SUMMARY

1. Pyridoxal can be oxidized by liver aldehyde oxidase.

2. The oxidation is competitively inhibited by antabuse.

3. The end product of the reaction has been isolated and identified as pyridoxic acid.

The authors have pleasure in expressing their sincere thanks to Dr H. M. Kalckar, who made it possible for them to carry out this work, and whose unfailing help and suggestions were a constant source of encouragement. Their thanks are also due to the Carlsberg Fond, Nordisk Insulin Fond and Kong Christian Xendes Fond for financial support in this work. They are indebted to Dr F. Carpenter and Dr R. Bonnichsen of the Medicinska Nobelinstitutet, Stockholm, for a generous gift of horse-liver aldehyde oxidase, to Dr E. Jacobsen and Dr J. Hald, Medicinalco Ltd., Copenhagen, for antabuse, and to Dr P. Möller, Institute of Biochemistry, Copenhagen, for pyridoxic acid.

REFERENCES

Gordon, A. H., Green, D. E. & Subramanyan, V. (1940). *Biochem. J.* **34**, 764.
Hald, J. & Jacobsen, E. (1948). *Acta Pharmacol.* **4**, 305.
Huff, I. W. & Perlzweig, W. A. (1944). *J. biol. Chem.* **155**, 345.
Kalckar, H. M., Kjeldgaard, N. O. & Klenow, H. (1950). *Biochim. Biophys. Acta*, **5**, 586.
Kjeldgaard, N. O. (1949). *Acta Pharmacol.* **5**, 397.
Knox, W. E. (1946). *J. biol. Chem.* **163**, 699.

The Amino-Acid Composition of Two Yeasts used to Produce Massive Dietetic Liver Necrosis in Rats

BY O. LINDAN AND ELIZABETH WORK
Medical Unit and Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

(*Received* 7 *July* 1950)

Although yeast consists mainly of protein and is easy and cheap to produce in bulk, it is not a good source of dietary protein. For example, Hock & Fink (1943*a*, *b*) showed that when low-protein diets containing different types of yeasts as the main source of protein were fed to young rats, the animals failed to grow normally and after some time died from acute liver lesions consisting of extensive necrosis and haemorrhages. The yeasts varied both in their growth-promoting properties and in their tendency to produce liver lesions (necrogenic properties); there appeared to be some relation between the sulphur/nitrogen ratio of the yeasts and their necrogenic properties, the more necrogenic diets having lower proportions of sulphur. Liver necrosis in rats has also been produced by feeding diets containing suboptimal amounts of other proteins, such as casein or soya-bean meal, as the main source of protein. The lesions appear to be caused by a condition similar to that occurring in rats fed the yeast diets, since development of necrosis on all these diets could be prevented by extra cystine or methionine (Weichselbaum, 1935; Hock & Fink, 1943*a*; Matet, Matet & Fridenson, 1947; Himsworth & Glynn, 1944*b*; György & Goldblatt, 1949).

Necrosis has been produced in this laboratory by using low-protein diets containing baker's yeast as the sole source of protein (Himsworth & Glynn, 1944*a*). Similar diets containing brewer's yeast have been shown by György to be considerably less necrogenic (György, 1948; György & Goldblatt, 1949). Since it seemed probable that the difference in types of yeast used in the diets accounted for the different results obtained in the two laboratories, samples of yeast were exchanged in order to compare their chemical composition and their nutritional properties for the same strain of rat.

The chemical investigation was confined to the amino-acid fractions, particularly sulphur-containing amino-acids. Tocopherol deficiency is another factor involved in the production of necrosis (Schwarz, 1944; Himsworth & Lindan, 1949), but since tocopherol is known to be absent from all types of yeasts (György, 1948, private communication) it could not have accounted for the differences observed. This paper describes the results of investigations on the nitrogen, sulphur and amino-acid contents of our baker's yeast and György's brewer's yeast. Because of the possibility that the extractable and therefore immediately available amino-acids and peptides might be of importance in the nutritional properties of the yeasts, separate investigations were made on the amino-acids present in the cell residues insoluble in 75% ethanol and on

the extractable amino-acids and peptides. The comparison of the nutritional properties of the two yeasts is reported in the following paper (Lindan & Work, 1951).

