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The Fate of ^{131}I-Labelled Homologous and Heterologous Thyroglobulins in the Rat, Dog, Monkey and Rabbit

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Iodine is readily fixed by the thyroid gland, where it is rapidly incorporated into thyroglobulin. If radioactive iodide is administered to an animal the gland may be damaged and total destruction can easily be accomplished. Thyroglobulin from the damaged tissue passes into the circulation, where its presence has been recognized by means of chromatographic and ultracentrifugal methods (Tong, Taurog & Chaikoff, 1952; Robbins & Rall, 1952; Brown & Jackson, 1954; Robbins, Peterman & Hall, 1954). Examination of the plasma of patients with thyroid carcinoma after treatment with large doses of ^{131}I (approx. 150–200 mc) has shown that a range of patterns of radioactive components occurs (Brown & Jackson, 1954). These appear to be related to a varying ability of different individuals to metabolize the thyroid protein liberated into the blood as a result of the destructive effects of the radioactive iodide. It seemed of interest therefore to study the metabolism of thyroglobulin in experimental animals. In the present paper, results are described from an examination of the fate of ^{131}I-labelled homologous and heterologous thyroglobulin in various species. A preliminary account of some of this work has already been published (Brown & Jackson, 1955).

EXPERIMENTAL

Preparation and administration of labelled thyroglobulin. At a suitable interval after the injection of carrier-free [^{131}I]iodide, animals were killed, the thyroid glands removed, ground with powdered glass with additions of ice-cold water and centrifuged. The precipitate, which invariably contained less than 5% of the total radioactivity of the gland, was discarded. The supernatant was stored at −20°. Over 99% of the radioactive iodine in these preparations was protein-bound, and paper-electrophoretic examination indicated the presence of a single radioactive component which, in the presence of plasma, was associated with the α-globulins.

Portions of the appropriate thyroglobulin solution containing 50–5000 μg. of protein/kg. body wt. were injected intravenously into animals and samples of venous blood obtained at suitable intervals. In rats, the tissue distribution of radioactivity after the administration of thyroglobulin was studied in groups of animals which were killed at various times. The tissues under investigation were rapidly removed and digested in LiOH solution (10 g. of LiOH, 1 g. of KI in 100 ml. of 20%, v/v, ethanol) and portions counted in a M6 liquid counter. Plasma and whole-blood samples (0·1 ml.) were dried on aluminium trays and their radioactivity was measured directly under a mica end-window counter.

Chromatography and fractionation procedures. Portions (10 μl.) of plasma, to which marker diiodotyrosine, triiodothyronine and thyroxine had been added, were analysed by ascending chromatography on Whatman no. 1 paper in butanol–dioxan–2N NH_3 (4:1:5, by vol.). The position of the marker substances was ascertained by developing with ninhydrin; the paper was then cut into small sections and the radioactivity measured under an end-window counter. In some instances, plasma samples were fractionated by a procedure which has already been described (Brown & Jackson, 1954). This involved the treatment of plasma with silver phosphate (hence estimation of iodide) and precipitation of the proteins with methanol (hence determination of the radioactivity present as thyroglobulin).

RESULTS

All the results described refer to ^{131}I-labelled thyroglobulin.

Metabolism of rat thyroglobulin

The clearance of this protein from the plasma of the rat, dog, monkey and rabbit that follows the intravenous administration of rat thyroglobulin is

