involvement with the carbohydrate moiety (cf. Porter, 1950). Although the acid lability of certain α -DNP-amino acids cannot be ignored (e.g. proline, Porter & Sanger, 1948), no evidence has been obtained for the presence of even traces of α -DNP-amino acids. Bailey (1951) could find only a trace of α -DNP-residue (1 mole/10⁶ g. of protein) in four-times-recrystallized ovalbumin.

The availability of lysine residues towards FDNB suggests that the native protein molecule is in the form of a coiled loop, as suggested by Anfinsen & Redfield (1956). The three types of lysine residue with differing reactivity towards FDNB might be accounted for in the following manner: (a) three ϵ -NH₂ groups which are in easily accessible sites in the native molecule; (b) a further nine groups which cannot react in the native state for steric reasons. As denaturation proceeds the molecule uncoils and these groups become progressively available for substitution. This would indicate that denaturation is a stepwise process, at least with ovalbumin, rather than the all-or-nothing reaction envisaged by Mirsky & Anson (1935); (c) as denaturation proceeds a point is reached (50–55 % ethanol; 0.5–0.75 M-guanidine) when all the remaining ϵ -NH₂ groups interact. This may be an indication of the existence of a cyclic portion of the molecule or that the remaining ϵ -NH₂ groups are in fairly close sequence. It has not been found possible to substitute a number of ϵ -lysine NH₂ groups intermediate between 12 and 20. Thus whereas 50 % ethanol exposes 12 groups to reaction, 55 % ethanol brings all the 20 groups into a reactive state.

SUMMARY

1. The reaction between ovalbumin and 1-fluoro-2:4-dinitrobenzene has been studied under various conditions.

2. Progressive denaturation before reaction enables three types of ϵ -amino groups with different affinities for 1-fluoro-2:4-dinitrobenzene to be detected.

3. Glucose inhibits denaturation by urea and prevents the substitution of ϵ -amino groups beyond the minimal three groups.

4. Ovalbumin contains no free terminal amino group.

5. All the lysine residues are bound in α -peptide bonds.

6. The significance of these results is discussed.

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Properties and Partial Purification of Kynureninase

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The work to be described originated in a general study of pyridoxal phosphate-linked enzymes. Jakoby & Bonner (1953a) obtained kynureninase from *Neurospora crassa* (Em 5256A), studied its properties and claimed to have purified it 70- to 100-fold. This work is being reported as some of

their results could not be corroborated. Its special purpose was to study the following problems: (1) To determine the optimum conditions for kynureninase activity. (2) To corroborate the general mechanism of action of pyridoxal phosphate-linked enzymes proposed by Metzler, Ikawa & Snell (1954). To do this it is essential to establish that metal ions are cofactors of these enzymes;

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consequently an attempt has been made to dissociate metal ions from kynureninase. (3) To examine whether kynureninase, like tryptophanase (Gunsalus, Galeener & Stamer, 1955) and some of the amino acid decarboxylases (Steensholt, Flikke & Joner, 1955), is an -SH group enzyme. (4) To attempt to purify the enzyme.

MATERIALS AND METHODS

Kynureninase was obtained from wild-type *N. crassa* (74Q-4b) by the technique of Jakoby & Bonner (1953a), with slight modifications.

Growth. The mycelium was produced by allowing *N. crassa* to grow under forced aeration in a 10 l. flask containing 5 l. of minimal medium (Beadle & Tatum, 1945) supplemented with 1.2 g. of DL-tryptophan. Growth was allowed to take place at 30° for 60 hr., the time necessary to reach a wet wt. of 50 g. Mycelium was stored in the deep freeze (-20°) for not more than 8 months.

