for the spectrophotometric study. The second factor results from the marked decrease of electron transport occurring at 0° since such electron transport seems to be necessary for non-osmotic mitochondrial swelling (Hunter *et al.* 1959).

In the light of all these findings it appears likely that there is a complex interaction between salicylate, EDTA and magnesium which affects the permeability of a mitochondrial membrane. Such membrane effects may be quite distinct from changes in oxidative phosphorylation.

SUMMARY

1. Sodium salicylate (5 mM) (but not sodium *p*-hydroxybenzoate or sodium chloride) increases by 60% the loss of K⁺ ions from washed rat-liver mitochondria suspended in 0.44M-sucrose for 10 min. at 0°.

2. Salicylate also induced swelling of rat-liver mitochondria prepared in the presence of EDTA.

3. The conditions of the experiments minimize a metabolic effect of salicylate. It is suggested that these effects of salicylate are primarily on the mitochondrial membrane.

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Carbohydrate Sulphatases of Marine Molluscs

ASSAY OF GLYCOSULPHATASES

By P. F. LLOYD, K. O. LLOYD AND OLIVE OWEN

Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire

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Sulphatases catalyse the reaction:

and methods of assay of such enzymes may be based upon measurement of unchanged substrate (I), desulphated residue (II), inorganic sulphate (III) or change of pH (IV). When (I) is a sugar sulphate and therefore (II) is the corresponding free sugar, the hydrolytic enzyme is usually described as a glycosulphatase. The assay of enzymes

of the sulphatase type has been discussed by Dodgson & Spencer (1957).

Soda (1936) and his co-workers, in the investigation of the glycosulphatases of Japanese molluscs, used methods of assay which included the indirect determination of unhydrolysed substrate (by gravimetric estimations of inorganic sulphate liberated on acid hydrolysis) and the measurement of liberated inorganic sulphate by potentiometric titration or nephelometrically. These methods are not capable of a high degree of accuracy unless

applied on a large scale. Soda also measured, by fermentation, the glucose liberated from glucose sulphate, but the method was involved and, again, relatively large amounts of substrate were required. Recently methods for the assay of sulphatases based on the turbidimetric determination of barium sulphate have been described (Dodgson, 1961; Rees, 1961). Colorimetric methods of sulphatase assay have been used by Dodgson & Spencer (1953) and by Lloyd (1959), and a small-scale version of each of these methods has been devised (Spencer, 1960). The method of Dodgson & Spencer (1953), in which precipitated benzidine sulphate is estimated colorimetrically, although it is time-consuming and demands very careful manipulation, has proved to be useful with a number of sulphuric esters. We have found, however, during studies on molluscan sulphatases which catalyse the hydrolysis of fucose sulphates, that the benzidine method gives low values for liberated sulphate.

In this paper we describe a more rapid method for the assay of the enzyme, which is based on the determination of L-fucose separated from unhydrolysed substrate by passage through a column of ion-exchange resin.

EXPERIMENTAL AND RESULTS

Glycosulphatase. The enzyme preparations were obtained by purification of an acetone-dried powder prepared from the viscera of *Patella vulgata* (Lloyd & Lloyd, 1961).

Preparation of L-fucose monosulphate. L-Fucose (1.95 g.) was dissolved in warm dry pyridine (25 ml.) and, after the solution had cooled, pyridine-sulphur trioxide (2.83 g.) (Sisler & Audrieth, 1946; Duff, 1949) was added. The mixture was kept at 35° for 33 hr., an equal volume of water added and the mixture stirred for 1 hr. Excess of barium hydroxide was added until the solution reached pH 10. After filtration and vacuum evaporation of the filtrate at 40° to small volume, addition of water and re-evaporation, the solution was neutralized with CO₂, decolorized with charcoal and evaporated to dryness. The presence in this crude product of L-fucose and L-fucose mono-, di- and tri-sulphates was demonstrated electrophoretically. The monosulphate component was separated by electrophoresis for 1½ hr. at 2400 v on strips of Whatman no. 3 paper, 18 cm. wide, with buffer prepared by adjusting the pH of 0.1M-acetic acid to 6.5 with pyridine. In ½ hr. under these conditions the components of the product of sulphation of L-fucose migrated to the following distances (cm.) from the starting line: L-fucose, 0.7; L-fucose monosulphate, 6.7; L-fucose disulphate, 10.9; L-fucose trisulphate, 12.8. Guide strips were cut from the edges and centre of each paper and the fucose sulphates were detected with aniline hydrogen phthalate. After the main papers had been dried, the areas containing fucose monosulphates were cut out and extracted with water, the aqueous solution was neutralized with sodium hydroxide or barium hydroxide and the respective salts were isolated by evaporation. These salts were white crystalline hygroscopic solids