shown in Fig. 1. In the rat and monkey the plasma thyroglobulin fell rapidly, whilst thyroxine and iodide appeared (Table 1). Injection of rat thyroglobulin into rats in which the thyroid gland had been ablated by treatment with ^{131}I some weeks before gave similar results. In the dog and rabbit, after an initial sharp drop, rat thyroglobulin disappeared more slowly, and only traces of radioactive metabolites were detected (Table 1). The removal of thyroglobulin from the circulation of all the species was independent of the amount of protein administered over a wide range; for example, essentially similar curves were obtained in the rat when amounts of radioactive protein ranging from that present in an entire rat gland down to 1% of this amount were injected. A second injection of rat thyroglobulin into the rabbit or dog led to its very rapid elimination from the plasma, presumably owing to the development of immunity. Further evidence of this is summarized in Table 2, where the change in distribution of radioactivity between plasma and cells in normal and immune animals is shown (cf. Almond, Francis, Hawkins & Wormall, 1954). Repeated injections of rat thyroglobulin into rats or monkeys, however, failed to increase the clearance rate of the protein from the blood. A preliminary study of the tissue distribution of radioactivity in rats after the injection of homologous thyroglobulin gave no indication of preferential localization.

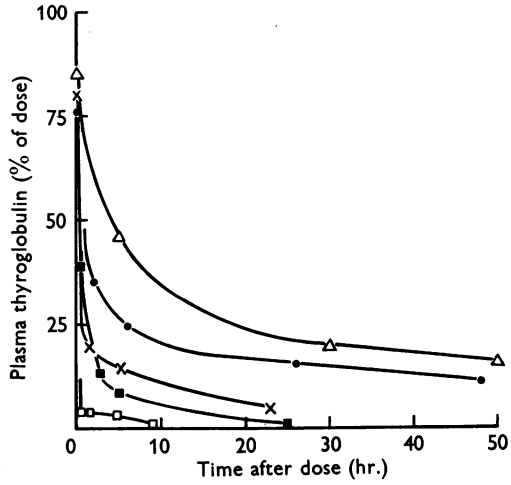

Fig. 1. Clearance of ^{131}I-labelled rat thyroglobulin from the plasma of the rat (×), dog (●), monkey (■) and rabbit (△) after one intravenous injection of the protein. The disappearance of rat thyroglobulin from the plasma of a dog previously immunized to this protein is also shown (□).

Metabolism of dog thyroglobulin

The disappearance of dog thyroglobulin from the plasma after intravenous injection into various species is shown in Fig. 2. Elimination of radioactivity was rapid in the dog, but was considerably slower in other species. The clearance of the protein in the dog was accompanied by the appearance of thyroxine, iodide and traces of iodotyrosines. The presence of iodinated tyrosine derivatives in the plasma is a further indication of the extra-thyroidal hydrolysis of homologous thyroglobulin since these amino-acids are not normally found in plasma. Apart from traces of thyroxine in the rat, no radioactive metabolites were detected after the in-

Table 1. *Distribution of radioactive components in the plasma of various species after the intravenous injection of rat thyroglobulin labelled with* ^{131}I

Recipient species	Time after injection (hr.)	Radioactive dose in plasma (%)	Distribution of radioactivity in plasma (%)			
			Iodide	Iodotyrosines	Thyroxine	Protein
Rat	1·5	20·4	3	1	1	95
	5·5	18·6	17	0	6	77
	23	5·8	6	0	18	76
	95	1·3	20	0	26	54
Monkey	0·16	39·2	0	0	1	99
	2·8	16·1	0	0	18	82
	5	11·1	5	0	19	76
	24	4·3	—	—	—	—
Dog	2	38·4	2	5	2	91
	6	27·2	4	3	3	90
	26	16·8	7	1	2	90
	48	12·0	5	2	2	91
	100	5·7	2	2	1	95
Rabbit	5	45·7	0	0	1	99
	30	19·3	0	0	1	99
	50	15·6	0	0	2	98
	74	10·5	0	0	2	98
	98	7·3	0	0	3	97
	122	3·9	—	—	—	—

Table 2. *Examples of the distribution of radioactivity between the plasma and cells in various species after the injection of* ^{131}I*-labelled thyroglobulins*

Thyroglobulin injected	Recipient species	Time after injection (hr.)	Radioactivity in plasma / Radioactivity in cells
Rat	Normal rabbit	4·5	3·3
		25	3·3
	Immune rabbit	3·5	0·82
		25	1·2
Rabbit	Normal monkey	1·5	5·9
		6·5	3·3
	Immune monkey	4·0	1·5
Dog	Normal rat	0·3	4·1
		2·0	4·4
		4·0	4·3
	Immune rat	0·5	1·5
		2·0	1·4
		4·0	1·7