EXPERIMENTAL

Materials. The following yeasts were analysed: dried baker's yeast (DCL no. *GB* 161) marketed by Distiller's Co. Ltd. London, and dried brewer's yeast (Fleischmann's type 50*B*) marketed by Standard Brands Inc., U.S.A., provided by Dr P. György. All our analyses were carried out on the same bulk samples of each yeast.

Elementary analysis. N was estimated by the standard micro-Kjeldahl method. Total S estimations were carried out after digestion with HNO_3 and $HClO_4$ according to Masters (1939); sulphate S was determined on material hydrolysed for 10 hr. with 6N-HCl. All estimations were carried out in duplicate on 2 g. samples, to avoid sampling errors due to inhomogeneity of material.

Amino-acid analysis. Most of the amino-acid assays, with the exception of cystine and methionine, were carried out by paper chromatography of yeast fractions prepared as follows: the yeast, finely powdered by grinding and sieving if necessary, was extracted with 75% (v/v) ethanol (10 ml./g. of dried yeast) for 24 hr. with shaking in a centrifuge tube. The ethanol was removed by centrifugation and replaced by an equal volume of fresh solvent; the process was repeated three times. The progress of extraction was followed by estimation of total N in the separate extracts.

Since the ethanol extracts could not be used directly for paper chromatography owing to interference from extracted substances other than amino-acids, the method of Awapara (1948) for preparing tissue extracts was used. The ethanol extracts were combined and shaken with 3 vol. of $CHCl_3$. The clear aqueous supernatant phase, which separated from the bulky ethanol-$CHCl_3$ phase on standing or after centrifugation, was concentrated *in vacuo* to a total N concentration of 2·5 mg./ml. This solution, which contained the bulk of the extractable amino-acids of the yeast, will be referred to as 'ethanol extract'.

Fractions of the ethanol extract and the ethanol-extracted yeast cell residues were hydrolysed with 6N-HCl at 105° for 24 hr. HCl was removed *in vacuo* and water added to the residue to give total N concentrations of 5 mg./ml.

The basic technique of paper chromatography was as described by Consden, Gordon & Martin (1944) and Dent (1948), with two-dimensional chromatograms run first in phenol (NH_3 atmosphere) and then in collidine-lutidine as the organic solvents. A modification of Dent's box for running chromatograms was used (Fig. 1); this allows six or eight chromatograms to be run from a single trough by suitable spacing of three or four glass rods on either side of the trough. The point of application (*X* in Fig. 1) of the solution to the paper square must be below the level of the glass rod when the papers are hanging from the trough.

The estimation consisted of finding the amount of amino-acid which gave on a chromatogram a spot of similar size and colour intensity to the corresponding spot obtained from the unknown solution when run under similar conditions (Dent, 1948; Work, 1949). Because of the occurrence of unpredictable variations in chromatograms, even in those run simultaneously, the spots were compared by eye, the accuracy of the method being limited by reproducibility. The error was reduced to $\pm 30\%$ by using the following technique: each pair of spots (standard and unknown) was compared at three intensities, the lowest being the minimum detectable amount and the others twice and four times this amount. The minimum detectable amounts were of the same order as those given by Pratt & Auclair (1948). Owing to wide differences in concentrations of individual amino-acids in the yeasts, amounts of yeast solutions were applied corresponding to levels of total N between 1·3 and 200 μg., ascending in geometrical progression. Repeated comparisons at each level were carried out.

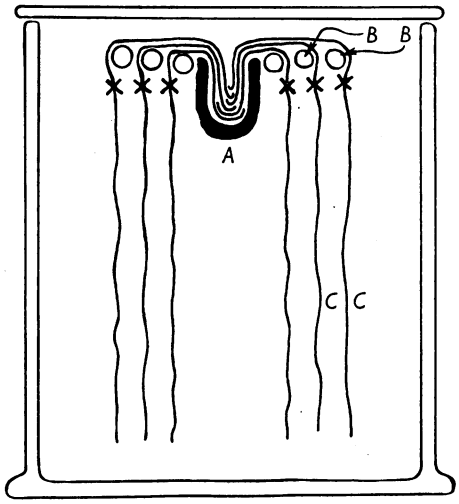

Fig. 1. Sectional diagram of arrangement for running multiple squares. *A*, trough with solvent; *B*, glass rods; *C*, filter-paper square; *X*, point of application of solution for chromatography.

