Intrinsic instability of di-iodotyrosine (see Fig. 1)

Quantities of 470 mg. (1 mmol.) of di-iodotyrosine dihydrate were dissolved rapidly by boiling in 25 ml. water containing the requisite amount of HCl or NaOH to give the desired pH. The solutions were then heated for 1 hr. on a boiling water-bath, cooled, treated with 12.5N-NaOH to make a final concentration of N and extracted once with 25 ml. of butanol. The aqueous layer was separated quantitatively and submitted to a brief vacuum distillation to remove butanol; it was then transferred to a boiling tube, being made up to 30 ml. with washings, and acidified when hot with 3 ml. glacial acetic acid.

At the end of the period of heating a great difference in the behaviour of di-iodotyrosine at the various pH values was already apparent; the solutions in the pH range 6-9 became dark brown, and at the stage of acidification threw down a dark-coloured amorphous precipitate which was removed by filtration before crystallization of residual di-iodotyrosine set in. In all cases crystallization was completed by keeping in the ice-chest overnight; the residues of unchanged di-iodotyrosine were then collected, dried *in vacuo* over P_2O_5 and weighed.

SUMMARY

1. The work of von Mutzenbecher (1939) on the formation of thyroxine by incubation of di-iodo-

tyrosine in alkaline solution is confirmed. It is shown that this conversion is prevented by exclusion of atmospheric oxygen or by addition of thiosulphate to the solution; the reaction is also profoundly influenced by the pH during incubation.

2. The effect of added oxidizing agents on the conversion of di-iodotyrosine to thyroxine has been studied; with the aid of hydrogen peroxide increased yields of thyroxine can be obtained in a much shorter time, the effect of pH being similar to that observed on simple aerobic incubation. The conversion can also be accelerated by addition of iodine. Reasons are given for supposing that in all cases observed the actual agent effective in bringing about the oxidation of di-iodotyrosine to thyroxine is free iodine, and attention is called to the biological implications of this hypothesis.

3. The thyroxine obtained by oxidation of *l*(+)-di-iodotyrosine, itself derived from *l*(-)-tyrosine, is shown to have an optical rotation identical with that of the natural product; this provides direct chemical proof of the configurative relationship of natural tyrosine and natural thyroxine.

4. Di-iodotyrosine in aqueous solution has been found to exhibit a remarkable degree of instability towards heat over the pH range 4-9; at higher acidity or alkalinity the amino-acid is comparatively stable.

REFERENCES

- Abderhalden, E. & Guggenheim, M. (1908). *Ber. deutsch. chem. Ges.* **41**, 1238.
 Barkdoll, A. E. & Ross, W. F. (1944). *J. Amer. chem. Soc.* **66**, 898.
 Block, P., Jr. (1940). *J. biol. Chem.* **135**, 51.
 Block, P., Jr. & Powell, G. (1943). *J. Amer. chem. Soc.* **65**, 1430.
 Foster, G. L., Palmer, W. W. & Leland, J. P. (1936). *J. biol. Chem.* **115**, 467.
 Harrington, C. R. (1928). *Biochem. J.* **22**, 1429.
 Harrington, C. R. (1944). *J. chem. Soc.* p. 193.
 Harrington, C. R. & Barger, G. (1927). *Biochem. J.* **21**, 169.
 Harrington, C. R. & Neuberger, A. (1936). *Biochem. J.* **30**, 809.
 Johnson, T. B. & Tewkesbury, L. B. (1942). *Proc. nat. Acad. Sci., Wash.*, **28**, 73.
 Keston, A. S. (1944). *J. biol. Chem.* **153**, 335.
 Leland, J. P. & Foster, G. L. (1932). *J. biol. Chem.* **95**, 165.
 Ludwig, W. & von Mutzenbecher, P. (1939). *Hoppe-Seyl. Z.* **258**, 195.
 von Mutzenbecher, P. (1939). *Hoppe-Seyl. Z.* **261**, 253.
 Neuberger, A. (1934). *Biochem. J.* **28**, 1982.
 Pummerer, R., Melamed, D. & Puttfarcken, H. (1922). *Ber. deutsch. chem. Ges.* **55**, 3116.
 Pummerer, R., Puttfarcken, H. & Schopflocher, P. (1925). *Ber. deutsch. chem. Ges.* **58**, 1808.
 Winnek, P. S. & Schmidt, C. L. A. (1935). *J. gen. Physiol.* **18**, 892.