which were free from fucose and from the di- and tri-sulphates of fucose. The barium and sodium salts of the L-fucose monosulphate component (mixture of isomers) had $[\alpha]_D^{25} - 54.8$ (c 0.5 in water), and $[\alpha]_D^{25} - 55.3$ (c 0.5 in water) respectively (Found: C, 23.4; H, 3.8; S, 9.8. C₁₂H₂₂O₁₆S₂Ba requires C, 23.3; H, 3.6; S, 10.3%).

Determination of sugars

Method A for 6-deoxyhexoses. This is a modification of the method of Dische & Shettles (1948). In a 15 ml. test tube was placed exactly 2 ml. of a solution of L-fucose (containing 7–150 µg. of fucose). The tube was cooled in ice-water and concentrated (AnalaR) sulphuric acid (4.00 ml.) was added slowly. The tube was withdrawn from the ice bath and, after being kept for about 5 min. at room temperature, it was heated to 100° for exactly 10 min. The tube was cooled to room temperature and a 3% (w/v) solution of cysteine hydrochloride (0.1 ml.) was added with a micro-pipette. After being stirred and kept for 3–5 hr. the solution was transferred to a 1 cm. glass cell and the extinction at 390 mµ determined against a reagent blank. The amount of L-fucose present was read from a linear calibration curve obtained by carrying out the above procedure with solutions containing known amounts of L-fucose.

Method B for sugars (general). This is a modification of the method of Dubois, Gilles, Hamilton, Ribers & Smith (1956). To the sugar solution (3.00 ml., containing 10–140 µg. of sugar, e.g. D-glucose or L-fucose) contained in a EEL colorimeter tube was added 5% (w/v) phenol solution (1.00 ml.). Concentrated (AnalaR) sulphuric acid (5.00 ml.) was added, with stirring, and after 25 min. the intensity of the colour developed was read on the colorimeter by using an Ilford 623 filter (transmission band 460–550 mµ). The instrument was set to zero by the use of a reagent blank. The amount of sugar present was read from a calibration curve (non-linear) obtained by assay of known amounts of the sugar.

Separation of free sugar from sugar sulphates

Ion-exchange method. Small chromatography columns (0.7 cm. internal diam. × 12 cm.) containing the acetate form (about 2 ml.) of De-Acidite FF or of Amberlite resin CG-400 (chromatographic grade, type 1, 100–200 mesh) were prepared. The lower end of each tube was drawn out to a capillary tube of internal diameter about 0.02 mm., such that water percolated through the column at a rate of about 0.2 ml./min.; the columns were supported by vented rubber bungs over 10 ml. tubes graduated at 4.00 ml.

A sample (0.2 ml.) of the digest or of the solution of sugar, sugar sulphate and enzyme (in recovery experiments) was added to the top of the column with a micro-pipette. The column was eluted with water until exactly 4 ml. of eluate had been collected. The sugar in the eluate was estimated by method A or B described above.

When standard solutions of L-fucose (containing 10–110 µg. of sugar/0.2 ml.), in 0.05M- or 0.2M-sodium acetate-acetic acid buffer at pH values in the range 4–7, were passed through the columns and estimated by method A, quantitative recoveries in the eluate were obtained. The recovery of L-fucose in the presence of L-fucose monosulphate was also quantitative.

Separation and determination of L-fucose under various conditions

In the presence of various amounts of both L-fucose monosulphate and of a partially purified preparation of L-fucose sulphatase from *P. vulgata*, L-fucose could be recovered quantitatively by the ion-exchange separation followed by sugar estimation by method A, as is shown in Tables 1 and 2.