While the majority of amino-acids were examined on two-dimensional chromatograms in the standard way, special techniques were adopted for certain of them. Tyrosine and histidine were estimated after running only in collidine-lutidine, the spots being developed with the Pauly reagent (Dent, 1948). Isoleucine and leucine were separated by using *tert.*-amyl alcohol in the presence of diethylamine vapour (Work, 1949); ornithine, which joins the lysine spot on two-dimensional chromatograms, was separated from lysine by running in the phenol direction in an atmosphere of acetic acid instead of NH_3 and was distinguished from hydroxylysine by its stability to periodate. Methionine and cystine were detected as methionine sulphone and cysteic acid respectively, after oxidation of the solution with H_2O_2 using NH_4^+ molybdate as catalyst (Dent, 1948); methionine and cystine could not be estimated quantitatively because of variable losses, probably caused by peroxide, and were therefore determined gravimetrically. Cystine S was estimated on 2 g. samples by the method of Lugg (1938); methionine S was calculated from the difference between total organic S and cystine S.

Glutathione analysis. The identification of glutathione on paper chromatograms was complicated by the fact that it formed three spots (see Results). These were examined separately by cutting out and eluting (Work, 1949) and the eluates were hydrolysed with 6N-HCl and examined for amino-acids. Although after H_2O_2 oxidation of glutathione

there was only one spot, the method could not be used for estimation, for the same reason as in the case of methionine and cystine. Iodometric titration was therefore used, reduced glutathione being estimated in ethanol-salicylosulphonic acid extracts of whole yeast (Astrup & Øhlenschläger, 1948). Electrolytic reduction of these extracts as described by Dohan & Woodward (1939), followed by iodometric titration, gave the level of total glutathione (oxidized and reduced). The glutathione contents of the ethanol extracts of yeast used for chromatography were also estimated by titration after electrolytic reduction.

RESULTS

Nitrogen distribution in yeast fractions

The nitrogen contents of successive 75% ethanol extracts of baker's yeast are shown in Table 1; four extractions were considered sufficient to remove practically all the soluble nitrogen from the yeast. The figures for the aqueous phase after chloroform treatment of the combined extracts indicate that a threefold concentration was achieved by this process with loss of only 13% of the nitrogen; this nitrogen was probably contained in substances other than amino-acids, since no amino-acids could be detected by chromatography of the acid-hydrolysed contents of the whole chloroform phase from 10 g. of yeast.

Table 1. *Nitrogen contents of successive 75% (v/v) ethanolic extracts from baker's yeast*

(10 g. yeast, 100 ml. 75% (v/v) ethanol in each extraction.)

Extract no.	Volume (ml.)	N content of extract (mg. N/ml.)	N content of extract Percentage of extractable N
1	85	0·682	59·0
2	85	0·284	24·5
3	83	0·113	9·5
4	80	0·086	7·0
Combined	333	0·296	100
Aqueous phase after treatment of combined extracts with 3 vol. of $CHCl_3$	92	0·940	87

Table 2. *Nitrogen distribution in the two yeasts*

	Baker's yeast	Brewer's yeast
Whole yeast (g. N/100 g. yeast)	7·35	8·40
Wt. lost after ethanol extraction (g./100 g. yeast)	28·0	22·0
75% ethanol extract, untreated (g. N/100 g. total N of yeast)	13·5	17·7
75% ethanol extract, 'aqueous phase' after chloroform treatment (g. N/100 g. total N of yeast)	11·7	16·1

Table 2 shows the nitrogen contents of the yeasts and of the various fractions used for chromatography.