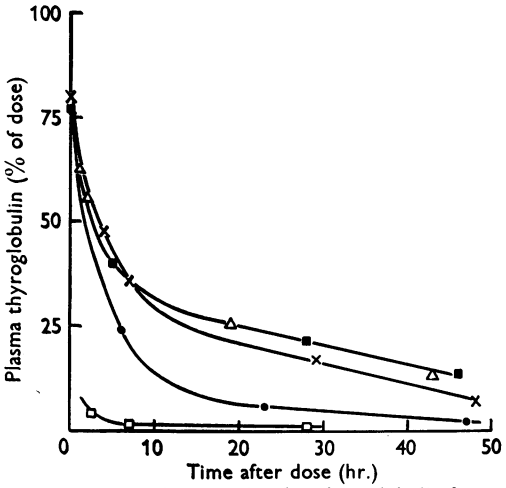

Fig. 2. Removal of radioactive dog thyroglobulin from the plasma of the dog (●), rat (×), monkey (■) and rabbit (△) after a single intravenous injection of the protein. A curve showing the response in the rats previously immunized to dog thyroglobulin is also shown (□).

jection of dog thyroglobulin into the other three species examined. This is emphasized by the constant percentage of protein present (Table 3).

Repeated injection of the protein into rats resulted in the development of immunity (Table 2 and Fig. 2). A comparison of the distribution of radioactivity in the tissues of normal and immune rats after the injection of labelled dog thyroglobulin revealed marked differences between the two groups (Table 4). The liver and spleen of the immune group contained considerably more radioactivity than the corresponding tissues of the normal animals. Similar observations were made in the rabbit by Almond *et al.* (1954) after the administration of foreign protein labelled *in vitro* with ^{131}I.

Metabolism of monkey thyroglobulin

Monkey thyroglobulin was rapidly metabolized in both monkey and rat, with the liberation into the plasma of iodide and thyroxine; it was more slowly removed from the plasma of the dog and rabbit (Fig. 3), with little evidence of breakdown products (Table 5). These results, taken in conjunction with those obtained with rat thyroglobulin, may indicate a structural resemblance between these two proteins.

Metabolism of rabbit thyroglobulin

In striking contrast to the fate of homologous thyroglobulins in the other species referred to above, rabbit thyroglobulin disappeared slowly from the blood of rabbits and radioactive metabolites were not detected (Fig. 4). To eliminate the possibility that the rabbit might show an unusual degree of specificity, even towards homologous thyroglobulin, the fate of thyroglobulin liberated from the thyroid gland by a destructive dose of ^{131}I was examined (Fig. 5). No radioactive metabolites were detected in the plasma, and the clearance of this thyroglobulin followed an exponential curve similar to that found in untreated rabbits; the mean half-life of the protein was about 55 hr. The injection of three successive doses (at weekly intervals) of rabbit thyroglobulin into rabbits failed to modify the shape of the curve, but this protein behaved like other heterologous thyroglobulins in the rat and monkey. A similar rate of clearance of rabbit thyroglobulin in the rat was

Table 3. *Distribution of radioactive components in the plasma of various species after intravenous injection of dog thyroglobulin*

Recipient species	Time after injection (hr.)	Radioactive dose in plasma (%)	Distribution of radioactivity in plasma (%)			
			Iodide	Iodotyrosines	Thyroxine	Protein
Dog	0·25	76·2	0	0	0	100
	6	30·0	10	7	3	80
	23	10·3	35	3	6	56
	47	5·6	57	0	7	36
	96	1·0	45	0	30	25
	125	1·2	60	0	22	18
	148	0·9	49	0	36	15
Rat	4	47·6	0	0	0	100
	7	36·4	0	0	2	98
	29	17·4	0	0	4	96
	48	7·4	1	0	3	96
Monkey	5	40·1	0	0	0	100
	28	21·6	0	0	0	100
	46	13·3	2	0	0	98
	123	4·1	—	—	—	—
Rabbit	1	62·5	0	0	0	100
	2	55·5	1	0	1	98
	19	25·5	1	0	1	98
	43	13·2	1	0	1	98
	93	4·5	0	0	0	100

