heterologous thyroglobulins and thyroxine in increasing the oxygen consumption are in progress.

SUMMARY

1. The metabolism of ^{131}I -labelled thyroglobulin from four mammalian species has been studied.

2. The rat, dog and monkey rapidly metabolize the homologous protein, with the formation of iodide, iodotyrosines and thyroxine. Rabbit thyroglobulin disappears slowly from the plasma of the rabbit after intravenous injection, and no metabolites have so far been detected in the blood.

3. The development of immune reactions to foreign thyroglobulins has been observed in all the species.

4. Heterologous thyroid protein is, in general, cleared from the plasma of non-immune animals of all four species without the appearance in the plasma of significant amounts of simple metabolites. This is not so, however, when monkey thyroglobulin is injected into rats and vice versa, for rapid metabolism takes place and thyroxine appears in the plasma.

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Glutamic Acid Decarboxylase in *Rhodotorula glutinis*

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Enzymic decarboxylation of L-glutamic acid to γ -aminobutyric acid in plants, animals and bacteria is well known, and the importance of this process in nitrogen metabolism has been reviewed (Schales, 1951). Recent observations (Roberts, Ayengar & Posner, 1953) on the transamination of γ -aminobutyric acid with α -oxoglutarate serve to indicate the possible role of glutamic acid decarboxylation in the 'flow of nitrogen'. γ -Aminobutyric acid was first detected in yeast by Reed (1950), who found it in refrigerated samples and autolysates of yeast extract (Difco). The occurrence of γ -aminobutyric acid in a yeast, *Rhodotorula glutinis*, and the glutamic acid decarboxylase activity of washed cell-suspensions of this organism were reported earlier (Krishnaswamy & Giri, 1953). In this paper, details of investigations carried out on this enzyme are briefly described.

METHODS

The organism. The yeast, *Rhodotorula glutinis* Harrison var. *rubescens* Saito (Lodder) was maintained on wort-agar slants by fortnightly subculture.

Cultivation of the organism. To obtain sufficient cell material the organism was cultivated in a synthetic liquid medium (Krishnaswamy & Giri, 1953). A portion (2 l.) of this medium, after sterilization, was seeded with an actively growing inoculum (obtained by making two serial transfers of the organism at 24 hr. intervals) and mildly aerated. The cells were harvested by centrifuging (after growth for 18 hr. at room temp.).

Preparation of the enzyme. In earlier experiments, washed cells suspended in suitable buffers were used directly. Cell-free extracts obtained by grinding the cells with Pyrex-glass powder and centrifuging off the cell-debris showed lower activity. Acetone powders were prepared by treating the cells with a large volume of cold acetone and filtering on a Büchner funnel, after vigorous stirring. After the material had dried, it was finely powdered and stored in a refrigerator. Fresh preparations were always used for the extraction of the enzyme which was done by suspending the acetone powder with 0.02M Na phosphate buffer, pH 8.0 (20 mg./ml.) for 2 hr. at 30°. The supernatant obtained after centrifuging (3500 r.p.m.) was slightly yellowish in colour and it was directly used.

Measurement of enzyme activity. The reaction mixtures, unless otherwise stated, consisted of 0.2 ml. of 0.1M L-glutamic acid solution, 0.8 ml. of M/15 acetate buffer,

pH 4.5, and 1.0 ml. of enzyme preparation, total volume being 2.0 ml. They were incubated at 30° and at specified intervals the reaction was arrested by adding ethanol (1 vol.). γ -Aminobutyric acid was estimated in 20- μ l. samples of the supernatant by circular-paper chromatography, and interaction with ninhydrin, followed by colorimetric comparison of the colour intensity with that of a standard solution of γ -aminobutyric acid treated in the same way (Giri, Radhakrishnan & Vaidyanathan, 1952). Appropriate blanks and controls which included sets for endogenous reaction were always run. Activity was expressed as μ g. of γ -aminobutyric acid formed under specified conditions.

RESULTS AND DISCUSSION

Isolation and estimation of γ -aminobutyric acid by paper chromatography

To 2 ml. of the cell-free extracts various quantities of γ -aminobutyric acid were added from a 0.2% solution, and the total volume was made up to 2.5 ml. Immediately an equal volume of ethanol was added to all the tubes and γ -aminobutyric acid in the supernatants was estimated by paper chromatography. The recovery of added γ -aminobutyric acid from extracts incubated for 1 hr. at 30° was also measured, and the percentage recovery ranged between 94 and 104.

Table 1. *Recovery of added γ -aminobutyric acid from enzyme extracts*

Expt. no.	γ -Amino-butyric acid in the preparation (μ g.)	γ -Amino-butyric acid added (μ g.)	γ -Amino-butyric acid found in mixture (μ g.)	Recovery (%)
1	45	250	285	96
2	45	500	536	98
3	46	1000	1077	103

Fig. 1. Formation of γ -aminobutyric acid from L-glutamic acid in the presence of glutamic acid decarboxylase.