Extraction of the enzyme. All procedures were carried out at approx. 4° except where otherwise stated. Wet mycelium (50 g.) was cut into small pieces and homogenized with 200 ml. of 0.1M-phosphate buffer, pH 7.8, for 5 min. in an Auto-Mix (M.S.E.). The homogenized suspension was divided equally between two bottles, each containing 300 glass beads (3 mm. diam.), and shaken on a Towers Extra-Rapid Shaker, model E, for 2 hr. The contents of the bottles were strained through cheese-cloth and centrifuged for 35 min. at 17 800 g (approximate gravitational field at the bottom of the tube). The volume of the supernatant was made up to 200 ml. with 0.1M-phosphate buffer, pH 7.8. An attempt to extract the enzyme with water was without success. The extract (200 ml.) was submitted to fractionation with ammonium sulphate. Amounts of ammonium sulphate of 36, 24.4, 8.4 and 9.2 g. were added consecutively to give 25, 42, 48 and 54% saturation respectively. The precipitate after each addition was removed by centrifuging at 17 800 g. The fractions precipitated at 42-48% and 48-54% were dissolved together in 20 ml. of water (4°) and dialysed for 2 hr. against 4 l. of distilled water. The precipitate which appeared during dialysis was removed by centrifuging and the enzyme solution was stored in a deep-freeze until required. The preparation was active for more than a month.

Purification procedure

An attempt was made to purify the enzyme by the technique of Jakoby & Bonner (1953a), with slight modifications. The optimum conditions for the partially purified enzyme were determined before purification was undertaken.

Specific activity. The activity of enzyme preparation in amounts enough to show a linear relationship when plotted against protein concentration was determined in a total volume of 2 ml. The incubation mixture was as follows: 0.5 ml. of 0.05M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7.7, 0.203 μ mole of pyridoxal phosphate and 4.8 μ moles of DL-kynurenine. Units of specific activity were defined as the μ moles of anthranilic acid formed/min. under standard conditions by 1 mg. of protein.

Preparation and purification of the enzyme. Jakoby & Bonner (1953a) extracted kynureninase from the lyophilized mycelium. A freeze-drier was not available when this work was started, therefore the enzyme was extracted by homogenizing the mycelium. When a freeze-drier became available it was noticed that the activity in the extract from the lyophilized mycelium was reduced by 50% compared with the activity in the extract from homogenized mycelium. The detailed procedure for extraction and fractionation of the enzyme has already been given and only the modifications and further steps in purification will be mentioned here.

(i) **Crude enzyme preparation.** Wet mycelium (100 g.) was homogenized with 300 ml. of 0.1M-phosphate buffer, pH 7.8, from which 235 ml. of cell-free extract was obtained containing 2256 mg. of protein with a specific activity of 0.013.

(ii) **Combined ammonium sulphate fractions.** Crude enzyme (235 ml.) preparation was submitted to fractionation with ammonium sulphate and the precipitates obtained between 42-48% and 48-54% of ammonium sulphate saturation were dissolved together in 40 ml. of 0.1M-phosphate buffer. This solution was dialysed against 4 l. of distilled water for 2 hr. and the precipitate which appeared was removed by centrifuging. The supernatant solution contained 228 mg. of protein with a specific activity of 0.05.

(iii) **Protamine sulphate treatment.** To 40 ml. of ammonium sulphate fraction was added 68.4 mg. of protamine sulphate, providing 30 mg. of protamine sulphate for each 100 mg. of protein. The precipitate was removed by centrifuging and the supernatant fluid dialysed for 2 hr. against 4 l. of distilled water. The precipitate, which appears during dialysis, was removed by centrifuging. The protein content of the supernatant was 132 mg. and its specific activity was 0.098.

(iv) **Ethanol treatment.** The enzyme solution (40 ml.) was treated with ethanol by the technique of Jakoby & Bonner (1953a). The precipitate obtained by raising the concentration of ethanol to 70% (v/v) was dissolved in 18 ml. of 0.05M-phosphate buffer, pH 7.7. All the precipitated material did not go into solution. The supernatant was dialysed against 4 l. of distilled water for 2 hr. and the precipitate removed by centrifuging. The enzyme preparation (18 ml.) contained 15 mg. of protein. The specific activity determined immediately was 0.032. The activity of the preparation tended to deteriorate rapidly. Thus the ethanol treatment resulted in a fall in the specific activity.

