The Purification and Properties of δ-Aminolaevulic Acid Dehydrase

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The work of Shemin and his colleagues on the incorporation into haem of glycine (Shemin & Rittenberg, 1945, 1946; Wittenberg & Shemin, 1949, 1950), acetate (Shemin & Wittenberg, 1951) and succinate (Shemin & Kumin, 1952), together with the work of Muir & Neuberger (1949, 1950) on the incorporation of glycine and acetate, led to the conclusion that the initial reaction in the biosynthesis of haem is the condensation of glycine with a derivative of α-oxoglutarate. At the other end of the biosynthetic pathway, it has been suggested that the first tetrapyrrole formed is probably closely related to uroporphyrin III (Turner, 1940; Radin, Rittenberg & Shemin, 1950; Neuberger, Muir & Gray, 1950). The isolation by Westall (1952) of porphobilinogen (PBG), first described by Waldenström (1934), provided a link between the aliphatic precursors and the tetrapyrrolic products. This substance, which was characterized as a monopyrrole by Cookson & Rimington (1953, 1954), Kennard (1953) and Cookson (1953), was found to be an effective precursor of porphyrins and haem both in avian red cells (Falk, Dresel & Rimington, 1953) and in green algae (Bogorad & Granick, 1953).

The discovery of the role of PBG as a porphyrin precursor was followed very shortly by independent reports from Shemin & Russell (1953) and from Neuberger & Scott (1953) that δ-aminolaevulic acid (ALA) is also an effective precursor of haem and of porphyrins. Dresel & Falk (1953) found that it is as efficient a porphyrin precursor as PBG in avian red cells, and presented evidence indicating the existence in this system of an enzyme catalysing the conversion of ALA into PBG. This enzyme is presumably also present in the soluble enzyme system forming haem from ALA described by Shemin, Abramsky & Russell (1954). It is the purpose of this communication to describe the purification and properties of this enzyme, which catalyses the reaction shown in Fig. 1, and which we have called δ-aminolaevulic acid dehydrase. A preliminary note has appeared already (Gibson, Neuberger & Scott, 1954), and some properties of the enzyme have been briefly described elsewhere (Gibson, 1955). Almost simultaneously with our first note, independent reports of work on the dehydrase were published by Granick (1954) and by Schmid & Shemin (1955; see also Shemin, Gatt, Schmid & Weliky, 1955). Another communication on this subject has also appeared (Schulman, 1955).

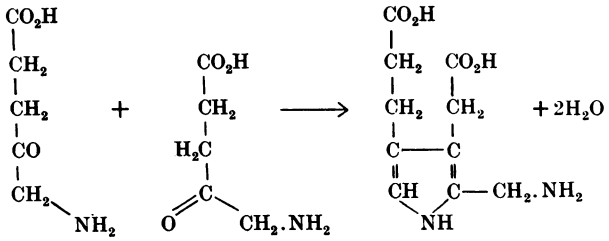

Fig. 1

EXPERIMENTAL

Preparation of compounds

δ-Aminolaevulic acid (ALA) was prepared as described previously (Neuberger & Scott, 1954). Porphobilinogen (PBG) was isolated according to Cookson & Rimington (1954) from the urine of rabbits which had received *N*-(2-*iso*propylpent-4-enoyl)urea (Sedormid), or from that of humans given an oral dose of ALA (Berlin, Gray, Neuberger & Scott, 1954). αδ-Diaminolaevulic acid and aminoacetone were prepared according to Harington & Overhoff (1933) and Gabriel & Pinkus (1893) respectively. 6-Amino-5-oxohexanoic acid was prepared by a method completely analogous to that used for preparing ALA (Route B of Neuberger & Scott, 1954). This method was as follows: γ-methoxycarbonylbutyryl chloride was prepared according to Naylor (1947) (b.p. 95–98°/10 mm.). A solution of this compound (25 g.) in dry ether (82 ml.) was added

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during 2 hr. with mechanical stirring to a solution of diazomethane (13 g.) in ether (520 ml.) at −5°. The mixture was treated exactly as described previously for the preparation of methyl δ-chlorolaevulate (Neuberger & Scott, 1954). The combined ether washings were dried ($MgSO_4$) and the solvent was removed, the yield of crude *methyl ε-chloro-δ-oxohexanoate* being 12·5 g. (46%). Another sample prepared in this manner was distilled *in vacuo* (b.p. 95–96°/0·071 mm.). (Found: C, 47·50; H, 6·21. $C_7H_{11}O_3Cl$ requires C, 47·05; H, 6·16%.) The crude material obtained above was added dropwise with stirring during 0·5 hr. to a solution of anhydrous potassiophthalimide (14 g.) in dimethylformamide (100 ml.). The mixture was heated at 60° for 1 hr., and the solvent was removed. The residue was twice recrystallized from water. The yield of *methyl ε-phthalimido-δ-oxohexanoate*, which had m.p. 96°, was 16 g. (79%). (Found: C, 62·50; H, 4·94; N, 4·80. $C_{15}H_{15}O_5N$ requires C, 62·28; H, 5·19; N, 4·84%.) This compound (11 g.) was then refluxed for 6 hr. with 7N-HCl (110 ml.). After cooling and removal of precipitated phthalic acid, the solvent was removed *in vacuo*. The residue was recrystallized once from dry methanol–ethyl acetate and once from dry methanol–ether; 4·2 g. (61%) of *6-amino-5-oxohexanoic acid hydrochloride* (m.p. 149–150°) were finally obtained. (Found: C, 39·90; H, 6·55; N, 7·73. $C_6H_{11}O_3N$,HCl requires C, 39·67; H, 6·61; N, 7·71%.)

Methods

Estimation of PBG. PBG was estimated throughout by the colour developed with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) in acid solution. A sample (1 or 2 ml.) of a suitable dilution of the solution to be estimated was mixed with an equal volume of 2% (w/v) Ehrlich's reagent in 5N-HCl; the absorption of the coloured product at 552 mμ. was read against a blank consisting of the unknown solution mixed with an equal volume of 5N-HCl. The absorption was followed until it reached a maximum, and the PBG present was calculated from this figure by using the data for the absorption of the Ehrlich compound of PBG given by Cookson & Rimington (1954). Thiol reagents, which interfere with the colour development, were oxidized as described below. The very slight blank absorption at 552 mμ. observed with a mixture of $CuSO_4$ and Ehrlich's reagent alone was sufficiently small under the conditions used in this work to be neglected in comparison with the experimental error.

Estimation of ALA. This was estimated by the picric acid method of L. Shuster (paper in preparation). The samples for estimation were deproteinized by addition of trichloroacetic acid (TCA) to a concentration of 5% (w/v).

Preparation of homogenates. Tissue homogenates were prepared by mixing the fresh tissue with twice its wet weight of ice-cold 0·15M-KCl and blending in a Potter–Elvehjem glass homogenizer for about 3 min. The homogenates were strained through muslin before use.