Some material (derived from the enzyme sample) which was not absorbed by the column reacted with the cysteine-sulphuric acid reagent to produce substances absorbing in the 296 m μ region. To eliminate this source of error, a control solution equivalent to the test solution, except that L-fucose was omitted, was submitted to the above procedure, and its extinction at 296 m μ measured. The difference between the apparent L-fucose values for test and control solutions gave the recovery values quoted. The apparent L-fucose values shown by the control solutions depended on the degree of purity and the proportion of the enzyme

Table 1. Recoveries of L-fucose from solutions containing various amounts of L-fucose monosulphate and sulphatase in 0.05M-acetate buffer, pH 5.5

L-Fucose solution (0.2 ml.), sodium L-fucose monosulphate solution (0.2 ml.) and sulphatase solution (0.2 ml.; 7.8 mg./ml.) were mixed, and immediately 0.2 ml. of the mixture was withdrawn and the L-fucose separated and determined.

Final concn. of sodium L-fucose monosulphate (M)	L-Fucose added (μ g.)	L-Fucose recovered (μ g.)	Recovery (%)
0.005	55.0	54.4	98.9
0.05	55.0	54.0	98.2
0.1	55.0	54.0	98.2

Table 2. Recoveries of L-fucose from solutions containing various amounts of L-fucose monosulphate and sulphatase in 0.5M-acetate buffer, pH 5.5

The method used was as described in Table 1.

Final concn. of sulphatase (mg./ml.)	Final concn. of sodium L-fucose sulphate (M)	L-Fucose added (μ g.)	L-Fucose recovered (μ g.)	Recovery (%)
5.0	0.005	5.0	7.0	140.0
5.0	0.005	18.3	18.5	101.1
5.0	0.005	25.0	26.0	104.0
5.0	0.005	48.5	47.5	98.0
5.0	0.005	60.0	59.0	98.3
3.0	0.003	6.8	4.0	58.8
3.0	0.003	13.7	13.0	94.0
3.0	0.003	55.0	53.0	96.4
3.0	0.003	82.0	82.0	100.0
3.0	0.003	110.0	110.0	100.0
1.1	0.003	55.0	54.0	98.2
3.6	0.003	55.0	57.0	103.6
5.35	0.003	55.0	54.0	98.2
7.1	0.003	55.0	55.0	100.0
10.7	0.003	55.0	55.0	100.0
3.0	0.003	55.0*	55.0	100.0

* In acetate buffer, pH 5.5, final concn. 0.2M.

preparation and was usually equivalent to between 1 and 4 μ g. of fucose for partially purified enzymes. Crude preparations such as the acetone-dried powder from *P. vulgata* gave control values of as much as 22 μ g. of apparent fucose.

Light-absorption measurements at 296 m μ were usually made 3–5 hr. after the addition of cysteine. Fig. 1 shows the variation with time of the extinction recorded for test and control solutions in a typical recovery experiment. If the solutions were kept overnight before spectrophotometric examination, lower extinction values were recorded.

The recovery of L-fucose after incubation of L-fucose monosulphates with sulphatase in 0.05M-acetate buffer at pH 4.0, 5.5 and 6.0 was quantitative. The control readings at these pH values were identical.

In a further series of experiments, salts (final concn. 5 mM) were added to solutions of L-fucose, L-fucose monosulphate and sulphatase. The control values were similar in each case and differed by no more than an apparent fucose value of 0.4 μ g. Table 3 shows that most of the added salts do not materially interfere with the recovery of fucose and that values within about 7% of the expected value of 30.2 μ g. were obtained. The results from other experiments which involved added salts were similar and indicated that the differences in recoveries could in most cases be attributed to the limitation of the method in the presence of added salts rather than to specific salt effects. Ferric chloride was an exception, since its addition leads to abnormally high fucose values (in three experiments recoveries of 112.3, 113.7 and 111.1% were obtained) even when it is present in low concentration (1 mM). Calcium chloride may also lead to high fucose values (recoveries of 108.9 and 109.3% were obtained).

Separation and determination of D-glucose under various conditions

Table 4 records the recovery (determined by method B) of D-glucose from the incubation of D-glucose 6-sulphate

Fig. 1. Variation of extinction with time. A portion (0.2 ml.) of a solution in 0.05M-acetate buffer, pH 5.5, of L-fucose (30.2 μ g.), L-fucose monosulphates (5 mM) and *P. vulgata* sulphatase (2.5 mg./ml.) was passed through an ion-exchange column and the eluate (4 ml.) collected. The eluate (2 ml.) was treated with sulphuric acid and cysteine and the extinction at 296 m μ (O) of the resultant solution was recorded with a Unicam SP. 500 spectrophotometer and 1 cm. cells. The extinction (●) of a control solution is also shown.