Method of measuring the enzyme activity. Tubes containing 2.4 μ moles of DL-kynurenine, 0.5 ml. of 0.05M-tris buffer, pH 7.7 (or 0.5 ml. of the buffer under test), and 0.25 ml. of enzyme in a final volume of 2 ml. were incubated at 37° for 1 hr. At the end of the incubation period the reaction was stopped by the addition of 4 ml. of ethanol and the precipitate removed by centrifuging. Each determination required one blank tube containing all the constituents in the reaction tube, with deproteinizing agent added at zero time, which was subsequently treated in the same manner as the reaction tube. The optical density of the supernatant solutions was measured in a 1 cm. cell at 310 m μ in a Unicam spectrophotometer model SP. 500. The amount of anthranilic acid formed was read from a calibration curve.

Protein estimation. The protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). The

calibration curve was prepared with crystalline bovine plasma albumin (The Armour Laboratories, Chicago, Ill., U.S.A.).

Dialysis. The technique of dialysis was that of Spiegelman, Reiner & Morgan (1947). Enzyme preparations were dialysed in cellophan bags rotated at an angle of 45° in the dialysis liquid; an air bubble was left in the bag to increase the circulation of its contents. The dialysis was done in the cold room ($4-5^\circ$).

Buffers. The following buffers were used: phosphate (Sørensen, 1909); citrate-phosphate (McIlvaine, 1921); borate (Clark, 1922); universal-buffer mixture (Johnson & Lindsey, 1939); 2-amino-2-hydroxymethylpropane-1:3-diol (tris) and veronal (Gomori, 1955).

Chemicals. DL-Kynurenine and analogues of vitamin B₆ (Roche Products Ltd.). *p*-Chloromercuribenzoic acid (Hopkin and Williams Ltd.). L-Cysteine, reduced glutathione and tris (L. Light and Co. Ltd., Colnbrook, Bucks.). Biotin, diethylbarbituric acid and sodium barbitone (British Drug Houses Ltd.). Anthranilic acid was recrystallized several times from hot water until the sample had a constant m.p. at 145° . Other chemicals used were A.R. grade.

Measurement of pH. The pH of buffers was determined with a Marconi type T.F. 511D pH meter, buffer tablets (Marconi Instruments Ltd.) being used as standard.

RESULTS

Time. The effect of variation of time (15–240 min.) on the enzyme reaction is shown in Fig. 1.

Effect of pH and buffers. The curves relating activity to pH and buffers (Fig. 2) show an optimum pH at 7.7 and also reveal that at pH 7.3 the activity in borate and universal-buffer mixture is 51.8 % of that in phosphate and citrate-phosphate buffers at the same pH. An experiment with a view to observing the comparative effect of

Fig. 1. Amounts of anthranilic acid formed with the time of enzyme reaction. Production of anthranilic acid is linear with time up to 1 hr.

0.0125M-tris, 0.0125M-veronal and 0.025M-phosphate buffer on the enzyme activity showed that the optimum pH of the enzyme activity in all the buffers was at 7.7; the activity in tris and veronal buffers was 41.5 % of that in phosphate buffer, comparison being made at pH 7.7. The increased activity in phosphate buffer may be due to the activation of the enzyme by some component of the phosphate buffer. Neither sodium chloride nor potassium chloride affected enzymic activity. Sodium phosphate, pH 7.8, at concentrations above 0.1 mM activated the enzyme, the maximum activation being 78.4 %, which was observed at 10 mM-phosphate in tris buffer, pH 7.8.

Substrate concentration. Fig. 3 shows the effect of varying the substrate concentration on the enzymic activity in the presence of 0.0406 μmole of pyridoxal phosphate/ml.; it shows that 2.4 μmoles of DL-kynurenine/ml. are required for optimum activity and DL-kynurenine is not inhibitory even at a concentration of 4.8 mM.

Coenzyme. The saturation curve (Fig. 4) shows that 0.0406 μmole of pyridoxal phosphate/ml. is needed for optimum activity and that it is not inhibitory at 0.2 mM concentration. The dissociation constant is 0.35 μM. Table 1 shows that the activating effects of pyridoxal phosphate and phosphate were not additive.