Chromatographic estimation of amino-acids

The results of the estimations of amino-acids in the ethanol extracts and in the insoluble residues of the yeasts are shown in Table 3. The figures for whole yeasts were mostly not obtained directly, but were calculated by summing the contents of the hydrolysed ethanol extracts and the insoluble residues.

Diagrammatic representations of typical chromatograms from each fraction are shown in Fig. 2; the level of nitrogen, representing the amount of solution applied to the chromatogram, being chosen to illustrate most clearly the characteristic picture obtained from each extract. Chromatograms of the acid-hydrolysed ethanol-insoluble residues (Figs. 2*A* and 2*a*) showed all the amino-acids normally found in protein hydrolysates, with ornithine present in addition in brewer's yeast. There were no striking differences between the two yeasts, except that baker's yeast had lower contents of arginine and threonine, and a higher content of phenylalanine.

The ethanol extract of baker's yeast (Fig. 2*B*) contained all the amino-acids of the hydrolysed cell residues, with the exception of methionine and cystine, which were not detectable after H_2O_2 oxidation. The relative proportions of the amino-acids bore no resemblance to those of the cell residues. The chromatograms showed in addition the following six spots: nos. 2, 3, 4, 11, 15 and 25. Spots nos. 2, 3 and 4 can be attributed to glutathione, and will be discussed later. Spot no. 11, which was brown in colour, was identified as asparagine; it disappeared after acid hydrolysis. Spot no. 15 may be a peptide with considerable stability to acid hydrolysis; hydrolysed eluates from paper cuts in the position of this spot contained aspartic acid, glutamic acid, glycine and alanine, besides some unchanged material. Spot no. 25 was acid-stable and corresponded in position to γ-aminobutyric acid; its identity was checked by its stability to copper treatment (Crumpler & Dent, 1949). No tryptophan was found in the extract.

The ethanol extract of brewer's yeast, although generally resembling that of baker's, differed from it in certain respects as shown in Table 3 and Fig. 2*b*. The most striking differences occurred in the contents of glutathione, arginine and threonine; α-aminobutyric acid (spot no. 17) was present, although absent from unhydrolysed baker's yeast extracts. The lower

Table 3. *Amino-acid and glutathione contents of yeast fractions*

(The amino-acid contents were estimated chromatographically, except for chemical estimations on S-containing compounds. 0, indicates amino-acid destroyed by hydrolysis; —, indicates amino-acid not detected.)

	Amino-acid content (g. amino-acid N/100 g. N of fraction or of whole yeast)							
	Baker's yeast				Brewer's yeast			
	Ethanol-insoluble residue hydrolysed	Ethanol extract	Ethanol extract hydrolysed	Whole yeast*	Ethanol-insoluble residue hydrolysed	Ethanol extract	Ethanol extract hydrolysed	Whole yeast*
Alanine	10	4	6	9	10	4	6	9
β-Alanine	—	—	0·3	0·04	—	—	—	—
α-Aminobutyric acid	—	—	0·3	0·04	—	0·1	0·3	0·05
γ-Aminobutyric acid	—	0·5	1	0·1	—	0·5	1	0·2
Arginine	5	2·5	5	5	10	8	10	10
Aspartic acid	8	1·5	5	8	8	3	5	7
Asparagine	0	3	0	0·4	0	3	0	0·5
Cystine	0·9†	—	3‡	1·25§	0·9†	—	1·5‡	1·5§
Glutamic acid	10	10	20	11	10	5	20	12
Glutamine	0	—	0	—	0	2	0	0·3
Glycine	8	0·1	3	7	8	0·5	3	7
Histidine	8	2	2	7	8	1	1	7
Isoleucine	4	0·8	0·8	3	4	0·5	0·5	3
Leucine	6	0·8	0·8	5	6	0·5	0·5	5
Lysine	9	2	4	8	9	2	4	8
Methionine	0·7§	—	—	0·83§	0·5§	—	—	0·81§
Ornithine	—	—	—	—	1	5	5	1
Phenylalanine	3	2	2	3	1·6	—	0·7	1
Proline	3	2	2	3	3	2	2	3
Serine	4	0·7	0·7	4	4	0·7	0·7	3
Threonine	5	2	2	4	7	—	1	6
Tryptophan	0	—	0	—	0	—	0	—
Tyrosine	3	1	1	3	2	0·5	0·5	2
Valine	8	2	2	7	8	2	2	7
Sum	96	37	61	90	101	42	65	94
Glutathione	0	9·5	0	1·5‖	0	4·5	0	1·7‖
Total N content of fraction (g./100 g. untreated yeast)	6·36	0·86	0·86	7·35	6·92	1·35	1·35	8·40