(sodium salt) with partially purified sulphatase (from *P. vulgata*) in acetate buffer. The D-glucose equivalents of the controls were 4–16 μg . It is clear that this method is both rapid and of wide applicability, but it yields, in its present form, only quasi-quantitative results.

Application to the assay of fucosulphatase

Equal volumes of substrate and enzyme solutions in acetate buffer were mixed and incubated. The concentration of substrate and conditions were such that 15–110 μg . of L-fucose was liberated

Table 3. *Recoveries of L-fucose from solutions containing sulphatase and L-fucose monosulphate in the presence of various salts*

Solutions of 0.2 M-sodium L-fucose monosulphate (0.1 ml.), L-fucose (0.1 ml.), enzyme (0.1 ml.) and salt solution (0.1 ml.) were mixed. Immediately, 0.2 ml. of the mixture was withdrawn and the L-fucose separated and determined. The final concentrations were: L-fucose, 30.2 μg ./0.2 ml.; enzyme, 3 mg./ml.; acetate buffer, 0.05 M (pH 5.5). The added compounds were present in a final concn. of 5 mM in every case, with the exception of FeCl_3 which had a final concn. of 1 mM.

Compound added	L-Fucose recovered (μg .)	Recovery (%)
Na_2SO_4	31.0	102.6
Na_2HPO_4	31.0	102.6
NaCN	32.2	106.6
$\text{Na}_2\text{B}_4\text{O}_7$	32.4	107.3
Sodium acetate	30.2	100.0
NaCl	29.8	98.7
Sodium citrate	29.4	97.4
NaF	28.5	94.4
Potassium acetate	30.8	102.0
BaCl_2	31.2	103.3
CuCl_2	30.0	99.3
CaCl_2	33.0	109.3
MgCl_2	31.0	102.6
EDTA (sodium salt)	29.4	97.4
FeCl_3	34.0	112.3

Table 4. *Recoveries of D-glucose from solutions containing sulphatase and sodium D-glucose 6-sulphate in 0.05 M-acetate buffer, pH 5.5*

Solutions of D-glucose (0.2 ml.), sodium D-glucose 6-sulphate (0.2 ml.) and sulphatase (0.2 ml.; 9 μg ./ml.) were mixed, and immediately 0.2 ml. of the mixture was withdrawn and the D-glucose separated and determined (method B).

Final concn. of sodium D-glucose 6-sulphate (mM)	D-Glucose added (μg .)	D-Glucose recovered (μg .)
5	141	126
5	71	62
5	46	50
5	28	22
50	71	68
50	47	46
50	28	25

during the period of assay. Alternatively the mixture may be diluted to give a sugar concentration within this range before addition of a 0.2 ml. sample to the column of ion-exchange resin. The determination of the free sugar in the eluate was made by method A. A suitable control value is obtained by following the procedure outlined above except that substrate and enzyme are incubated separately and are mixed immediately before they are put on the ion-exchange column.

Check of the method. The method was checked by assay of both L-fucose and inorganic sulphate liberated from sodium L-fucose monosulphates by the glycosulphatase preparations from *P. vulgata*. (a) In the first experiment sodium L-fucose monosulphate (0.6 ml.; 0.02 M) and *P. vulgata* sulphatase solution (0.6 ml.; 5.32 mg./ml.) were incubated at 35°. Periodically the inorganic sulphate released by enzyme action was determined by the benzidine method (Dodgson & Spencer, 1953) and L-fucose by the method described above. The values obtained (Table 5) by the two methods were not in agreement. Therefore determinations of inorganic sulphate were made on acetate-buffered solutions which contained, in addition to enzyme, amounts of L-fucose, sodium L-fucose monosulphate and inorganic sulphate corresponding in amounts to succeeding stages in the enzymic hydrolysis. The latter results showed that in the presence of L-fucose sulphates application of the benzidine method of free sulphate estimation yielded low values; the deviation varied with the experimental conditions but was about half of the true value in each case. When the appropriate corrections were made the results derived from the determination of free sulphate (Table 5) were in agreement with those calculated from the L-fucose determinations. (b) In the second experiment equal volumes of sodium L-fucose monosulphate (0.01 M) and of another sulphatase solution (8.6 mg./ml.), both in 0.05 M-acetate buffer, were mixed and incubated

Table 5. *Determination of the degree of hydrolysis of sodium L-fucose monosulphates by Patella vulgata sulphatases*

The liberated L-fucose and sulphate were determined by the benzidine method (hydrolysis 1), and by the BaSO_4 turbidimetric method (hydrolysis 2). Details are given in the text.