Table 4. *Distribution of radioactivity in the tissues of normal and immune rats after the intravenous injection of radioactive dog thyroglobulin*

Time after injection (hr.)		Dose (%) in 1 g. of tissue / Dose (%) in 1 g. of blood			
		Liver	Spleen	Kidney	Lung
0·5	Normal	0·39	0·12	0·33	0·39
	Immune	4·62	2·45	1·02	0·83
2·0	Normal	0·26	0·15	0·26	0·33
	Immune	0·88	1·19	0·89	0·59
4·0	Normal	0·24	0·13	0·38	0·41
	Immune	0·93	0·59	1·07	0·45

Table 5. *Distribution of radioactive components in the plasma of various species after intravenous injection of monkey thyroglobulin*

Recipient species	Time after injection (hr.)	Radioactive dose in plasma (%)	Distribution of radioactivity in plasma (%)			
			Iodide	Iodotyrosines	Thyroxine	Protein
Monkey	2·5	37·0	5	2	3	90
	5	24·7	4	1	11	84
	24	5·4	20	0	23	57
Rat	6	15·1	6	17	4	73
	24	5·9	15	22	6	57
	50	1·6	—	—	—	—
Rabbit	2·5	35·0	7	0	0	93
	7	21·8	4	0	3	93
	25	12·7	5	0	5	90
	48	7·3	0	0	3	97
Dog	3·5	17·7	2	0	0	98
	23	11·6	2	0	0	98
	47	7·5	4	0	3	93

reported by Scott, Bostick, Shimkin & Hamilton (1949). An immune response was obtained in the monkey after three successive injections of rabbit thyroglobulin; the relation between the radioactivity in the plasma and cells after the first and fourth injection of the rabbit protein into the same monkey is shown in Table 2. However, four injections of rabbit thyroglobulin into rats did not induce immunity.

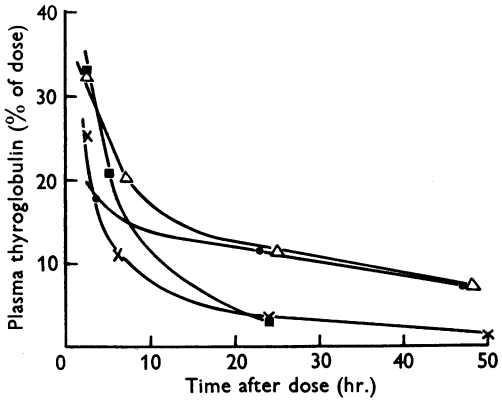

Fig. 3. Clearance of ^{131}I-labelled monkey thyroglobulin (injected intravenously) from the plasma of the monkey (■), rat (×), dog (●) and rabbit (△).

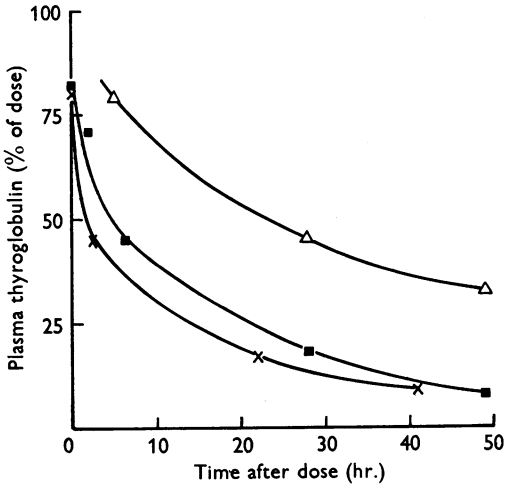

Fig. 4. Removal of intravenously administered ^{131}I-labelled rabbit thyroglobulin from the plasma of the monkey (■), rat (×) and rabbit (△). In the rabbit there is no initial fall in plasma radioactivity such as occurred in the other two species (see also Figs. 1–3).