Estimation of enzyme activity. It has been found that PBG is not further metabolized appreciably even in the presence of crude tissue preparations if it is incubated *in vacuo*, and this has been made use of in estimating dehydrase activity. The standard conditions which were used in most of the experiments described here were as follows: the enzyme preparation, together with M/15 phosphate buffer (pH 6·8) and 0·01M glutathione (GSH) or cysteine (CySH), in a total fluid volume of 3·0 ml., was incubated for 1 hr. at 38° in a Thunberg tube *in vacuo*. (Phosphate buffers used throughout the work described here were made by mixing solutions of KH_2PO_4 and Na_2HPO_4 in suitable proportions.) This serves to activate the enzyme (see below, under Results). At the end of this period 0·33 ml. of 0·1M ALA hydrochloride was added from the side arm of the Thunberg tube, and incubation was continued for a period of up to 1·5 hr. The protein was then precipitated by pouring the contents of the tube into 1 ml. of colloidal Fe_2O_3 ('Dialysed Iron', approx. 5% w/v Fe_2O_3; British Drug Houses Ltd.) in a 15 ml. centrifuge tube. Saturated aq. $CuSO_4$ (2 drops) was added, and the tube stoppered and shaken vigorously for about 10 sec. This oxidized the thiol and discharged the colloid. After the precipitate had been removed by centrifuging, the amount of PBG in the supernatant was estimated with Ehrlich's reagent as described above.

With purified preparations addition of the colloidal Fe_2O_3 was unnecessary. The procedure for stopping the reaction was to pour the incubation mixture directly into a centrifuge tube containing 2 drops of saturated aq. $CuSO_4$, shake vigorously as before and remove the precipitate of copper phosphate by centrifuging. The PBG in the supernatant was then estimated. It was found that the action of the dehydrase was inhibited immediately on adding the $CuSO_4$, possibly by oxidation of its essential SH groups.

By using this method over 90% of the PBG could be recovered if the PBG concentration exceeded about 10^{-5}M. Table 1 shows a recovery experiment carried out at two

Table 1. *Recovery of PBG*

Contents of Thunberg tubes: 0·067M phosphate buffer, pH 6·8, enzyme and GSH as indicated; liquid volume 3·0 ml. Activation at 38° as indicated; then PBG, in 0·1 ml., added from side arm. Rabbit liver homogenized in 0·15M-KCl (2·0 ml./g. wet weight) as described in the text. Purified dehydrase prepared as described in the text (*A*, 37·3; *N*, 1·46). After incubation, tubes containing liver homogenate deproteinized with colloidal Fe_2O_3 and $CuSO_4$; those with purified enzyme deproteinized with $CuSO_4$ alone.

Enzyme	GSH (M)	Activation time (hr.)	Incubation time (hr.)	PBG added (μmole)	PBG recovered (μmole)	PBG recovered (%)
Homogenate (0·5 ml.)	—	—	—	0·087	0·083	96
	10^{-2}	—	—	0·087	0·089	102
	10^{-2}	1·0	1·0	0·087	0·079	91
	10^{-2}	—	—	0·0087	0·0075	86
	10^{-2}	1·0	1·0	0·0087	0·0060	69
Purified dehydrase (0·01 ml.)	10^{-2}	—	—	0·087	0·082	94
	10^{-2}	1·0	1·0	0·087	0·080	92

concentrations of PBG, with either rabbit-liver homogenate or the purified dehydrase. With the homogenate at the higher concentration, $2{\cdot}8 \times 10^{-5}$ M, more than 90% of the added PBG was recovered even after 1 hr. incubation, although greater losses occurred at one-tenth of this concentration. In this experiment colloidal Fe_2O_3 was used as protein precipitant. With the purified enzyme and $2{\cdot}8 \times 10^{-5}$ M PBG, recovery was again greater than 90%; in this experiment colloidal Fe_2O_3 was not added.

RESULTS

Distribution

ALA dehydrase has been found in a variety of organisms and tissues in addition to chicken erythrocytes, in which it was first reported by Dresel & Falk (1953) and from which it has been prepared by Granick (1954), and duck erythrocytes, which Schmid & Shemin (1955) used as starting material for their preparation. The activities in some of these organisms and tissues are shown in Tables 2 and 3. Although there is some variation among the figures recorded there, it is clear that the enzyme is widely distributed in nature. Apart from haemopoietic tissues, there is considerable activity in the liver and kidney of rats and rabbits, and in the liver of pigeons. In addition, a number of the other tissues of the rabbit have a small but definite activity. With the exception of yeast, all the micro-organisms tested have activities at least comparable with that of liver. Some activity is also present in extracts of spinach leaf and in pea seedlings, although this is much less than has been found in micro-organisms and in the most active mammalian tissues.

Table 2. *General distribution of ALA dehydrase*

Tissues of rat and pigeon, and pea seedlings, homogenized in 0·15 M-KCl (2 ml./g. wet weight). Spinach leaf ground with sand in the cold, extracted with 0·15 M-KCl (5 ml./g. wet weight of leaf). *Bacterium cadaveris* (NCTC 6578) and yeast extracts made by mixing with equal weight of Al_2O_3, freezing, grinding in mortar while thawing, and extracting with 0·15 M-KCl (5 ml./g. wet weight of cells). Acetone-dried *Corynebacterium diphtheriae* (Park-Williams no. 8, Toronto) extracted with water (5 ml./g. dried cells) for 2 hr. at 0°.

Contents of Thunberg tubes: 0·067 M phosphate buffer, pH 6·8, 0·01 M GSH, 1·0 ml. of homogenate, extract or cell suspension; liquid volume 3·0 ml. Activation for 1 hr. at 38°; then 0·33 ml. of 0·1 M ALA added from side arm. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$.

In this and subsequent tables and figures ALA is to be taken to denote the hydrochloride.

Organism or tissue	Activity (μmole PBG/hr./g. wet wt.)
Rat liver	0·70
Rat kidney	0·24
Rat Harderian gland	0·15
Pigeon liver	0·88
Pigeon breast muscle	0·02
Spinach leaf (extract)	0·06
Pea seedlings	0·01
Brewer's top yeast (whole cells)	0·03
Brewer's top yeast (extract)	0·07
	(μmoles PBG/hr./100 mg. dry wt.)
Bact. cadaveris (whole cells)	0·03
Bact. cadaveris (extract)	0·19
Corynebact. diphtheriae (extract of acetone powder)	1·03

Table 3. *ALA dehydrase of normal rabbit tissues*

Fresh rabbit tissues homogenized in 0·15 M-KCl (2 ml./g. wet weight). Contents of Thunberg tubes: 0·067 M phosphate buffer, pH 6·8, 0·01 M GSH, 1·0 ml. of tissue homogenate; liquid volume 3·0 ml. Activation for 1 hr. at 38°; then 0·33 ml. of 0·1 M ALA added from side arm. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$.

Tissue	Activity (μmoles PBG/hr./g. wet wt.) Rabbit no. 1	Rabbit no. 2	Rabbit no. 3	Rabbit no. 4
Liver	0·99	1·14	0·95	1·05
Kidney	0·28	—	0·30	0·32
Bone marrow	—	0·26	0·23	0·24
Spleen	0·10	—	0·10	0·11
Blood	—	0·05	0·05	0·06
Heart muscle	—	0·04	—	0·04
Brain	—	0·06	—	—
Lung	—	—	—	0·07
Skeletal muscle	—	—	—	0·01

The distribution of ALA dehydrase activity in the tissues of four normal rabbits is shown in Table 3. The most striking finding is the large activity of liver, which is more than double that of any of the other tissues tested. Kidney and bone marrow are also rich in the enzyme, but the activities of the other tissues are low. It is probable that the figures in Table 3 represent a measure of the real concentration of the enzyme in the various tissues, since they agree satisfactorily with the activities which can be extracted from the acetone-dried tissues and are also unaffected by the nature or ionic strength of the medium in which the tissue extracts are made. The distribution of ALA dehydrase activity has also been studied in animals made anaemic or porphyric by administration of *N*-acetylphenylhydrazine and of Sedormid respectively (Table 4). There is a threefold increase in activity in blood and spleen of anaemic animals with no effect on liver; but in the porphyric animal the activities of liver and kidney show a twofold rise, while those of the blood and spleen are unaffected. The activity of bone marrow shows no significant variation from normal in either condition, and that of lung is at most very little affected by the experimental porphyria.