Fig. 2. Effect of pH and buffers on the enzyme activity. Buffers: ○, borate; Δ, universal-buffer mixture; ●, citrate-phosphate; ▲, phosphate.

Fig. 3. Effect of substrate concentration on the enzyme activity. Activity was determined in the presence of pyridoxal phosphate (0.0406 μ mole/ml.). DL-Kynurenine (2.4 μ moles/ml.) is necessary for optimum activity.

Fig. 4. Pyridoxal phosphate-saturation curve. The assay was conducted under standard conditions except for the variation of pyridoxal phosphate. Pyridoxal phosphate (0.0406 μ mole/ml.) is necessary for optimum activity.

Table 1. Effect of pyridoxal phosphate and sodium phosphate (pH 7.8) on the enzyme when tested separately and combined together

Activity was determined in tris buffer, pH 7.8.

Expt. no.	Sodium phosphate (mM)	Pyridoxal phosphate (μ mole/ml.)	Activity (% of original)
1	0	0	100
2	10	0	166.2
3	0	0.0609	203.2
4	10	0.0609	203.2

Metal ions. Tryptophanase and glutamic acid decarboxylase, which are pyridoxal phosphate-linked enzymes, are activated by some univalent cations (Happold & Struyvenberg, 1954; Happold, 1955). The chlorides of sodium, potassium, ammonium and rubidium at concentrations from mM to 0.1M were without effect on kynureninase activity. The effect of varying concentrations of some bivalent metal ions both in the presence and absence of 0.203 μ mole of pyridoxal phosphate was also determined. Cobalt chloride, magnesium sulphate, ferrous sulphate and zinc sulphate at concentrations above 0.01 mM inhibited the enzyme activity both in the presence and absence of pyridoxal phosphate. Calcium chloride activated both in the presence and absence of pyridoxal phosphate at concentrations above 0.01 mM, and the optimum activity was observed at mM; the activation varied from 5 to 56 % with different batches of the enzyme preparation. Manganese sulphate, in the presence of pyridoxal phosphate, activated kynureninase at concentrations from 5 μ M to 0.5 mM; the optimum activation was 33.6 %, which was obtained at 10 μ M concentration. At concentrations greater than 0.5 mM this salt inhibited and in the absence of pyridoxal phosphate it was inhibitory at concentrations greater than 10 μ M.

A large number of techniques have been used for dissociating metal ions from enzymes (Smith, 1948; Dagley & Sykes, 1956). Attempts to dissociate the metal ions from kynureninase by dialysing the enzyme against (a) 0.02M-ethylenediaminetetra-acetic acid (EDTA) adjusted to either pH 7.2 or 4.5 for 24 hr., (b) 0.1M-phosphate-citrate buffer, pH 7.6, for 12 hr., (c) distilled water for 48 hr. were all only partially successful. The enzyme dialysed against EDTA (a) or phosphate-citrate (b) was activated by Mn^{2+} ions to the same extent as the undialysed enzyme. The enzyme dialysed against distilled water and the undialysed preparation were activated by Mn^{2+} ions to the extent of 27.2 and 15.5 % respectively.

Effect of analogues of vitamin B₆. Before the addition of the substrate, the enzyme, buffer and analogue of vitamin B₆ (25 μ g./ml.) were incubated for 45 min. at 37°. Pyridoxal did not affect the enzyme activity; pyridoxamine inhibited by 14 %; and pyridoxamine phosphate and pyridoxal phosphate activated the enzyme by 104 and 150 % respectively. The results of varying the concentration of pyridoxamine phosphate are shown in Table 2.

Pyridoxamine phosphate transaminase catalyses the conversion of pyridoxamine phosphate into pyridoxal phosphate (Beechey & Happold, 1955). The activation of kynureninase by pyridoxamine phosphate may be due to the presence of pyridox-

amine phosphate transaminase. However, the presence of such an enzyme could not be demonstrated in the kynureninase preparation by the technique of Beechey & Happold (1955).