After decarboxylation of glutamic acid by the enzyme, γ -aminobutyric acid was isolated by circular-paper chromatography on a preparative scale (Giri, 1954, 1955) from the reaction mixture and the product obtained was compared with a synthetic sample (m.p. and mixed m.p. 192°, nitrogen, 13.1%).

Properties of the enzyme

Experiments showed that the yeast decarboxylase had similar properties to those of bacterial decarboxylase. Glutamic acid decarboxylation was found to be quantitative at pH 4.5 and 30° (Figs. 1-3);

Fig. 2. Effect of pH on the activity of L-glutamic acid decarboxylase.

Fig. 3. Effect of temperature on the activity of L-glutamic acid decarboxylase.

the enzyme was unstable above 30°. The Michaelis constant was found to be $2.75 \times 10^{-3} \text{M}$. As with other decarboxylases, dialysis reduced the activity and only pyridoxal phosphate was effective in restoring the activity.

Effect of inhibitors. The enzyme was found to be sensitive to silver, cyanide and mercury ions. Hydroxylamine was the most prominent of the carbonyl-trapping reagents. The enzyme did not show any abnormal sensitiveness to copper (the common sulphhydryl inhibitor), iodoacetate, or fluoride. In all these respects, the enzyme resembled the bacterial decarboxylase (Gale, 1946).

Reversibility of the inhibition caused by hydroxylamine with pyridoxal phosphate. In one set of reaction mixtures, hydroxylamine was added immediately after the substrate, and in another the enzyme was pre-incubated with the inhibitor for 5 min. at 30°, followed by the addition of the substrate. Pyridoxal phosphate was added to the control (without inhibitor) as well as to other reaction mixtures at the fifteenth minute. It was observed that the addition of pyridoxal phosphate resulted in the reversal of inhibition, γ -aminobutyric acid formation in all the reaction mixtures reaching the optimum level. These observations may serve as additional evidence for an earlier suggestion (Roberts, 1952) that the aldehyde group of pyridoxal phosphate may be the most likely point of attack of the inhibitor as well as of the substrate, the attachment of the latter taking place through the α -amino group, forming a Schiff's base.

Attempts to purify the enzyme

Glutamic acid decarboxylase has not yet been obtained in a purified state. Either acetone powders or crude extracts have been used directly by previous workers (Taylor & Gale, 1945; Roberts & Frankel, 1950, 1951). Attempts to purify the enzyme by fractional precipitation with organic solvents such as acetone and ethanol failed to give satisfactory recoveries of the enzyme. Freshly prepared acetone powder (300 mg.) was suspended in 15 ml. of 0.02M phosphate buffer, pH 8.0 (preparation A). The suspension was kept for 2 hr. at 30° with frequent stirring and the supernatant (12 ml.) obtained after centrifuging off the sediment was preserved (preparation B). Acetic acid (3%) was added slowly to the supernatant until the pH reached 5.5. A portion (6 ml.) of a suspension containing 10 mg. of alumina C_γ /ml. was added and the mixture was allowed to stand for 15 min. The adsorbent was centrifuged down and twice eluted with 6 ml. of M/15 phosphate buffer (pH 7.0) and the eluates were combined (preparation C). The combined eluates were precipitated with $(\text{NH}_4)_2\text{SO}_4$ (50 g./100 ml.), the pre-

cipitate was dissolved in 10 ml. of water and the salt concentration increased to 55% saturation. The precipitate thus obtained was dissolved in 10 ml. of water, the solution dialysed for 24 hr. in the cold and the volume made up to 15 ml. (preparation D). The activity of these preparations was determined as before, 40 μg . of pyridoxal phosphate being added in every case. The results are shown in Table 2.

Table 2. *Progress of purification of L-glutamic acid decarboxylase*

Activity is expressed as μg . of γ -aminobutyric acid formed/10 min. at 30°/ml. of enzyme preparation.

Stage of purification (see text)	Activity	Dry wt. (mg./ml.)	Activity/mg.	Degree of purification
A	1192	20.4	58.42	—
B	998	9.2	108.47	1.9
C	650	4.3	151.16	2.6
D	296	1.4	211.42	3.6

SUMMARY

1. Glutamic acid decarboxylase has been detected in a yeast, *Rhodotorula glutinis*, and its properties were studied by the application of circular-paper chromatography.

2. Decarboxylation was quantitative at pH 4.5 and 30°. The Michaelis constant was $2.75 \times 10^{-3} \text{M}$. Pyridoxal phosphate could restore the activity of dialysed preparations and reverse the inhibition caused by hydroxylamine.

3. Adsorption of the enzyme (precipitated by acidification of crude extracts) on alumina C_γ and fractional precipitation of the eluted enzyme with $(\text{NH}_4)_2\text{SO}_4$ have been carried out in an attempt to purify the enzyme.

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