The result of an experiment to determine the distribution of ALA dehydrase in the particulate fractions of a rat liver is shown in Table 5. No activity was found to be associated with either the mitochrondria or the microsomes, while all the activity was recovered in the soluble fraction. The small activity found in the nuclear fraction was probably due to the presence of unbroken erythrocytes.

Table 4. *Effect of experimental anaemia and porphyria on ALA dehydrase in rabbit tissues*

Anaemia produced by daily intravenous injection of *N*-acetylphenylhydrazine (1·5 mg./kg. body weight) for 3 days; animals killed on fifth day, when blood-haemoglobin concentration was reduced by 40–50%. Porphyria produced by daily administration by stomach tube of Sedormid (200 mg./kg. body weight); animals killed on sixth day, 24 hr. after first appearance of PBG in urine. Tissues homogenized in 0·15M-KCl (2 ml./g. wet weight). Contents of Thunberg tubes, incubation conditions and protein precipitation as in Table 3.

Tissue	Activity (μmoles PBG/hr./g. wet wt.) Rabbit no. 5	Rabbit no. 6	Rabbit no. 7	Rabbit no. 8	Rabbit no. 9
Anaemic					
Liver	1·05	1·14			
Bone marrow	0·24	0·27			
Spleen	0·36	—			
Blood	0·16	0·18			
Porphyric					
Liver			2·04	2·20	2·30
Kidney			0·84	—	—
Bone marrow			0·22	0·23	—
Spleen			0·09	0·09	—
Blood			0·03	0·03	—
Lung			0·12	—	—

Table 5. *ALA dehydrase in particulate fractions of rat liver*

Fresh rat liver homogenized in 0·25M sucrose (9 ml./g. wet weight of liver) and strained through muslin. Nuclear and mitochondrial fractions prepared according to Schneider & Hogeboom (1950); microsomal and soluble fractions according to Schneider (1948). Incubations at 38°; 0·067M phosphate, pH 6·8, 0·01M GSH, 1 ml. of liver fraction; liquid volume, 3·0 ml. Activation for 1 hr.; 0·33 ml. of 0·1M ALA added from side arm. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$.

Liver fraction	Activity (μmole PBG/hr./g. wet wt. original liver)
Whole homogenate	0·71
Nuclear fraction	0·02
Mitochrondria	0·00
Microsomes	0·00
Soluble fraction	0·74

Purification

The dehydrase has been purified about 270-fold, an aqueous extract of acetone-dried ox liver being used as starting material. The preparation procedure is described below for a typical batch. All operations were carried out as far as possible in the cold. All solutions used after step 8 were made up with A.R. reagents in ion-free water. Activity, *A*, is expressed as μmoles of PBG formed/hr./ml. of enzyme solution under the standard conditions described above. Protein nitrogen, *N*, was determined by the Kjeldahl procedure of Hiller, Plazin & Van Slyke (1948), and is expressed as mg. of protein N/ml. of enzyme solution.

Preparation of acetone-dried powder (*step* 1). The liver of an ox was cut into thin slices and frozen in solid CO_2 immediately after removal from the carcass. The frozen material was allowed to thaw and was put through a mechanical mincer. The minced liver was homogenized in a Waring Blendor with 4–5 times its volume of acetone at −5° to −10°. The slurry was filtered on a Büchner filter; the precipitate was washed with acetone at −5°, followed by ether at −5°, and dried in a stream of air at 4°. A batch of 10 lb. of liver yielded about 1 kg. of dry powder, which had lost no dehydrase activity. Such powders are stable for 6–8 weeks if stored at −5°.

Extraction of the dehydrase (*step* 2). Acetone-dried ox liver (500 g.) was stirred mechanically for 2 hr. at 0° with 10 l. of water. The mixture was strained through muslin. *A*, 0·245; *N*, 2·85; *A*/*N*, 0·087; determined with a portion of the extract previously centrifuged until clear.

Purification by heating (*step* 3). Portions of about 500 ml. of the extract were heated with stirring until the temperature reached 50°. The heat source was then removed and the temperature allowed to rise to 53°. The time taken for the temperature to rise from 4° to 53° was about 3 min. The portion of extract was stirred for 2 min. after the temperature had reached 53°, during which time it rose to 56–58°; it was then poured into a flask in ice and cooled as quickly as possibly until the temperature had fallen below 35°. After all the extract had been treated in this way the precipitate was filtered off. Vol. of filtrate, 5600 ml.; *A*, 0·259; *N*, 2·09; *A*/*N*, 0·124.

First precipitation with $(NH_4)_2SO_4$ (*step* 4). To the filtrate after heating were added 1400 g. of $(NH_4)_2SO_4$, to bring it to 40% saturation. The precipitate was filtered off and discarded. A further 590 g. of $(NH_4)_2SO_4$ were added to the filtrate (5900 ml.) to bring it to 55% saturation. The precipitate was filtered off and dissolved in 1000 ml. of water. *A*, 0·850; *N*, 3·50; *A*/*N*, 0·243.

Adsorption of impurities on $Ca_3(PO_4)_2$ *gel* (*step* 5). To the solution of enzyme (1000 ml.) from step 4 (which contained 4·90 mg. of NH_3-N/ml.) were added 50 ml. of 3M sodium acetate buffer, pH 5·6 (9 parts of $CH_3.CO_2Na$ to 1 part of $CH_3.CO_2H$), and 50 ml. of $Ca_3(PO_4)_2$ gel suspension (12·2 mg./ml., prepared according to Singer & Kearney (1950), using ion-free water throughout). The precipitate was discarded. *A* of supernatant, 0·835; *N*, 3·00; *A*/*N*, 0·278.

First adsorption of the enzyme on $Ca_3(PO_4)_2$ *gel and elution* (*step* 6). A further 50 ml. of $Ca_3(PO_4)_2$ gel suspension were added to the supernatant from step 5. The mixture was centrifuged and the precipitate set aside. This was repeated three times. The four precipitates were combined and eluted successively with (i) 100 ml. of 0·05M phosphate

buffer, pH 7·4; (ii) 200 ml. of 0·05M phosphate buffer, pH 7·4, 10% saturated with respect to $(NH_4)_2SO_4$; (iii) 200 ml. of 0·05M phosphate buffer, pH 7·4, 15% saturated with respect to $(NH_4)_2SO_4$. The activities and protein-nitrogen contents of the eluates were:

Eluate	*A*	*N*	*A/N*
1st	0·51	0·75	0·67
2nd	1·35	0·38	3·61
3rd	0·39	0·16	2·35

Second precipitation with $(NH_4)_2SO_4$ (*step* 7). To the second eluate from step 6 (195 ml.) 57·5 g. of $(NH_4)_2SO_4$ were added, to bring it to 55% saturation. The precipitate was dissolved in 40 ml. of water. *A*, 6·2; *N*, 1·14; *A/N*, 5·41.