(An Addendum has been added on p. 212.)

Note on the Nutritive Value of the Nitrogenous Substances contained in Dried Yeast (*Torulopsis lipofera*)

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The yeast was a dried specimen of *Torulopsis lipofera*, a species having a high fat content, provided by Dr A. Kleinzeller (1944). Its composition was: moisture less than 1, fat 18.6, nitrogen 5.85, 'crude' protein (N $\times$ 6) 35.1%. Analyses by Stutzer's

method showed that about 10% of the nitrogen was non-protein in character.

The following experiments were designed to show the nutritive value of the nitrogenous substances, protein and non-protein, contained in the yeast, as

measured by their capacity to support growth in young rats, and the supplementary value, if any, possessed by these nitrogenous substances for the proteins contained in wheat flour.

EXPERIMENTAL

The young rats (Lister Institute strain of pied rats) of average weight 52–54 g. received the experimental diets a few days after weaning. The diets were arranged to be adequate in all respects except for their content of protein, which was suboptimal and was the factor limiting growth. The rate of weight increase of litter-mates was compared on the following three diets, all of which contained about 2·1 % nitrogen or 12–13 % crude protein (calculated on a dry-weight basis). The nitrogen in diet 1 was derived entirely from whole-wheat flour (representing 100 % of the grain); of that in diet 2 about three-quarters was derived from whole-wheat flour and about one-quarter from the yeast; that in diet 3 was derived entirely from the yeast. The composition of the diets is shown in Table 1. Diet 1 contained 7 parts of fat as hardened arachis oil, diet 2 only

Table 1. Composition of the diets used

	Parts by weight		
	Diet 1	Diet 2	Diet 3
Whole-wheat flour	107	80	—
Yeast	—	10·3	38
Pure maize starch	—	17	69
Hardened arachis oil	7	5·1*	—†
Salt mixture	2	2	2
(McCollum no. 185)			
Cod-liver oil	1	1	1
Average N content	2·14	2·12	2·08
Average protein content‡	12·8	12·7	12·5
(% of dry matter)			

* 1·9 parts of fat supplied by the yeast.
† 7·1 parts of fat supplied by the yeast.
‡ N × 6.

5·1 parts and diet 3 none, to make allowance for the fat supplied by the yeast in these two diets. Each diet contained 2 parts of McCollum's no. 185 salt mixture to correct any mineral deficiency and 1 part of cod-liver oil to provide vitamins A and D. A daily ration of 1 ml. of a protein-free yeast extract (equivalent to 0·5 g. dried brewers' yeast) was given separately to each rat to provide an excess of B vitamins.

The tests lasted for 6 weeks. During the fourth and fifth weeks of each experiment, measurements were made of the intake of food and of the weight of faeces passed and of their nitrogen content. From these results calculations could be made of the coefficient of (apparent) digestibility of the protein in each diet, as well as of the ratio of body-weight increase to g. protein eaten, the ratio adopted by Osborne, Mendel & Ferry (1919) to express nutritive value of protein. These figures are set out in Table 2.

RESULTS

At the end of 6 weeks, the rats receiving the wheat-flour diet 1 reached an average body weight of 127 g., while the average weight of those on the

Table 2. Protein utilization by rats receiving diets 1, 2 and 3

Diet	Source of protein	Protein* content of diet (% of dry matter)	Average weekly†										Coeffi- cient‡ of digesti- bility of N	Growth- eaten/unit promoting value§ of pro- tein	Food eaten/unit weight increase (g. dry matter/g.)
			No. of rats	Mean body weight (g.)		Weight increase (g.)	Food intake (g. of dry matter)	N intake (g.)	Protein* intake (g.)	Faeces passed (g. of dry matter)	N in faeces (g.)	N digested (g.)			
				Initial	Final										
1	Whole-wheat flour	12·8	5	52	127	11·8	64·6	1·37	8·22	6·70	0·26	1·11	81·1	1·44	5·5
2	75% from whole-wheat flour; 25% from yeast	12·7	6	54	133	13·2	64·9	1·38	8·28	6·79	0·29	1·08	78·6	1·59	4·9
3	Yeast	12·5	5	54	85	5·3	45·7	0·94	5·64	5·53	0·21	0·74	78·0	0·94	8·6

* N × 6.