Hydrolysis	Time of incubation (hr.)	L-Fucose liberated (%)	Sulphate liberated (%)	
			Found	Corrected
1	4	16.2	7.0	14.3
	12	22.6	12.1	24.6
	24	30.7	14.7	31.2
2	9	9.0	8.3	—
	48	26.8	23.8	—
	96	31.0	30.0	—

at 35°. Suitable controls were used. Liberation of L-fucose was followed as in method (a) above, and sulphate was determined by a slight modification of the turbidimetric method developed by Rees (1961). A sample (0.5 ml.) of the reaction mixture was treated with aqueous trichloroacetic acid (0.1 ml.; 40%, w/v) and water (1 ml.) in a centrifuge tube. The tube was heated at 85° for 15 min., then cooled to 25° and centrifuged. The supernatant (1 ml.) was withdrawn and, after transfer to a tube held in a thermostatically controlled water bath (25°), it was treated with barium chloride solution (2 ml.; 33.5 g./l.). The solutions were mixed and after 5 min. the turbidity of the solution was measured at 500 m μ (Unicam SP. 500 spectrophotometer) against water. A control was made in the usual manner and the amount of sulphate liberated was determined by reference to a calibration curve obtained by estimating known amounts of sulphate in the presence of substrate and enzyme. The results, which are in good agreement, are shown in Table 5.

DISCUSSION

The method of assay of glycosulphatases, acting on L-fucose sulphates, described here has been used by us for detailed studies on enzymes from marine molluscs. Clearly the method should lend itself to the study of any sulphatase which utilizes a monosulphated methylpentose or related oligosaccharide as its substrate, and, further, since the colorimetric method involved (Dische & Shettles, 1948) is effective even in the presence of a considerable excess of other sugars, its potential value for investigations on competing substrates is apparent.

Used in conjunction with an assay method dependent on the determination of free sulphate, the new method offers an attractive means of following many enzymic desulphation processes. From the results of the two methods it is possible to determine whether the enzymic reaction is a hydrolytic or a transesterification (Suzuki, Takahashi & Egami, 1957) process, always provided that the free sugar does not undergo further change as the result of other enzyme action. In the present work it is demonstrated that the fucosulphatase of *P. vulgata* is a hydrolytic enzyme.

The reason for the incomplete precipitation of sulphate as benzidine sulphate in the presence of L-fucose monosulphates is not known, though it appears likely that benzidine sulphate interacts with one or more of the isomeric fucose monosulphates to form soluble complexes. Dodgson & Spencer (1954) have observed that, in the presence of D-glucose 6-sulphate, benzidine sulphate precipitation is quantitative, but only partial precipitation occurs from solutions which contain D-glucose

3-sulphate. The interaction of benzidine sulphate and related substances with carbohydrate sulphates may provide the basis for a method for separating and identifying the latter.

Acetate buffers have been used throughout our investigations, but, as the method was not adversely affected by the presence of many common salts, the method will probably be of equal value when most other buffer solutions are chosen. Also, the methods described in this paper may be used in studies on enzyme mechanism to determine whether desulphation of a given polysulphated carbohydrate, sugar or polysaccharide proceeds in a single step (or rapid contiguous steps) or piecemeal, since only completely desulphated carbohydrates will pass through the ion-exchange column.

SUMMARY

1. A method for determining small amounts of L-fucose liberated from sodium L-fucose monosulphates by enzyme preparations from *Patella vulgata* is described.

2. By this method L-fucose was recovered quantitatively from solutions of various buffer molarities and pH values which also contained L-fucose monosulphates and enzymes.

3. The presence of small amounts of Fe³⁺ ions led to high recovery values for L-fucose. Otherwise the method was unaffected by the presence of small amounts of common anions and cations.

4. A general method for determining sugars in the presence of sugar sulphates is also briefly described. In its present form the method is insensitive.

5. When the micro-benzidine method of sulphate determination was used to follow the enzymic desulphation of L-fucose monosulphates, the values obtained were only about half the true values.