Second adsorption of the enzyme on to $Ca_3(PO_4)_2$ *gel and elution* (*step* 8). To the solution from step 7 (containing 3·85 mg. of NH_3-N/ml.) were added 2·0 ml. of 3M acetate buffer, pH 5·6. $Ca_3(PO_4)_2$ gel suspension (12·2 mg./ml.) was then added in portions of 2 ml. until all the activity had disappeared from the supernatant. This required twelve additions. The combined precipitates were eluted successively with (i) 10 ml. of 0·05M phosphate buffer, pH 7·4; (ii) 10 ml. of 0·05M phosphate buffer, pH 7·4, 10% saturated with respect to $(NH_4)_2SO_4$; (iii) 10 ml. of 0·05M phosphate buffer, pH 7·4, 15% saturated with respect to $(NH_4)_2SO_4$; (iv) 10 ml. of 0·05M phosphate buffer, pH 7·4, 20% saturated with respect to $(NH_4)_2SO_4$. The activities and nitrogen contents of the eluates were:

Eluate	*A*	*N*	*A/N*
1st	1·1	0·19	5·7
2nd	5·5	0·40	13·8
3rd	9·7	0·50	19·4
4th	6·1	0·34	18·0

It has been found that the efficiency of adsorption in both step 6 and step 8, especially the latter, varies very much from preparation to preparation. It is known that the presence of $(NH_4)_2SO_4$ in a concentration of more than about 10 mg. of NH_3-N/ml. prevents ALA dehydrase from being adsorbed on $Ca_3(PO_4)_2$ gel under these conditions, but, even when the $(NH_4)_2SO_4$ concentration is much lower than this, adsorption is very variable. It is possible that $Ca_3(PO_4)_2$ gel prepared by another method would give more reproducible results.

Third precipitation with $(NH_4)_2SO_4$ (*step* 9). The third and fourth eluates from step 8 (8·0 ml. of each) were combined and mixed with 2·0 ml. of 3M acetate buffer, pH 5·6. Then 13·0 ml. of saturated $(NH_4)_2SO_4$ solution were added, to bring the mixture to 48% saturation. After standing overnight at 4°, the precipitate was removed by centrifuging and dissolved in 4·0 ml. of ion-free water. *A*, 37·5; *N*, 1·46; *A/N*, 23·3.

The purified material obtained by this method is a pale-yellow clear solution, the amount of colour varying from preparation to preparation. The colour is not lost on dialysis under a variety of conditions. The purified enzyme is stable in solution at 4°, only about 20% of the activity being lost after 2 weeks.

Product of the reaction

Isolation of crystalline PBG. A mixture of 100 ml. of 0·1M phosphate buffer, pH 6·8, 30 ml. of 0·03M GSH (neutralized to pH 7·0 with NaOH), 50 ml. of 0·095M ALA (neutralized to pH 7·0 with NaOH), 76 ml. of partially purified ALA dehydrase [first $(NH_4)_2SO_4$ precipitate; *A*, 0·625; *N*, 2·42] and 40 ml. of water were put in a 1 l. flask, which was evacuated and incubated *in vacuo* for 18 hr. at 38°. At the end of this period the protein was precipitated by addition of 100 ml. of colloidal Fe_2O_3 and 10 ml. of saturated $CuSO_4$. The precipitate was removed by centrifuging and washed three times with about 50 ml. of water. The combined supernatant and washings, which contained Ehrlich-positive material equivalent to 53·0 mg. of PBG, were treated according to the procedure of Cookson & Rimington (1954) for the isolation of crystalline PBG. The yield of crystalline material was 20 mg.; on the basis of the colour formed with Ehrlich's reagent in acid solution this material was 97% pure PBG. The crystalline material was also subjected to paper chromatography, using the butanol–acetic acid solvent system of Campbell, Work & Mellanby (1951). Its R_F value in this solvent was identical with that of crystalline PBG isolated both from rabbit urine and from human urine.

Balance experiment. Table 6 shows an experiment in which the disappearance of ALA and the appearance of PBG were followed simultaneously, in the presence of purified dehydrase. The quotient of ALA lost over PBG formed is close to the theoretical value of 2 at different times after the start of the reaction. In addition, in the experiment described in Table 6, 85% of the ALA was converted into PBG, indicating that the equilibrium of the reaction is in favour of PBG formation.

Table 6. *Balance experiment*

Incubation at 38°: 0·067M phosphate buffer, pH 6·7, 0·08 ml. of purified ALA dehydrase (=116 μg. of protein N), 0·014M GSH; fluid volume 11·8 ml. Activation for 1 hr.; 0·2 ml. of ALA solution added (=2·34 μmoles of ALA). Samples (1·0 ml.) for ALA determination deproteinized by addition of 0·3 ml. of 20% TCA; samples (2·0 ml.) for PBG estimation poured into 2 drops of saturated $CuSO_4$ soln.

Time (min.)	Total ALA (μmoles)	ALA lost (μmoles)	Total PBG (μmoles)	ALA lost / PBG formed
0	2·34	—	0	—
45	1·06	1·28	0·67	1·91
90	0·63	1·71	0·89	1·92
150	0·35	1·99	1·02	1·95

Properties of the purified dehydrase

The solution of purified enzyme obtained above was subjected to electrophoresis in sodium diethylbarbiturate–HCl buffer, pH 8·42, ionic strength 0·2 at 2°. The protein migrated as one component over a period of 2·5 hr. Scanning photographs of the ascending and descending boundaries are shown in Fig. 2. The mobility has not been determined.

Sulphydryl nature of the enzyme

After the fourth or fifth step in the purification no activity is obtained unless the preparation is previously activated with a sulphydryl reagent. GSH and CySH are both effective activators (Gibson, 1955), but ascorbic acid and $Na_2S_2O_4$ do not activate; this is shown in Table 7. BAL has been found to activate the enzyme, but thioglycollic acid does not do so. The time course of the activation by GSH is shown in Fig. 3, from which it appears that activation is virtually complete in 1 hr. The effect of concentration of GSH on the activation of the purified enzyme is shown in Fig. 4. A concentration of 0·01 M GSH produces nearly maximal activation under these conditions.

Table 8 shows the inhibition by two thiol inhibitors of a crude preparation of dehydrase. This preparation, which was partially active without a thiol activator, was completely inhibited by 10^{-3} M *p*-chloromercuribenzoate or iodoacetamide. However, the inhibition was partially reversed by the addition of an equimolar amount of GSH.