* Sum of the values obtained for ethanol-insoluble residue and for hydrolysed ethanol extract.
† Calculated by difference; whole yeast cystine N minus cystine N in whole yeast glutathione.
‡ Cystine N in glutathione of ethanolic extract.
§ Estimated on whole yeast.
‖ Estimated on ethanol-salicylosulphonic acid extract of whole yeast.

glutathione content of the ethanol extract of the brewer's yeast was confirmed by iodometric titration. The unidentified spot no. 15 of the ethanol extract of the baker's yeast was not found, but a weak spot corresponding to glutamine was present.

Acid hydrolysis of the ethanol extracts (Figs. 2*C*, 2*c*) caused marked increases in the strengths of spots given by aspartic acid, glutamic acid, glycine and alanine; cysteic acid appeared. The glutathione, glutamine and asparagine spots disappeared, accounting, in part at least, for the increases noted. In baker's yeast spot no. 15 decreased in strength, and four additional spots appeared: no. 17 (α-aminobutyric acid), no. 18 (β-alanine) and nos. 9 and 20. Spot no. 20 resembled, both in colour (yellow) and in position, the spot given by thiazolidine-4-carboxylic acid (Consden, Gordon & Martin, 1946), but was shown to be different from this substance, as chromatograms run with thiazolidinecarboxylic acid added to the hydrolysate gave two neighbouring yellow spots. Spot no. 20 was not present in hydrolysed brewer's yeast extract. Spot no. 9 which was stronger in chromatograms in hydrolysed brewer's yeast extract than in those from baker's yeast extract, corresponded in position to either diaminopimelic acid (Work, 1950) or ethanolamine-*O*-phosphoric acid. It was shown to be different from diaminopimelic acid by electrodialysis, when it moved with the acidic fraction; it may, therefore, be ethanolamine phosphoric acid, but its identity was not confirmed.

Paper chromatography of glutathione. Pure natural and synthetic glutathione, dissolved in water or 75% ethanol, did not give reproducible chromatograms; usually there were three spots (nos. 2, 3 and 4, Fig. 2*B*), but they were not constant in position or strength. Spot no. 2 was often elongated in the direction of the glycine spot (spot no. 8), and was the only one present after heating an aqueous solution of glutathione for 15 min. at 80°. Spot no. 4 varied from a single spot below aspartic acid (spot no. 5) to a streak between

spots nos. 4 and 3; this streak was sometimes divided into two separate spots. Spot no. 3 was not always present, but after oxidation of the glutathione with H_2O_2 and NH_4^+ molybdate it was the only spot and had much increased in strength. The behaviour of spot no. 3 resembled that of cysteic acid, it was sometimes present after running an ordinary chromatogram, but it was always increased in strength after peroxide oxidation. By analogy with cysteic acid, one may attribute spot no. 3 to an oxidized form of glutathione, possibly containing sulphur as a sulphonic acid group.

Ethanol extracts from baker's yeast gave spots nos. 2–4 which varied in the same way as spots from solutions of pure glutathione. These spots, after elution with water at 2° from several chromatograms of the ethanol extract, were examined separately. Running chromatograms of the eluates showed that spot no. 2 gave spots nos. 2 and 3, spot no. 3 gave nos. 3 and 4, while spot no. 4 did not give any one identifiable spot. Hydrolysed eluates from spot no. 3 contained glutamic acid, cysteic acid (from cystine) and glycine, while eluates from spots nos. 2 and 4 contained glutamic acid, glycine and alanine but no cystine. The origin of the alanine in these hydrolysates must be attributed to cystine or cysteic acid. Brewer's yeast extracts produced the same three spots attributable to glutathione, but they were of lower strengths.