† Amounts per rat, from estimations made during weeks 4 and 5.

‡ $\frac{\text{Nitrogen intake} - \text{nitrogen in faeces}}{\text{Nitrogen intake}}$.

§ g. wt. increase/g. protein eaten (Osborne *et al.* 1919).

† Amounts per rat, from estimations made during weeks 4 and 5.
§ g. wt. increase/g. protein eaten (Osborne *et al.* 1919).

* N × 6.
† 100 (Nitrogen intake—nitrogen in faeces) / Nitrogen intake

yeast diet 3 was only 85 g.; those on diet 2, in which yeast and whole-wheat flour were combined, were slightly heavier than those on diet 1, the average body weight being 133 g.

The rats receiving diets 1 and 2 ate more food than those on diet 3 and made better use of it; the intake of dry food corresponding to 1 g. increase in body weight was respectively 5.5, 4.9 and 8.6 g. The nutritive values of the proteins in the three diets, measured by the weight increase per g. protein eaten (Osborne *et al.* 1919) were in a corresponding order, the figures being respectively, 1.44, 1.59 and 0.94. The digestibility coefficient calculated for the proteins in the three diets 81, 79 and 78, did not differ materially from one another.

These figures show that, whereas the proteins contained in the yeast were much less efficient for support of growth of young rats than those contained in whole wheat, yet a mixture of the two in

Fig. 1. Average growth curves of 5-6 young rats receiving: ----- diet 1, protein derived from whole-wheat flour; ——— diet 2, 75 % of protein derived from whole-wheat flour, and 25 % from yeast; diet 3, protein derived from yeast. All diets contained about 13 % 'crude' protein (on a dry-weight basis).

a diet, in the proportion of 1 part of yeast proteins to 3 parts of wheat proteins, had a nutritive value as great as, possibly slightly greater than, that of whole wheat (see Fig. 1). In other words, the mixture of amino-acids in the proteins of the yeast had a marked supplementary effect for those contained in whole wheat.

This result confirms the results of many previous workers who have found that yeast proteins, though possessing a relatively inferior nutritive value when given as sole source of nitrogen in a diet (Still & Koch, 1928; Klose & Tevold, 1944), yet, when combined with those of cereals, form a mixture which enhances the value of the latter (Light & Frey, 1943; Macrae, El Sadr & Sellers, 1942; Kon & Markuse, 1931).

Klose & Tevold (1944) traced the nutritive defect in yeast proteins to a deficiency of the essential amino-acid methionine. A few years earlier, Daft, Sebrell & Lillie (1941) had shown that the necrosis of the liver in rats fed on diets containing only 4 % casein could be prevented by increasing the proportion of casein to 30 % of the diet or the methionine content of the diet by an amount (0.7 %) corresponding to that of the additional casein. Recently, Himsworth & Glynn (1944 *a, b*) have found that liver necrosis occurs in rats if the protein of the diet is derived from bakers' yeast (*Saccharomyces cerevisiae*), and the content of 'crude' protein is 7 % or even higher. This necrosis could be prevented by adding 8 % of casein, or the equivalent amount of methionine, to the diet.

We also have observed severe necrosis and fatty degeneration of the liver of rats fed on diets containing 4-6 % casein as source of protein but, in the present series of experiments, no histological lesions were found in the liver of a rat which had been fed on diet 3 for 40 days. After 80 days there was apparent a certain degree of degeneration, limited to the cells adjacent to the portal areas; the degree of abnormality was, however, slight. In diet 3 the proportion of protein in the diet, 13 % of the dry weight, was relatively high, and it is also possible that the proteins of *Torulopsis lipofera* are less deficient in methionine than those of the species of yeast used by Himsworth & Glynn.

The proteins contained in wheat and other cereals, compared with those of yeast, are relatively rich in the sulphur-containing amino-acids methionine and cystine, and poor in lysine, an amino-acid essential for growth. The proteins of brewers' yeast, and presumably of other types of yeast also, while relatively poor in methionine, are well supplied with lysine. These facts offer an explanation of the complementary action shown by combinations of these two classes of proteins for maintenance of growth in young animals.

SUMMARY

1. The nitrogenous substances in the yeast *Torulopsis lipofera*, when employed as sole source of nitrogen in an otherwise complete diet, supported growth of young rats at a rate much inferior to that of litter-mates on a diet containing the same proportion of nitrogen derived from whole-wheat flour.