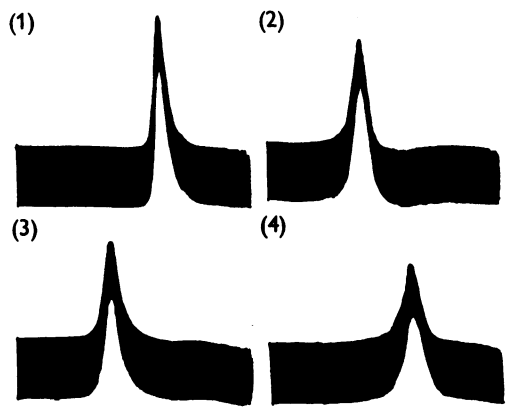

Fig. 2. Electrophoresis scanning photographs of ascending and descending boundaries; Perkin–Elmer Tiselius apparatus, model 38. Protein concn. 0·91% (w/v) in sodium barbitone–HCl buffer, pH 8·42, *I* 0·2 (0·02 M sodium barbitone, 0·0053 N-HCl, 0·18 M-NaCl). Field strength 1·9 v/cm. (1) Ascending boundary, 90 min.; (2) descending boundary, 90 min.; (3) ascending boundary, 150 min.; (4) descending boundary, 150 min.

Table 7. *Activation of ALA dehydrase*

Incubations at 38°; 0·067 M phosphate buffer, pH 6·8, 0·1 ml. of partially purified ALA dehydrase (step 4; *A*, 0·880; *N*, 3·90), activator as indicated; liquid volume 3·0 ml. Activation for 45 min.; 0·33 ml. of 0·1 M ALA added from side arm. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$.

Activator	Activity (μmole PBG/hr.)
None	0·02
$Na_2S_2O_4$ (0·009 M)	0·00
Ascorbic acid (0·009 M)	0·01
GSH (0·011 M)	0·44
CySH (0·007 M)	0·31

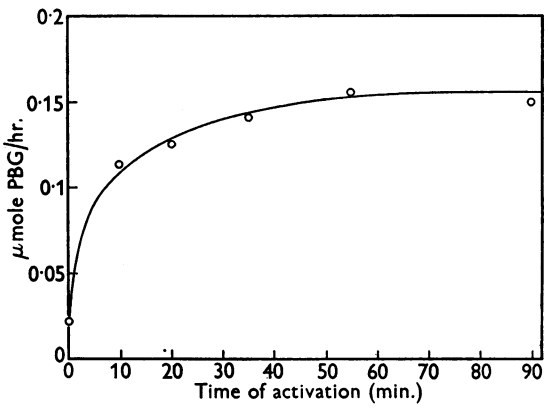

Fig. 3. Activity and time of activation. Incubations at 38°: 0·067 M phosphate buffer, pH 6·7, 0·01 M GSH, 0·01 ml. of purified enzyme (=14·6 μg. of protein N); liquid volume 3·0 ml. Activation for periods as shown; then 0·33 ml. of 0·1 M ALA added. Reaction stopped after 10 min. by pouring into 2 drops of saturated $CuSO_4$.

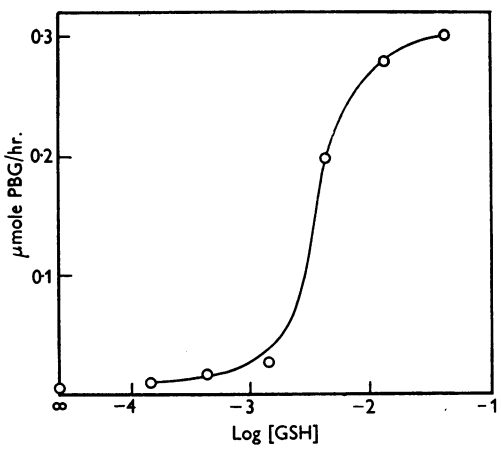

Fig. 4. Activity and concentration of GSH. Incubations at 38°: 0·067 M phosphate buffer, pH 6·7, 0·01 ml. of purified enzyme (=14·6 μg. of protein N), GSH concentration as shown; liquid volume 3·0 ml. Activation for 1 hr.; 0·33 ml. of 0·1 M ALA added. Reaction stopped with $CuSO_4$.

Kinetics

Progress curve. The progress curve in Fig. 5 was obtained with a partially purified preparation of the dehydrase. The amount of PBG formed was proportional to the time at least up to 2 hr. at 38°.

Table 8. *Inhibition of ALA dehydrase by thiol inhibitors*

Incubations at 38°: 0·067 M phosphate buffer, pH 6·8, 1·0 ml. of heat-treated extract of acetone-dried ox liver (=2·02 mg. of protein N), inhibitors and GSH as indicated; liquid volume 3·0 ml. Activation for 1 hr.; 0·33 ml. of 0·1 M ALA added from side arm. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$.

Glutathione (M)	*p*-Chloromercuribenzoate (M)	Iodoacetamide (M)	Activity (μmole PBG/hr.)	Inhibition (%)
10^{-2}	—	—	0·270	—
—	—	—	0·154	0
—	10^{-3}	—	0·002	99
10^{-3}	10^{-3}	—	0·122	21
—	—	10^{-3}	0·005	97
10^{-3}	—	10^{-3}	0·064	61

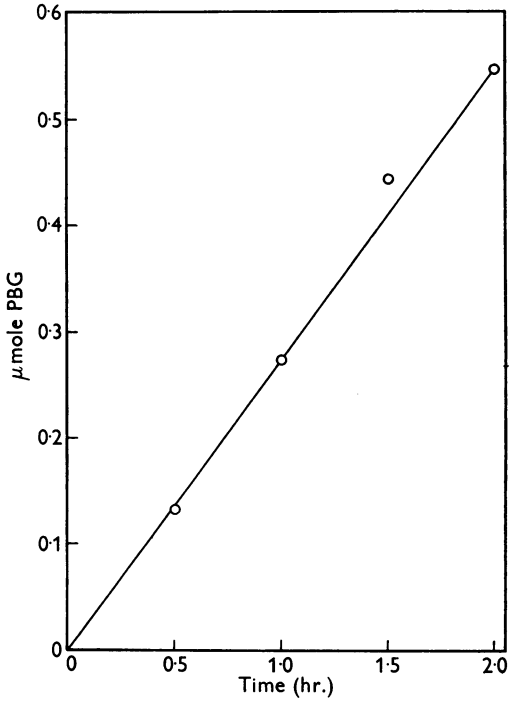

Fig. 5. Progress curve. Incubations at 38°: 0·067 M phosphate buffer, pH 6·8, 0·2 ml. of partially purified dehydrase (step 4; *N*, 2·65), 0·01 M GSH; liquid volume 3·0 ml. Activation for 1 hr.; 0·33 ml. of 0·1 M ALA added. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$. Each point represents a separate incubation.

Enzyme concentration and initial velocity. The initial reaction rate was found to be proportional to the concentration of dehydrase over a 24-fold range, at pH 7·0 and 38°. This is shown in Fig. 6.

pH and initial velocity. The variation of initial velocity with pH is shown in Fig. 7. There is a fairly marked optimum at pH 6·7, with a sharp decrease on the acid side and a more gradual slope on the alkaline side. This agrees with the observation of Granick (1954) on the pH–activity curve of

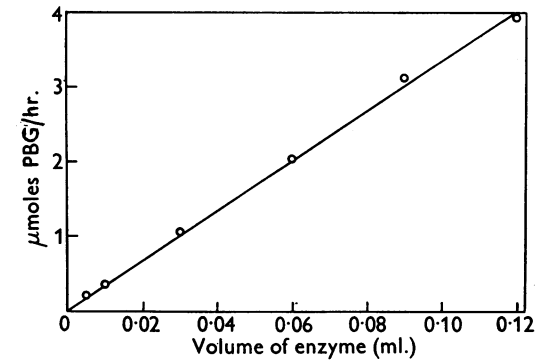

Fig. 6. Activity and enzyme concentration. Incubations at 38°: 0·067 M phosphate buffer, pH 6·8, 0·05 M CySH, purified ALA dehydrase (*N*, 2·30) as shown; liquid volume 7·4 ml. Activation for 1 hr.; then 0·1 ml. of 0·1 M ALA added. Samples of 2 ml. removed into 2 drops of saturated $CuSO_4$ solution at varying times, and initial reaction rates calculated from PBG formed.