Evidence for the presence of functional sulphhydryl groups. Steensholt *et al.* (1955), Gunsalus *et al.* (1955) and Sher & Mallette (1954) have shown that glutamic acid decarboxylase, tryptophanase and lysine decarboxylase, all of which are pyridoxal phosphate-linked enzymes, require the integrity of -SH groups in their structure for their activity. *p*-Chloromercuribenzoate specifically inhibits enzymes, dependent for activity on the presence of -SH groups (Hellerman, Chinard & Deitz, 1943). Sher & Mallette (1954), for example, inactivated lysine decarboxylase by *p*-chloromercuribenzoate and were able to maintain the activity at 80% of the original activity by incubating the enzyme with 100-fold excess of cysteine for 20 min. before the addition of *p*-chloromercuribenzoate. The

other enzymes inactivated by *p*-chloromercuribenzoic acid have been shown to be reactivated by reduced glutathione (Barron, 1943). Iodoacetic acid is also known to inactivate enzymes depending for activity upon reduced -SH groups (Hopkins, Morgan & Lutwak-Mann, 1938). The activity of this compound was tested as follows: 50 ml. of 0.5M-iodoacetic acid was adjusted to pH 7.7 with sodium hydroxide and the volume made up to 100 ml. The enzyme preparation was then incubated at room temperature with iodoacetate (12.5 mM), pyridoxal phosphate (0.203 μ mole) and buffer for 2 hr. before determining the activity. Iodoacetate inhibited the enzymic activity by 80%.

Reversibility of p-chloromercuribenzoic acid inactivation by reduced glutathione and L-cysteine. The enzyme preparation was incubated at room temperature with varying concentrations of *p*-chloromercuribenzoic acid, pyridoxal phosphate (0.203 μ mole) and buffer for 2 hr. before determining the enzymic activity. *p*-Chloromercuribenzoic acid inhibited activity at concentrations above 10 μ M and there was 93% inhibition at 0.1 mM.

Reduced glutathione was incubated with the enzyme, pyridoxal phosphate (0.0813 μ mole) and buffer for 20 min. before the addition of *p*-chloromercuribenzoic acid. The concentrations of glutathione and *p*-chloromercuribenzoic acid and their effect on the enzymic activity are presented in Table 3, which shows that reduced glutathione counteracts the inhibitory effect of *p*-chloromercuribenzoic acid and that the activity is maintained at 99.4% of its initial value. *p*-Chloromercuribenzoic acid alone inhibits the enzymic activity by 89.7% in this experiment.

A solution of L-cysteine hydrochloride was neutralized to pH 7.0 with sodium hydroxide and diluted to the required concentration. A concentration of L-cysteine at 10 mM inhibited the kynureninase activity by 47% (Jakoby & Bonner, 1953b). The inhibition was thought to be due to the formation of a cyclic compound between L-cysteine and pyridoxal phosphate. It was therefore considered desirable to add pyridoxal phosphate in excess to minimize the inhibitory effect of L-cysteine. L-Cysteine was incubated with the enzyme, buffer and pyridoxal phosphate (0.325 μ mole) for 20 min. before the addition of chloromercuribenzoic acid. The concentration of L-cysteine and chloromercuribenzoic acid and their effect on the enzymic activity are given in Table 4. L-Cysteine did not inhibit the enzymic activity, whereas the 90% inhibition caused by chloromercuribenzoic acid was almost completely reversed by incubation of the enzyme with cysteine for 20 min. before the addition of *p*-chloromercuribenzoic acid.

Table 2. *Effect of varying the concentration of pyridoxamine phosphate on the enzymic activity*

Expt. no.	Pyridoxamine phosphate (μ mole/ml.)	Activity (% of original)
1	0	100
2	0.0202	197.3
3	0.0404	236.8
4	0.0607	250.0
5	0.1012	276.3

Table 3. *Effect of reduced glutathione on the inhibitory effect of chloromercuribenzoic acid*

Pyridoxal phosphate (0.0813 μ mole) was present.