2. When, however, one-quarter of the nitrogen in the diet was derived from the yeast and three-quarters from whole-wheat flour, the rate of weight increase and utilization of nitrogen was slightly greater than when all the nitrogen was obtained from the whole-wheat flour.

3. This complementary action is presumably due to the relatively high proportion in yeast protein of

lysine, an amino-acid essential for growth, which is present in cereal proteins in relatively low amount.

We wish to thank Sir Charles Martin, who kindly examined the histological specimens, and Mr T. C. Fletcher, who kindly prepared them. Our thanks are also due to Mr G. W. Flynn for his help in the preparation of the diets and in the care of the experimental animals.

REFERENCES

- Daft, F. S., Sebrell, W. H. & Lillie, R. D. (1941). *Proc. Soc. exp. Biol., N.Y.*, **48**, 228.
 Himsforth, H. P. & Glynn, L. E. (1944a). *Clin. Sci.* **5**, 93.
 Himsforth, H. P. & Glynn, L. E. (1944b). *Clin. Sci.* **5**, 133.
 Kleinzeller, A. (1944). *Biochem. J.* **38**, 480.
 Klose, A. A. & Tevold, M. L. (1944). *Proc. Soc. exp. Biol., N.Y.*, **56**, 98.
 Kon, S. K. & Markuse, Z. (1931). *Biochem. J.* **25**, 1476.
 Light, R. F. & Frey, C. N. (1943). *Cereal Chem.* **20**, 645.
 Macrae, T. F., El Sadr, M. M. & Sellers, K. C. (1942). *Biochem. J.* **36**, 4604.
 Osborne, T. B., Mendel, L. B. & Ferry, E. L. (1919). *J. biol. Chem.* **37**, 223.
 Still, E. U. & Koch, F. C. (1928). *Amer. J. Physiol.* **87**, 225.

The Mechanism of Polysaccharide Production from Sucrose. 2

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Different polysaccharides can be produced from either α -glucose-1-phosphate or sucrose by the action of cell-free enzymes. From α -glucose-1-phosphate there have been obtained amylose, amylopectin, and glycogen (Hanes, 1940; Hassid, Cori & McReady, 1943; Haworth, Peat & Browne, 1944; Cori & Cori, 1943), whereas from sucrose the formation of a glucosan, dextran (Hehre & Sugg, 1942; Hehre, 1943) and a fructosan, levan (Hestrin, Avineri-Shapiro & Aschner, 1943), have been reported.

There is a structural similarity between the two substrates which are employed for these polymerizations. The relation of the substrates has been emphasized further by the recent demonstration that α -glucose-1-phosphate and fructose enter into dynamic equilibrium with sucrose and phosphoric acid in the presence of an enzyme which has been isolated from a suitable bacterial species (Doudoroff, 1943; Hassid, Doudoroff & Barker, 1944; Doudoroff, Hassid & Barker, 1944). The known syntheses of different polysaccharides can, in the light of these observations, be fitted into a single scheme:

The group name *polymero-synthase* provides us with a generic description of the enzyme entities which mediate the polymeric steps of the scheme

presented above. The elements of water are not included in the balance sheet of the listed reactions. The action of the *polymero-synthases* on their substrates can therefore be described as a *direct polymeric degradation* (Leibowitz & Hestrin, 1945). *Levansucrase* is the name which has been given to the polymero-synthase which degrades suitable aldoso-fructosides (sucrose, raffinose) polymerically into aldose (glucose, melibiose) and levan.

Levansucrase has been obtained in cell-free solution in concentrations suitable for kinetic studies from an organism which has been designated as *Aerobacter levanicum* (Hestrin *et al.* 1943).

Our levansucrase preparation catalyzes the following transformation (Hestrin & Avineri-Shapiro, 1943, 1944):

m and n being molar quantities. The action of the preparation is thus two-fold, hydrolytic and polymeric. Elsewhere evidence has been advanced which shows that the polymeric reaction (m -term of equation (1)) is not a sequence of the hydrolytic reaction (n -term of equation (1)) (Hestrin, 1944). The present paper defines the conditions of pH, substrate concentration, enzyme concentration and reaction time in which levansucrase acts with maximum efficiency in terms of production of polymer.

It is to be assumed that information concerning