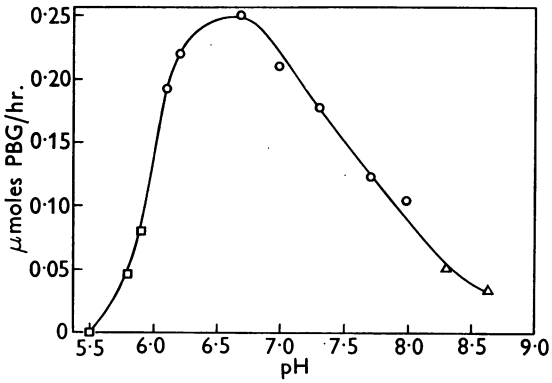

Fig. 7. Activity and pH. Incubations at 38°: buffer as indicated, 0·01 M GSH, 0·01 ml. of purified ALA dehydrase (=14·6 μg. of protein N); liquid volume 3·0 ml. Activation for 1 hr.; 0·33 ml. of 0·1 M ALA added. Reaction stopped with $CuSO_4$. □, 0·1 M Acetate buffer; ○, 0·067 M phosphate buffer; △, 0·1 M barbitone buffer.

ALA dehydrase from chicken erythrocytes. The curve in Fig. 7 was obtained at a concentration of ALA of 10^{-2} M; since this is about 60 times the value of 'K_m' the curve probably represents a close approximation to the effect of pH on the maximal initial velocity.

Substrate concentration and initial velocity. Fig. 8 is a plot of the reciprocal of the initial velocity against the reciprocal of the concentration of ALA, at pH 6·7 and 38°. The plot is linear within experimental error. The value of 'K_m' determined from the intercept on the abscissa is $1{\cdot}6 \times 10^{-4}$ M. The maximum initial velocity (determined from the ordinal intercept) was 18·1 μmoles of PBG/hr./mg. of protein-N, under these conditions.

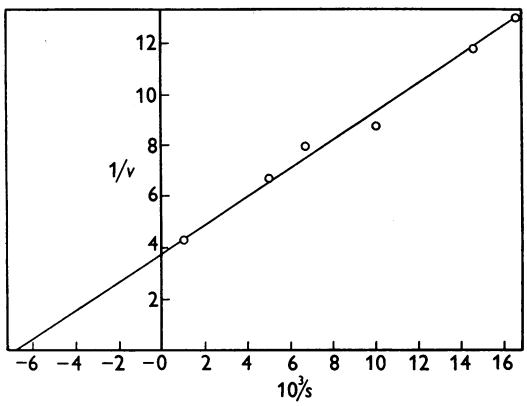

Fig. 8. Activity and substrate concentration. Incubations at 38°: 0·067 M phosphate buffer, pH 6·7, 0·01 M GSH, 0·01 ml. of purified ALA dehydrase (=14·6 μg. of protein N); liquid volume 2·8 ml. Activation for 1 hr.; ALA added as shown, in 0·2 ml. of water. Reaction stopped with $CuSO_4$. Results plotted as $1/v$ and $1/s$; v (initial reaction rate) expressed as μmoles of PBG formed/hr.; s expressed as molar concentration of ALA.

Specificity

No Ehrlich-positive compound is formed when ALA dehydrase is incubated with aminoacetone, αδ-diaminolaevulic acid or 6-amino-5-oxohexanoic acid (Table 9). The possibility that any of them is in fact a competitive inhibitor has not been investigated in detail, but it can be inferred from Table 9 that probably none of them has a high affinity for the enzyme. It is also possible that mixed porphobilinogens could be formed from ALA in the presence of any of the compounds, but this again has not been looked into.

Inhibition

A variety of compounds have been tested for their effect on ALA-dehydrase activity. The results obtained with several metals are summarized in Table 10; a crude preparation was used because it does not require thiol activation. The metals fall into three groups, consisting of (1) Cu^{2+}, Hg^{2+} and Ag^{+}, which inhibit completely at a molarity of 10^{-3}; (2) Zn^{2+}, which inhibits about 50%, and Pb^{2+}, which inhibits slightly at 10^{-3} M concentration; (3) other metals, which do not inhibit at all at this concentration. The first groups of metals

Table 9. *Action of ALA dehydrase on compounds related to ALA*

Contents of Thunberg tubes: 0·067 M phosphate buffer, pH 6·8, 0·01 M GSH; liquid volume 3·0 ml. For the experiment with aminoacetone, 0·01 ml. of purified enzyme (*A*, 45·2; *N*, 2·30) was used; for that with αδ-diaminolaevulic acid, 0·1 ml. of partially purified enzyme (step 6; *A*, 1·50; *N*, 4·30); and for that with 6-amino-5-oxohexanoic acid, 0·5 ml. of a heat-treated extract (step 3; *A*, 0·220; *N*, 1·90). Activation for 1 hr. at 38°; then contents of side arm added as indicated. Reaction stopped with $CuSO_4$.

Contents of side arm (ml. 0·1 M)		Activity
ALA	Aminoacetone	(μmole PBG/hr.)
0·33	—	0·442
—	0·33	0·000
0·33	0·33	0·372
	αδ-Diamino-	
ALA	laevulic acid	
0·03	—	0·146
—	0·03	0·000
0·03	0·03	0·125
0·03	0·09	0·109
	6-Amino-5-	
ALA	oxohexanoic acid	
0·1	—	0·108
—	0·2	0·001
0·1	0·2	0·073

Table 10. *Effect of some metals on crude ALA dehydrase*

Contents of Thunberg tubes: 0·067 M phosphate buffer, pH 6·8, 1·0 ml. of heat-treated extract of acetone-dried ox liver (step 2 of purification procedure; *A* (without thiol activator), 0·114; *N*, 2·35); and 10^{-3} M metal as indicated; liquid volume 3·0 ml. After equilibration at 38°, 0·33 ml. of 0·1 M ALA added from side arm. Reaction stopped with colloidal Fe_2O_3 and $CuSO_4$.

Metal added	Percentage of maximal activity
Cu^{2+}	0
Hg^{2+}	0
Ag^{+}	0
Zn^{2+}	55
Pb^{2+}	80
Fe^{3+}	100
Mg^{2+}	100
Mn^{2+}	100
Ca^{2+}	100
Ba^{2+}	100
Sr^{2+}	100

would be expected to inhibit the dehydrase if it were a thiol enzyme. The inhibition by Zn^{2+} has also been observed with more purified preparations. This is shown in Table 11, where the effects of Fe^{2+} and Mg^{2+} on the same preparation are described. It can be seen that Zn^{2+} inhibits to a large extent at $3{\cdot}3 \times 10^{-4}$ M, whereas Mg^{2+} and Fe^{2+} have very little effect at this concentration.