Distribution of sulphur and sulphur-containing amino-acids in yeasts

Table 4 summarizes the results on the content and distribution of sulphur in the yeasts. The differences in the total sulphur contents of the two yeasts were greater than those in organic sulphur owing to the virtual absence of sulphate from baker's yeast. The latter contained 0·35 % organic sulphur, 60 % of which was cystine sulphur. The corresponding values for brewer's yeast were 0·44 % organic sulphur and 65 % cystine sulphur. Allowing for the cystine content of glutathione, 36 % of the organic sulphur of baker's yeast and 40 % of that of brewer's yeast may be calculated to be present as protein cystine. Methionine, calculated as the difference between organic sulphur and total cystine sulphur, also makes up roughly similar percentages of organic sulphur in the two yeasts (40 and 35 % for baker's

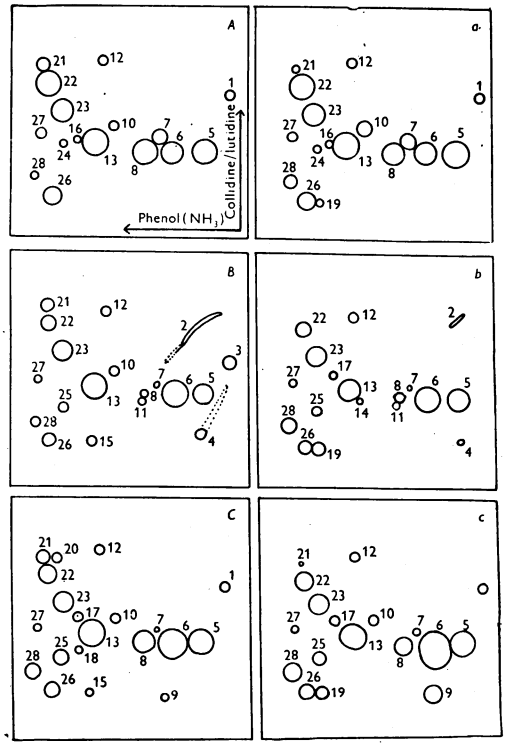

Fig. 2. Chromatograms from yeast fractions. (Solvents: 1, Phenol/NH_3 and 2, collidine/lutidine.) *A*, *B* and *C*, baker's yeast; *a*, *b* and *c*, brewer's yeast. *A*, *a*, acid-hydrolysed ethanol-insoluble residue, equiv. to 50 μg. N. *B*, *b*, ethanol extract, equiv. to 100 μg. N. *C*, *c*, acid-hydrolysed ethanol extract, equiv. to 100 μg. N. 1, Cysteic acid; 2, 3 and 4, glutathione; 5, aspartic acid; 6, glutamic acid; 7, serine; 8, glycine; 9, unknown; 10, threonine; 11, asparagine; 12, tyrosine; 13, alanine; 14, glutamine; 15, unknown; 16, histidine; 17, α-aminobutyric acid; 18, β-alanine; 19, ornithine; 20, unknown; 21, phenylalanine; 22, leucine and isoleucine; 23, valine and methionine; 24, methionine sulphoxide; 25, γ-aminobutyric acid; 26, lysine; 27, proline; 28, arginine.

Table 4. *Sulphur distribution in yeasts*

	Sulphur (g./100 g. dry yeast)		Sulphur (g./100 g. organic S)	
	Baker's yeast	Brewer's yeast	Baker's yeast	Brewer's yeast
Total S	0·36	0·55	—	—
Sulphate S	0·01	0·11	—	—
Organic S	0·35	0·44	—	—
Total cystine S	0·21	0·29	60	65
Total glutathione S (oxidized + reduced)	0·08	0·11	24	25
Protein-cystine S	0·13	0·18	36	40
Methionine S (organic S – total cystine S)	0·14	0·16	40	35
Ethanol-extractable S	0·09	0·08	—	—
Ethanol-extractable glutathione S (oxidized + reduced)	0·06	0·05	17	11
Ratio, total S:total N	1:20	1:15	—	—
Ratio, organic S:total N	1:21	1:19	—	—

and brewer's respectively). Because of the different organic sulphur contents of the two yeasts, the differences in the concentration of the sulphur-containing amino-acids, particularly cystine, become more obvious when the results are expressed in terms of dry yeast.