Expt. no.	Addition	Molarity (mM)	Activity (% of original)
1	None	—	100
2	Reduced glutathione	20	97.3
3	<i>p</i> -Chloromercuribenzoic acid	0.1	10.3
4	Reduced glutathione + <i>p</i> -chloromercuribenzoic acid	20 0.1	99.4

Table 4. *Effect of L-cysteine on the inhibitory effect of chloromercuribenzoic acid*

Pyridoxal phosphate (0.325 μ mole) was present.

Expt. no.	Addition	Molarity (mM)	Activity (% of original)
1	—	0	100
2	L-Cysteine	1.5	102.5
3	<i>p</i> -Chloromercuribenzoic acid	0.1	10.2
4	L-Cysteine + <i>p</i> -chloromercuribenzoic acid	1.5 0.1	97.4

DISCUSSION

It has been shown that the increased enzymic activity in phosphate as compared with that in borate, tris, universal and veronal buffer is due to the activating effect of the phosphate radical. Jakoby & Bonner (1953*a*) observed the same degree of kynureninase activity in phosphate, tris and borate buffers. This difference has been satisfactorily explained since it was observed that phosphate did not activate kynureninase in the presence of added pyridoxal phosphate. Jakoby & Bonner (1953*a*) determined the effect of different buffers on the enzymic activity in the presence of added pyridoxal phosphate.

Jakoby & Bonner (1953*b*) stated that the inhibition by higher concentrations of the substrate, in the presence of optimum amounts of added pyridoxal phosphate, was due to the formation of a 'kynurenine-pyridoxal phosphate complex'. The formation of this complex lowered the effective pyridoxal phosphate concentration and the complex itself was thought to act as an analogue type of inhibitor. Although D-kynurenine did not act as substrate, it was as inhibitory as its enantiomorph; L-kynurenine inhibited at concentrations above 0.4 mM. In the present study DL-kynurenine in the presence of an optimum amount of pyridoxal phosphate was found not to inhibit the enzymic activity even at 4.8 mM. No satisfactory explanation could be given for the difference between the present results and those of Jakoby & Bonner (1953*b*). However, it may be mentioned that DL-kynurenine has been used as substrate here, whereas Jakoby & Bonner (1953*b*) used L-kynurenine sulphate.

Jakoby & Bonner (1953*a*) found that pyridoxal phosphate inhibited kynureninase activity at concentrations above 4 μ M and that the dissociation constant derived by determining the coenzyme concentration at half-maximum velocity was 0.6 μ M. The present work shows that pyridoxal phosphate even at 0.2 mM is not inhibitory and the dissociation constant of the coenzyme has a value of 0.35 μ M.

Jakoby & Bonner (1953*a*) claimed that Mg^{2+} ions at a concentration of 2 mM activated kynureninase at all stages of purification, the activation being of the order of 15–45%, depending upon the preparation, and Ca^{2+} ions also activated at some stages of purification; the response was highly variable. The present work shows that Mn^{2+} and Ca^{2+} ions activate the enzyme. It seems probable that kynureninase obtained from different strains of wild-type *N. crassa* is not activated by the same metal ions.

Of the various analogues of vitamin B₆ tested, only pyridoxal phosphate and pyridoxamine

phosphate activated the enzyme. Jakoby & Bonner (1953*a*) reported that pyridoxamine phosphate did not activate kynureninase. The possibility that pyridoxamine phosphate might also act as a coenzyme of kynureninase should also be borne in mind—especially in view of the fact that kynurenine contains a carboxyl group. Meister, Sobar & Peterson (1954) reported that purified pig-heart glutamic-aspartic apotransaminase is activated by pyridoxal phosphate and pyridoxamine phosphate.

Evidence has been presented for the first time that kynureninase possesses functional -SH groups. Some of the other pyridoxal phosphate-linked enzymes, such as glutamic acid decarboxylase, leucine decarboxylase and tryptophanase, possess functional -SH groups.