The other compounds which have been tested for inhibition of the dehydrase are either thiol inhibitors or metal-chelating agents. Of the four thiol inhibitors studied, the effect of two has already been described (Table 8). The third such compound, sodium arsenite, was found not to inhibit the enzyme at all at molarities from 10^{-5} to 10^{-2}; this was true at all stages of purification, whether activated with GSH or not. Finally, sodium fluoride was found not to inhibit at concentrations up to 10^{-2} M. Several metal-chelating agents have been tested for their effect on the enzyme, but only three have been found to have any effect. The inhibition of the purified enzyme by disodium ethylenediaminetetraacetate is shown in Fig. 9, where it is found that 50% inhibition occurs at a molarity of about $5{\cdot}5 \times 10^{-6}$. The other chelating agents which affected ALA dehydrase were 8-hydroxyquinoline-5-sulphonic acid and 8-hydroxy-7-iodoquinoline-5-sulphonic acid, which both inhibited a heat-treated acetone-powder extract about 80% at 10^{-5} M concentration. This was also true in the presence of excess of Fe^{3+}; it seems possible that the inhibition may be due to the presence of the sulphonic acid group rather than to any chelating properties of these compounds. In support of this, 8-hydroxyquinoline, 8-hydroxyquinaldine, quinaldinic acid and picrolonic acid were all found to have no effect on the same preparation. In addition, it was found that neither KCN nor NaN_3 caused any inhibition of these preparations at molarities up to 10^{-2}.

ALA dehydrase has been found to be completely inactive in aminotrishydroxymethylmethane (tris) buffer, although it is active in mixtures of tris and phosphate, or of tris and arsenate, at the same pH value. This effect is shown in Table 12, from which it can be seen that even when the tris concentration is nine times that of the phosphate or arsenate, there is no diminution in activity. However, the enzyme does not have a specific requirement for phosphate or arsenate, being active in bicarbonate buffer as well as in acetate or veronal (see Fig. 7).

Table 11. *Inhibition of purified dehydrase by* Zn^{2+}, Fe^{2+} *and* Mg^{2+}

Contents of Thunberg tubes: 0·067 M phosphate buffer, pH 6·8, 0·05 ml. purified dehydrase (=36·7 μg. of protein N), 0·01 M GSH, metal as indicated; liquid volume 3·0 ml. Activation for 1 hr.; then 0·33 ml. 0·1 M ALA added from side arm. Reaction stopped with $CuSO_4$.

Metal added	Concentration (M)	Activity (μmole PBG/hr.)	Inhibition (%)
None	—	0·180	0
Zn^{2+}	$3{\cdot}3 \times 10^{-4}$	0·049	73
Zn^{2+}	10^{-3}	0·015	92
Fe^{2+}	$3{\cdot}3 \times 10^{-4}$	0·174	3
Fe^{2+}	10^{-3}	0·052	71
Mg^{2+}	$3{\cdot}3 \times 10^{-4}$	0·168	7
Mg^{2+}	10^{-3}	0·082	55

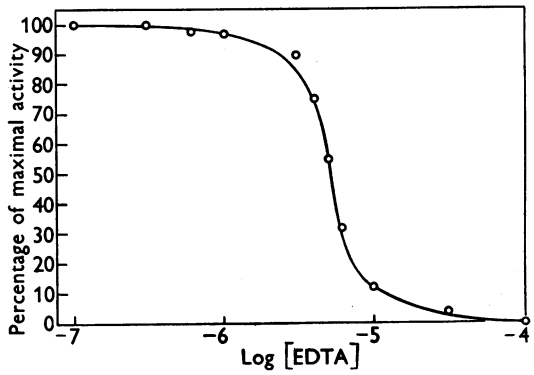

Fig. 9. Inhibition of ALA dehydrase by disodium ethylenediaminetetraacetate (EDTA). Incubations at 38°: 0·067 M phosphate buffer, pH 6·8, 0·05 ml. of purified enzyme (*A*, 1·36; *N*, 0·19), 0·01 M GSH, EDTA as indicated; liquid volume 3·0 ml. Activation for 1 hr.: then 0·33 ml. of 0·1 M ALA added. Reaction stopped with $CuSO_4$.

Table 12. *Effect of tris buffer on ALA dehydrase*

Contents of Thunberg tubes: buffer as indicated, 0·07 ml. of purified enzyme (=13·3 μg. of protein N), 0·01 M GSH; liquid volume, 3·0 ml. Activation for 1 hr. at 38°; then 0·33 ml. of 0·1 M ALA added from side arm. Reaction stopped with $CuSO_4$.

Buffer, pH 7·3			Activity
Phosphate	Arsenate	Tris	(μmole PBG/hr.)
$6{\cdot}67 \times 10^{-2}$ M	—	—	0·062
$6{\cdot}67 \times 10^{-3}$ M	—	$6{\cdot}0 \times 10^{-2}$ M	0·061
—	$6{\cdot}67 \times 10^{-2}$ M	—	0·034
—	$6{\cdot}67 \times 10^{-3}$ M	$6{\cdot}0 \times 10^{-2}$ M	0·036
—	—	$6{\cdot}67 \times 10^{-2}$ M	0·002

DISCUSSION

Distribution of dehydrase activity

If δ-aminolaevulic acid and porphobilinogen are both on the main pathway of biosynthesis of porphyrins, it is to be expected that the enzyme catalysing the conversion of one into the other will be widely distributed in nature. The distribution studies reported here bear out this view. All the tissues and organisms tested have a metabolism which is at least partly aerobic and possess a cytochrome spectrum of some type, and hence should be able to synthesize porphyrins; and each of them has been found to possess δ-aminolaevulic acid-dehydrase activity to a greater or less extent. It also seems that the amount of such activity may be broadly related to the extent of porphyrin synthesis in the tissue concerned. In the rat and rabbit there is more activity in tissues in which haem or porphyrins are produced in quantity, such as bone marrow and rat Harderian gland, than in other tissues, such as muscle, brain or lung. This is shown also by the increased activity in the spleen of anaemic rabbits, since it is known that considerable haemopoiesis occurs in this organ in anaemia (Jordan, 1942). The increased activity of the blood in this condition is most probably due mainly to the large proportion of reticulocytes which it contains. The large activity of the liver in rabbits, rats and pigeons reflects the importance of this tissue in porphyrin metabolism, a point which has been emphasized recently by Goldberg & Rimington (1955) (see also Prunty, 1945; Watson, Schwartz & Hawkinson, 1945). The increase in dehydrase activity of both liver and kidney after administration of Sedormid is in keeping with the conclusion reached by Goldberg & Rimington (1955) that the porphyrins excreted in this condition arise from increased formation rather than from reduced breakdown of porphyrins.

It seems probable that *in vivo* the dehydrase normally exists in solution in the cytoplasm, at least in liver. As shown in Table 5, all the activity of a rat-liver homogenate can be recovered in the soluble fraction of the cytoplasm, whereas none is found in the particulate fractions, and at most only a trace in the nuclei. The complete stability of the enzyme to acetone drying is in keeping with this deduction.