6. The new method has been checked by following the release of L-fucose during the hydrolysis of L-fucose monosulphates by the sulphatases of *P. vulgata*. The values obtained corresponded to those obtained for the release of sulphate by application of the micro-benzidine (corrected) and turbidimetric methods.

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The Inhibition of Pea-Seedling Diamine Oxidase by Chelating Agents

BY J. M. HILL AND P. J. G. MANN

Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts.

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Werle & Pechmann (1949) described an enzyme in extracts of higher plants that catalysed the oxidation of 1,4-diaminobutane, 1,5-diaminopentane and histamine, and suggested that it corresponds to the diamine oxidase (diamine-oxygen oxidoreductase, EC 1.4.3.6) of animal tissues. Kenten & Mann (1952) and Mann (1955) showed that the plant enzyme also catalyses the oxidation of phenylalkylamines and of aliphatic monoamines, and suggested the name 'plant amine oxidase' for the enzyme; this name was used later by Mann & Smithies (1955*a, b*), Clarke & Mann (1957, 1959) and Mann (1960, 1961). Since Fouts, Blanksma, Carbon & Zeller (1957) showed that animal diamine oxidase also catalyses the oxidation of monoamines and phenylalkylamines, this distinction between the two enzymes on the basis of substrate specificity seems unjustified and the plant enzyme is therefore called diamine oxidase in the present work. The isolation, from pig kidney, by Kapeller-Adler & Macfarlane (1962) of a histaminase acting specifically on histamine suggests that the use of the trivial name histaminase for diamine oxidases is incorrect.

Several workers have reported that the diamine oxidase of pea and clover seedlings is inhibited by chelating agents and have therefore suggested that the enzyme may be a metalloprotein. The enzyme is inhibited by sodium diethyldithiocarbamate, salicylaldoxime and potassium ethyl xanthane (Mann, 1955), and also by diphenylthiocarbazono and 8-hydroxyquinoline (Werle & Hartung, 1956) and by 2,2'-bipyridyl and 1,10-phenanthroline (Suzuki, 1959). Mann (1961) described a method yielding highly purified preparations of pea-seedling diamine oxidase and reported that the copper content increased during purification to 0.08–0.09% in the final preparations. The results of spectrographic analysis of one of the final preparations suggested that zinc was the only other trace

element present in possibly significant amounts. The removal of copper, by precipitation with sodium diethyldithiocarbamate, resulted in complete loss of the catalytic activity; most of the original activity was restored by the addition of Cu^{2+} ions, but not of Zn^{2+} ions, to the copper-free preparation. It was therefore suggested that the diamine oxidase is an enzyme containing copper.

The present work was started to find out if the inhibition of the enzyme by chelating agents is due to the presence of copper in the enzyme.

MATERIALS AND METHODS

Buffers. Orthophosphate buffers were prepared from KH_2PO_4 and KOH and freed from traces of copper by extraction with diphenylthiocarbazono in CCl_4 , as described by Hewitt (1952), and then freed from traces of diphenylthiocarbazono by repeated extraction with CCl_4 . Pyrophosphate buffers were prepared from $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ and $\text{K}_4\text{P}_2\text{O}_7$.

Catalase. Crystalline ox-liver catalase (EC 1.11.1.6) was prepared as described by Clarke & Mann (1959).

Diamine oxidase. Diamine-oxidase preparations were made from pea seedlings by the method of Mann (1961). The assay was based on the initial rate of uptake of O_2 by reaction mixtures containing diamine oxidase, catalase (25 $\mu\text{g.}$), 10 mm-1,4-diaminobutane and 67 mm-orthophosphate buffer, pH 7, in a total volume of 3 ml. A unit of diamine oxidase is defined as the amount which will catalyse the oxidation of 1 μmole of 1,4-diaminobutane/min. at 28°, giving an initial rate of uptake of O_2 of 11.2 $\mu\text{l./min.}$ The specific activity is defined as units/mg. of protein. The protein content was calculated from the total nitrogen content by using the factor 6.25. The two preparations used contained 800 and 830 units/ml., 1920 and 2000 $\mu\text{g.}$ of N/ml., and 0.09 and 0.084% of copper, respectively. The specific activity of both preparations was 67. This corresponds to a value of 280000 for the specific activity (units/mg. of N) calculated from the units previously defined by Mann (1961).

The preparations were stored at -10° and were thawed about once weekly to take samples. The loss of activity was