DISCUSSION

Previous work has established that the destruction of thyroid tissue by the administration of ^{131}I to rats is soon followed by the appearance in the circulation of labelled thyroglobulin, which is then speedily removed (Brown & Jackson, 1954). It has now been shown that the homologous thyroglobulin is removed at a similar rate by both normal and thyroidectomized rats, so that this process is independent of the thyroid gland. Metabolites in the form of thyroxine and iodide (the latter probably arising from deiodination of iodotyrosines) appear in the plasma. Clearly an effective process exists for the specific removal and hydrolysis of homologous thyroglobulin. Administration of the protein over a wide dose range has shown that its

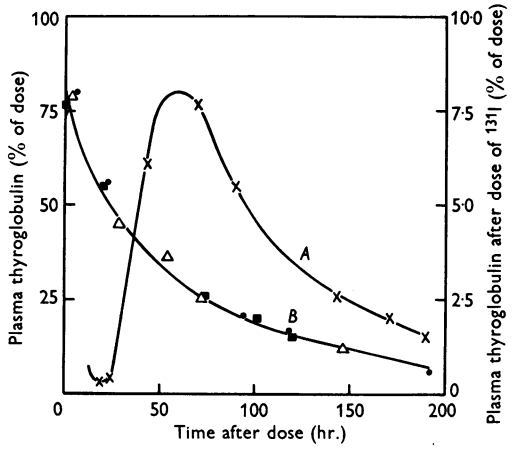

Fig. 5. Radioactive-protein content of the plasma of a rabbit (curve *A*) after a dose of ^{131}I sufficient to destroy its thyroid gland (20 mc). Beyond the peak the curve is identical with that obtained from two other rabbits (△, ●; curve *B*) given an intravenous injection of ^{131}I-labelled rabbit thyroglobulin. The filled squares (■) on curve *B* represent the corresponding points on curve *A*, transposed by modifying the abscissa.

rate of elimination from plasma is independent of the amount injected, and preliminary experiments reveal that liver and splenic tissue can hydrolyse thyroglobulin *in vitro* with the formation of thyroxine and iodotyrosines. It seemed not unreasonable, therefore, to reinvestigate the possibility that thyroglobulin itself might be secreted normally by the gland and then hydrolysed to form thyroxine in the extra-thyroidal tissues. The nature of the circulating thyroid hormone has been a subject for speculation for many years. Hektoen, Carlson & Schulhof (1923), using immunological methods, obtained evidence for the presence of thyroglobulin in the thyroid lymphatics and veins of the goitrous dog; but Lerman (1940), applying similar techniques to man, failed to obtain unequivocal evidence for the presence of thyroglobulin in the thyroid veins under normal

conditions, although trauma to the gland during operation was followed by leakage of the thyroid protein into the circulation. The current view is that thyroxine is secreted as such by the thyroid gland and that thyroglobulin or derived peptides have no physiological role outside the gland (Roche & Michel, 1954).

The possibility that homologous thyroglobulin might normally be metabolized in the extra-thyroidal tissues seemed to be strengthened by our observation that rats were unable to metabolize the thyroglobulins of the dog and rabbit, although they rapidly hydrolysed rat thyroglobulins. In fact, rats gave a typically immune response to dog thyroglobulin after two successive injections of this protein (Fig. 2). Similarly, in the dog, homologous thyroglobulin was rapidly degraded into iodide, iodotyrosines and thyroxine, but no such breakdown occurred after administration of the thyroid protein from the rat, rabbit or monkey.