Product of the reaction

The experiments described here, together with others reported by Granick (1954) and Schmid & Shemin (1955), leave no doubt that the product of the reaction catalysed by δ-aminolaevulic acid dehydrase is porphobilinogen. The isolation of a crystalline substance, indistinguishable from porphobilinogen obtained by other means, from an incubation mixture containing δ-aminolaevulic acid and the dehydrase has been described above; and both these groups of workers have reported almost identical experiments. Schmid & Shemin (1955) have found that porphobilinogen formed from [^{14}C]δ-aminolaevulic acid in the presence of their enzyme preparation has double the molar specific activity of the aminolaevulic acid, as expected. In Table 6, it is shown that the disappearance of δ-aminolaevulic acid catalysed by the purified dehydrase is accompanied by the appearance of an equivalent amount of porphobilinogen as judged from the Ehrlich colour. From all these lines of evidence it can be concluded that δ-aminolaevulic acid dehydrase catalyses the reaction formulated in Fig. 1.

Properties of the enzyme

In its behaviour during purification, and in experiments, not reported here, on the effect of dialysis, the dehydrase appears to possess the properties of a euglobulin. It is precipitated completely by 55% saturated ammonium sulphate; it is also precipitated by dialysis against solutions of low ionic strength in the pH range 4·5–6·5, but is brought into solution readily at ionic strengths above about 0·02. The electrophoresis pattern in Fig. 2 might indicate that under a particular set of conditions there was only one component present in one batch of the purified enzyme. However, the A/N quotient of this batch was not as high as has been obtained in at least two other experiments. It seems likely that there was present some other material, possibly an inactive form of the same protein, which cannot be separated from the dehydrase by electrophoresis under the conditions used.

The kinetic studies described here present no exceptional features, apart from the asymmetry of the pH–activity curve. The value of 'K_m', the theoretical interpretation of which constant seems open to question (Gibson, 1955), is in the usual range of values reported for this constant, and indicates a moderately high affinity of the enzyme for its substrate.

There is little doubt that the dehydrase is a thiol enzyme. The fact that addition of a thiol reagent is necessary in order to secure any activity after the third or fourth step in the purification, together with the inhibition by *p*-chloromercuribenzoate and iodoacetamide, are strong evidence that the enzyme possesses SH groups which are essential for activity. The reversal by glutathione of the inhibition by these two compounds and the inhibition by copper, mercury and silver are further confirmation. The thiol nature of the enzyme has also been recognized by Schmid & Shemin (1955). Attention should be drawn to the

ease with which ALA dehydrase undergoes reversible oxidation. Extreme care must be exercised in the early stages of purification if oxidation is to be avoided, and no preparation has been obtained which was active without thiol reagents after adsorption on calcium phosphate gel. Rather a high concentration, about 0·02 M, of glutathione or cysteine is necessary in order to secure maximal activity, even after activation for one hour. However, reduction proceeds quite rapidly at a given concentration of activator, over 80% of the activity being present after incubation for 20 min. in the presence of the thiol reagent.

It has not yet proved possible to obtain any conclusive evidence as to the existence of a prosthetic group of δ-aminolaevulic acid dehydrase. Attempts to split off a co-factor by dialysis at pH values between 4·5 and 8·5 and at ionic strengths from zero to 0·2 have so far been fruitless. There is no indication of the presence of a light-absorbing prosthetic group from the ultraviolet absorption spectrum of the purified enzyme (Gibson, 1955). The inhibition by ethylenediaminetetraacetate, which is complete at 10^{-4} M and nearly 50% at 5×10^{-6} M, might indicate that the enzyme contains a bound metal, since it is unlikely that inhibition would be found at such low concentrations unless some form of chelation were involved. However, the negative results obtained with other chelating agents must throw some doubt on this conclusion. No activating effect has been found at any time with any compound other than a thiol reagent.

The effect of tris buffer on the enzyme is interesting but obscure. The experiment described in Table 12 recalls the observation of Lampen & Wang (1952) that the pyrimidine nucleosidase of *Lactobacillus pentosus* was rapidly inactivated in tris buffer but was protected from this inactivation by arsenate or phosphate. However, if the same is true of aminolaevulic acid dehydrase, the effect in this case cannot be due solely to the valency of the buffer anion as postulated by Lampen & Wang for the pyrimidine nucleosidase, since the dehydrase is active in bicarbonate, acetate and diethyl barbiturate. In fact tris is the only buffer in which the dehydrase has so far been found to be totally inactive.

SUMMARY

1. The purification of an enzyme catalysing the condensation of δ-aminolaevulic acid to porphobilinogen is described. The enzyme has been purified 270-fold when acetone-dried ox liver was used as starting material. The purified material behaved as one component on electrophoresis at pH 8·4.

2. The product of the reaction has been isolated and identified as porphobilinogen, and evidence was obtained that no other Ehrlich-positive compound is formed.

3. The enzyme is widely distributed in nature and is probably present in all cells having an aerobic metabolism. In mammals activity is highest in the liver, but kidney and bone marrow are also fairly active. The activity of liver appears to be confined to the soluble part of the cytoplasm.

4. In anaemia produced by phenylhydrazine the activity of the spleen and blood was found to be increased threefold. Experimental porphyria produced by Sedormid was associated with a twofold rise in activity in the liver and kidney.

5. After the fourth stage in the purification the enzyme required cysteine or glutathione for activity. The activity of crude preparations which do not have this requirement was abolished by thiol inhibitors. These and other facts strongly indicate that the enzyme requires thiol groups for activity.

6. Ethylenediaminetetraacetate has been found to inhibit strongly, but no requirement for a metal had so far been established. The enzyme has been found to be inactive in the presence of aminotrishydroxymethylmethane under certain conditions.

7. The enzyme does not act on aminoacetone, 6-amino-5-oxohexanoic acid and αδ-diaminolaevulic acid.

8. The kinetics of the enzyme-catalysed reaction have been investigated. The pH optimum at 38° is 6·7 and K_m at pH 6·7 and 38° is $1·4 \times 10^{-4}$ M.

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Zone Electrophoresis in Starch Gels: Group Variations in the Serum Proteins of Normal Human Adults

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The theoretical advantages of zone electrophoresis over free-boundary electrophoresis are well known: freedom from quantitatively important boundary anomalies, the possibility of preparing electrophoretically discrete proteins, and adaptability to small quantities of material. However, the two zone-electrophoresis methods at present in general use do not give a resolving power as great as the free-boundary method of Tiselius; the resolving power of the filter-paper-electrophoresis method of Kunkel & Tiselius (1952) is reduced by the difficulty of putting the sample to be investigated on the paper in a sufficiently narrow zone and by adsorption effects, and obligatory serial analyses limit the resolving power obtainable with the starch-grain method of Kunkel & Slater (1952) because of the difficulties of making sufficiently thin serial sections. A method of zone electrophoresis was therefore sought which would combine the advantages of the low adsorption characteristic of the starch-grain method with the convenience of protein detection by staining characteristic of the filter-paper method. At the same time, by introducing the sample in a sufficiently narrow zone, it was hoped to obtain a resolving power as good as that obtained in the classical Tiselius method.

The method evolved comprises zone electrophoresis with a starch gel as the supporting medium, and proves to have a resolving power in many cases superior to that of the Tiselius method. The method has been successfully applied to crystalline β-lactoglobulin, which can be resolved into its two main components (Smithies, O., unpublished work), to cabbage-enzyme preparations (Dixon, G. H. & Smithies, O., unpublished