SUMMARY

1. Production of anthranilic acid is linear with time up to 1 hr.

2. The pH optimum of kynureninase was 7.7 and the optimum activity was observed in phosphate buffer, which was shown to be due to the activating effect of phosphate radical. Phosphate did not activate the enzyme in the presence of added pyridoxal phosphate.

3. DL-Kynurenine even at a concentration of 4.8 mM in the presence of an optimum amount of pyridoxal phosphate did not inhibit the enzymic activity.

4. Pyridoxal phosphate at a concentration of 0.2 mM did not inhibit the enzymic activity.

5. Mn^{2+} ions activated kynureninase only in the presence of added pyridoxal phosphate, whereas Ca^{2+} ions activated both in the presence and absence of added pyridoxal phosphate.

6. Of the various analogues of vitamin B₆ tested, only pyridoxal phosphate and pyridoxamine phosphate activated the enzyme. Pyridoxamine was slightly inhibitory.

7. Evidence has been presented for the presence of functional -SH groups in kynureninase.

8. Kynureninase was purified eightfold.

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The Metabolism of DL-1-Deoxy-1-Fluoroglycerol

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The study of fluorine-substituted metabolites may give information about the pathways of normal metabolism or lead to the discovery of compounds suitable for therapeutic or pesticidal use. The compound 3-fluoro-DL-propane-1,2-diol (DL-1-deoxy-1-fluoroglycerol) was first described by Gryskiewicz-Trochimowski (1947), and its physical properties were given by Taylor & Kent (1956). Dr R. M. C. Dawson (personal communication) observed that the compound was toxic to mice and induced citrate accumulation, particularly in heart and kidney, and to a lesser extent in liver. Since the present work was started, Pattison & Norman (1957) have reported that DL-1-deoxy-1-fluoroglycerol is toxic to the mouse, and have suggested that it is oxidized *in vivo* to fluoroacetate or fluoroacetaldehyde, on the grounds of a comparison of its toxicity with that of various possible metabolites of DL-1-deoxy-1-fluoroglycerol or of their derivatives.

MATERIALS AND METHODS

DL-1-Deoxy-1-fluoroglycerol (FG) and epifluorohydrin were synthesized by Drs Taylor and Kent and generously presented to us by them. [Dr Kent found (for FG): C, 38.0%; H, 7.6%. Calc. C, 38.3; H, 7.45%.] Sodium

fluoroacetate was purchased from the Monsanto Chemical Co. The disodium salt of adenosine triphosphate (ATP) was used, purchased from the Pabst Laboratories, Milwaukee, Wisc., U.S.A. Citrate was determined by the method of Taylor (1953), except that the step of drying the light petroleum was omitted. Phosphate was determined by the method of Rockstein & Herron (1951). Acid-labile phosphate was determined following 10 min. hydrolysis in N-HCl at 100°. Glycolysis was measured according to LePage (1948). Kidney particles were prepared and assayed, with fumarate as substrate, as described by Peters & Wakelin (1953, 1957); brain particles were used as described by these authors. Metabolic products were examined for organic acids, by two-dimensional paper and by column chromatography.

Paper chromatography. The two-dimensional hydroxamate system of Bergmann & Segal (1956) was used; the first solvent was absolute ethanol-pyridine-water-ammonia (60:20:6:14, by vol.) and the second was absolute ethanol-dioxan-water-acetic acid (60:20:19:2, by vol.). At the suggestion of Dr D. B. Lindsay, the free acid for chromatography was obtained by evaporating a solution of the sodium salt to dryness, taking up in 75 μl . of 30% H_3PO_4 , adding to a micro-column (0.6 cm. $\times$ 1.5 cm.) of anhydrous Na_2SO_4 topped with Celite 545, and washing through with ether.

Column chromatography. The material to be chromatographed was made alkaline and evaporated to dryness on the steam bath, then taken up in 0.5 ml. of 0.5N- H_2SO_4 , and chromatographed by the silica-gel-column procedure of Bulen, Varner & Burrell (1952), eluting only with butanol-chloroform (5:95, v/v). Successive 2 ml. fractions were

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