However, there are two objections to the hypothesis that homologous thyroglobulin is normally secreted by the gland. In the first place, the behaviour of monkey thyroglobulin in the rat and of rat thyroglobulin in the monkey was indistinguishable from that of the homologous proteins. A more serious objection is the inability of the rabbit to degrade its own thyroglobulin in the same way that homologous thyroid protein is metabolized in the rat, dog and monkey, especially as the administration of a tracer dose of ^{131}I to the rabbit is followed by the appearance of labelled thyroxine in the plasma. The persistence of homologous thyroglobulin in rabbit plasma without the appearance of metabolites is very similar to the behaviour of heterologous thyroglobulin on first injection into other species, with the exception of the rat–monkey relationship referred to above.

The possibility that a high degree of individual specificity exists towards homologous thyroglobulin in rabbits seems very unlikely, since thyroid protein released into the blood after a destructive dose of ^{131}I disappeared in precisely the same manner as the injected material in other rabbits (Fig. 5). It is interesting that this result parallels that obtained from a patient with carcinoma thyroid after a therapeutic dose of ^{131}I (Brown & Jackson, 1954, case 3). This patient differed from many others in being unable to hydrolyse his own thyroglobulin, which disappeared slowly from the plasma without the formation of thyroxine.

After the first injection of homologous or heterologous thyroglobulins into the rat, dog and monkey (Figs. 1–4) a precipitous fall in the plasma radioactivity occurs during the first 1–2 hr. Three factors are probably involved in this phase: the fixation of radioactive material by erythrocytes (about 20%); the removal of thyroglobulin by metabolism; and, finally, its diffusion into the extravascular space in association with the normal plasma proteins. Paper electrophoresis has shown that thyroglobulin moves with the α-globulin fraction of plasma, and the penetration of the plasma proteins into the extravascular spaces has been demonstrated in the rabbit (Humphrey & McFarlane, 1954). Curiously enough, rabbit thyroglobulin behaves quite differently from that of the rat, dog and monkey. There is neither an initial rapid fall in the blood level nor evidence of hydrolytic breakdown in the rabbit, so that after 5 hr. most of the radioactive protein is still present in the blood (Fig. 4). This behaviour suggests that, in spite of its intimate association with the plasma proteins, rabbit thyroglobulin does not accompany these into the extravascular space. The failure of rabbit thyroglobulin to be metabolized in the rabbit might be accounted for in this way. It is established, however, that rabbit thyroglobulin leaves the plasma steadily with a half-life of about 55 hr., which is considerably shorter than that found for ^{14}C-labelled globulin in the rabbit (Humphrey & McFarlane, 1954).

Repeated injections of homologous thyroglobulin into rabbits failed to modify its rate of removal from the plasma, i.e. no immune type of response was induced. This was not so when heterologous thyroglobulin was used in these animals, for an immune reaction was readily elicited; similar responses to heterologous thyroglobulins occurred in the other species examined, apart from the rat–monkey relationship to which reference was made above. An alteration in the distribution of radioactivity between the plasma and cells always accompanied the immune response to a foreign thyroglobulin (Table 2); this change was found by Almond *et al.* (1954) to be associated with antigen–antibody reactions in the blood. Differences in tissue distribution of radioactive material when dog thyroglobulin is administered to normal and immune rats (Table 4) also provide additional evidence that the response is a true antigen–antibody reaction (compare Almond *et al.* 1954). This is in agreement with the conclusions of Hektoen *et al.* (1923), Stokinger & Heidelberger (1937) and Lerman (1940) that some thyroglobulins can act as antigens.

Gaddum (1929–30), Schulhof (1930) and Clutton, Harington & Yuill (1938) showed that injected ox, human or dog thyroglobulin was able to increase the metabolic rate of rats. Our results, however, suggest that if hydrolysis of the protein into thyroxine is a prerequisite for its action, thyroglobulin from the rat or monkey, but not that from the dog or rabbit, should be capable of increasing the metabolic rate of rats. Experiments designed to test the relative efficiencies of homologous and

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 0·02 M 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 0·1 M L-glutamic acid solution, 0·8 ml. of M/15 acetate buffer,