Extraction of whole yeast with 75% ethanol evidently did not extract all the glutathione, since the ethanol extracts showed lower glutathione contents than did the salicylosulphonic acid extracts. In the salicylosulphonic acid extracts, 71% of the glutathione of baker's yeast and 62% of that of brewer's yeast was present in the reduced form.

When considering the sulphur contents of the yeasts in relation to their total nitrogen contents, the higher nitrogen content of the brewer's yeast is of importance. Because of this, the ratio between organic sulphur and total nitrogen is almost the same in the two yeasts (1:21 and 1:19). The total sulphur:total nitrogen ratios differ (1:23 and 1:15), due to the high sulphate sulphur content of brewer's yeast.

DISCUSSION

Our figures for the amino-acid composition of the yeasts are based mainly on a paper-chromatographic method of estimation. This method, although not accurate (±30%), is very specific and has the advantage of being applicable to nearly all amino-acids except cystine and methionine. Acid hydrolysis was used to split the protein, and so tryptophan was not estimated, since it is destroyed by this treatment.

Various methods have been suggested for the quantitative estimation of amino-acids by paper chromatography (Block, 1948; Fisher, Parsons & Morrison, 1948; Woiwod, 1948; Naftalin, 1948; Martin & Mittelmann, 1948; Polson, 1948; Landua & Awapara, 1949). With the exception of those of Block and Polson, these methods are only applicable to simple amino-acid mixtures and could not be used in the present case (see Martin, 1949). The methods of Block and Polson are essentially similar to those employed here, except that Block makes his measurements with mechanical aids. Polson claims greater accuracy (±10%) than we have achieved and has given his figures for the amino-acid content of *Escherichia coli* to two places of decimals. Neither of these authors emphasized the necessity of using very pale spots near the minimal detectable level for comparison, a condition which we found essential.

Cystine and methionine, of particular interest in liver necrosis, were estimated gravimetrically (Lugg, 1938) because both chromatographic and microbiological methods of estimation proved unreliable. In the gravimetric method, the assumption is made that cystine and methionine comprise the bulk of the organic sulphur components. This assumption, although justifiable for pure proteins, cannot be strictly correct in the case of yeast, which is known to contain small amounts of other sulphur-containing compounds. The extent to which the presence of these compounds influenced our results is unknown; we are only able to say that taurine, which is detectable on chromatograms, was not found in our yeasts. A justification for our use of the method lies in the fact that the figures obtained for methionine are not unduly high, and so could not have been much affected by other sulphur-containing compounds not oxidized under conditions used in Lugg's method.

Published figures for the amino-acid contents of yeasts show some variations between different strains, but no gross or regular differences between baker's and brewer's yeast. Table 5 gives a comparison of our results with some of those reported in the recent literature obtained by chemical or microbiological methods. The present method of estimation is the only one which has produced figures for all the amino-acids present except tryptophan. Attention should be drawn to the differences between the results reported here on the sulphur, cystine and methionine contents of baker's yeast and those of Dent (1947), estimated by the same method on a different batch sample of the same strain of yeast. The main difference lies in the level of organic sulphur, which was much higher (0·5 g. sulphur/100 g. yeast) in Dent's sample; since methionine was estimated by difference, the methionine content was consequently also higher. It is now obvious that analytical figures are not identical on different batches of the same strain of yeast; György's figures for sulphur and nitrogen in both our baker's and his brewer's yeasts also differ from our figures (Table 5). A possible cause of these differences could be variations in the composition of the growth medium for the yeast: D.C.L. baker's yeast is grown on molasses, a substance whose composition might be expected to be relatively inconstant. Barton-Wright (1949) reports that the methionine content of baker's and brewer's yeasts can be altered by changes of the methionine content of the growth medium. It is therefore possible that variations in the organic sulphur content of the D.C.L. baker's yeast could, in part, be attributed to variations in methionine content.

