The organic acids in shoot extracts were fractionated on a silicic acid-gel column (see text). The results are expressed as a percentage of the total ^{14}C activity recovered from *Oxalis* shoots.

Compound injected	Distribution of total ^{14}C activity recovered from shoots (%)							Percentage of ^{14}C loaded on column and recovered as specific acids
	Glyoxylic acid	Glycollic acid	Oxalic acid	Malic acid	Citric acid	Isocitric acid	Tartaric acid	
D-[$^{14}C_6$]Glucose*	1·08	1·07	1·12	2·08	2·13	1·86	1·26	61·6
D-[$^{14}C_6$]Glucose†	14·33	13·52	21·41	4·03	3·78	5·55	3·14	81·7
[$^{14}C_2$]Glyoxylic acid monohydrate	26·85	21·03	21·12	6·78	1·65	1·30	1·48	93·3
[1-^{14}C]Glycollate	10·16	55·10	12·92	0·66	1·14	1·04	0·76	86·1
[$^{14}C_2$]Oxalic acid	1·29	5·85	81·16	1·78	1·68	0·99	1·19	94·8
Sodium hydrogen [^{14}C]carbonate	1·78	0·57	0·88	42·81	7·41	3·26	—	95·5
[$^{14}C_2$]Glycine	0·62	0·11	0·11	0·27	0·07	0·07	0·01	97·7
L-[$^{14}C_3$]Serine	0·05	0·10	0·05	0·04	0·04	0·02	—	97·3

* After 1 hr. † After 24 hr.

organic acids and the amino acids (59·3 and 40·7% respectively; Table 2). Tables 3 and 4 confirm the qualitative findings (Table 1) and show that there was preferential labelling of malic acid (42·8%), citric acid (7·4%) and isocitric acid (3·3%) among the organic acids (Table 4), and of glutamic acid (17·4%) and aspartic acid (15·1%) among the amino acids (Table 3). The pattern of labelling of the amino acids from sodium hydrogen carbonate is rather similar to that from D-glucose (see Tables 1 and 3) and can be accounted for by transamination of oxaloacetic acid and of α-oxoglutaric acid. Tables 1 and 4 give no evidence of rapid incorporation of label from sodium hydrogen carbonate into glycollic acid, as occurs in photosynthetic tissues (Schou *et al.* 1950; Kearney & Tolbert, 1961), and no evidence that glycollic acid is formed directly from malic acid, as may occur in tomato slices (Link, Klein & Barron, 1952). However, the distribution of label between isocitric acid (3·3%) and glyoxylic acid (1·8%) is consistent with cleavage of isocitric acid to glyoxylic acid and succinic acid, catalysed by isocitratase; that between glyoxylic acid (1·8%), glycollic acid (0·6%) and oxalic acid (0·9%) indicates oxidation of glyoxylic acid to oxalic acid and reduction to glycollic acid.

SUMMARY

1. The pathway of oxalic acid synthesis in white shoots of *Oxalis pes-caprae* has been investigated by administering ^{14}C-labelled glucose, glyoxylic acid, glycollic acid, oxalic acid, sodium hydrogen carbonate, glycine and serine.

2. The products that became labelled after injection of these compounds were examined qualitatively by paper chromatography and radioautography, and quantitatively by column chromatography.

3. Experiments with D-[$^{14}C_6$]glucose indicated that the carbon for synthesis of oxalic acid was derived from the sugar pool. The results obtained with sodium hydrogen [^{14}C]carbonate indicated that malate was not a direct precursor of oxalate; glycollate was not rapidly labelled from sodium hydrogen carbonate.

4. Although [$^{14}C_2$]glycine and L-[$^{14}C_3$]serine both gave rise to some label in oxalic acid, the relatively low incorporation suggests that these are unimportant physiologically in the synthesis of oxalic acid.

5. The results indicate that glycollic acid and glyoxylic acid are immediate precursors of oxalic acid in *Oxalis* shoots, and are consistent with formation of glyoxylic acid by cleavage of isocitric acid catalysed by isocitratase.

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The Effect of Colchicine on the Acid-Soluble Ribonucleotides of Normal and Regenerating Rat Liver

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Although colchicine has long been known to act as a mitotic poison, the nature of its action is not understood. The results of Lettré & Albrecht (1951), showing that the effect of colchicine on mitosis in tissue culture could be reduced by the addition of adenosine triphosphate (ATP) to the medium, have drawn attention to the role of ATP in this process, and it has been suggested that it causes the mitotic spindle to contract by direct action on its constituents. Further, Benitez, Murray & Chargaff (1954) have shown that when ATP is added with colchicine to tissue cultures there is a decrease in the degree of mitotic arrest, although these workers found that the percentage of cells in the various phases of division in the presence of ATP was the same as that found in cultures to which no ATP had been added. The above evidence appeared to give ATP an important role in cell division and it seemed possible that, if the action of colchicine were primarily on the level of production of this compound, then the difference would manifest itself in the acid-soluble pool of ribonucleotides. For this reason measurements were made of the content of the various acid-soluble ribonucleotides in normal rat liver and in the livers of rats treated with colchicine. Further, in order to exaggerate such a difference, if one existed, measurements were also made of the effect of colchicine on the acid-soluble ribonucleotides in the livers of partially hepatectomized rats at a time after the operation when the maximum rate of cell division would be expected to occur. Finally, a comparison was made between the effect of colchicine and Myleran, another mitotic poison, on the acid-soluble ribonucleotides.

METHODS

Animals. Adult male albino rats weighing about 200 g. were used. Those that were partially hepatectomized had the median and left-lateral lobes of the liver removed under ether anaesthesia as described by Higgins & Anderson (1931).

Colchicine. Colchicine, purchased from British Drug Houses Ltd., was dissolved in water (concn. 500 μg./ml.) immediately before use, and administered intraperitoneally in a dose of 1 mg./kg. body wt. It was given to partially hepatectomized animals 24 hr. after operation, i.e. at about the time when the maximum rate of mitosis in hepatic parenchymal cells would be expected.

Small portions of the livers of some of the partially hepatectomized animals were examined histologically; numerous arrested mitoses were seen in the animals treated