Chromatograms from the ethanol-insoluble cell residues of both yeasts gave no unidentifiable spots, nor even any unusual ones except for small amounts of ornithine in brewer's yeast. The ethanol extracts contained all the amino-acids of the cell residues, except for cystine and methionine. They also contained α- and γ-aminobutyric acids, amino-acids which have so far not been identified in proteins, but are known to occur in extracts from animals and plants (Dent, 1948) and from bacteria (Work, 1949 and unpublished observations). Baker's yeast

Table 5. *Comparison of analytical results on yeasts with published values*

(Except where stated, figures are calculated as g. of amino-acid N/100 g. total N)

Type of yeast ...	Baker's D.C.L.			Baker's	Brewer's (Fleischmann's)		Brewer's		
Author and reference	Present results	Dent (1947)	György (1948)	Block & Bolling (1945)	Present results	György (1948)	Block & Bolling (1945)	Edwards *et al.* (1946)	Barton-Wright (1949)
Method of estimation	Chromatographic, *Chemical	Chemical	—	Chemical, †Microbiological (mean of two values)	Chromatographic, *Chemical	—	Chemical, †Microbiological (mean of three values)	Chemical (mean of two values)	Microbiological
Arginine	5	—	—	7·5	10	—	10	26·2	—
Cystine	1·3*	1·0	—	0·7	1·5*	—	0·7	—	—
Histidine	7	—	—	4·8	7	—	4·8	5·0	4·5
Isoleucine	3	—	—	4·1†	3	—	3·9†	4·0	3·0
Leucine	5	—	—	4·8†	5	—	5·1†	5·2	3·7
Lysine	8	—	—	8·7	8	—	8·4	8·75	8·2
Methionine	0·8*	2·0	—	1·6	0·8*	—	1·6	1·3	0·9
Phenylalanine	3	—	—	2·2	1	—	2·3	1·8	1·8
Threonine	4	—	—	4·2	6	—	4·1	3·7	—
Tryptophan	—	—	—	1·4	—	—	1·6	0·6	—
Tyrosine	3	—	—	1·8	2	—	1·7	3·1	—
Valine	7	—	—	3·5†	7	—	3·5†	—	4·8
Total N, g./100 g. yeast	7·4	7·0	6·8	7·2	8·4	7·8	8·1	—	—
Total S, g./100 g. yeast	0·36	0·53	0·65	0·36	0·55	0·86	0·4	—	—
Ratio, total S:total N	1:20·6	1:13·2	1:10·5	1:20	1:15·3	1:9·1	1:20·3	—	—

ethanol extract showed an unknown spot (no. 15) which may be an acid-stable peptide. After hydrolysis, the ethanol extract gave in addition spots from β-alanine, ethanolamine-*O*-phosphoric acid (spot no. 9, not confirmed rigidly) and an unidentified substance (spot no. 20). Ethanolamine phosphoric acid was present in higher concentrations in hydrolysed ethanol extract from brewer's yeast.

Both ethanol extracts contained glutathione, which gave multiple spots, not yet fully identified. Each spot after elution hydrolysed to glutamic acid, glycine and cystine or alanine (derived from cystine), but the proportions of the amino-acids in each fraction were not determined. The streaky nature of two of the spots suggests that they were caused by changes occurring during development of the chromatogram. The reason is unknown for the higher glutathione contents of the salicylosulphonic acid extracts of the yeasts compared with the ethanol extracts. The iodometric titration method may estimate other reducing substances in addition to glutathione. Unfortunately, no independent check on glutathione content by glyoxalase coenzyme estimation could be made, because after neutralization of the salicylosulphonic acid extract the titration figure dropped rapidly—owing probably to oxidation of the glutathione. The considerable difference (30 % or more) in the levels of total and reduced glutathione do not agree with the findings of Astrup & Øhlenschläger (1948), who state that the titration figure never rose by more than 10 % after electrolytic reduction.

The conclusions we can draw about the composition of baker's and brewer's yeasts are that the brewer's yeast is about 14 % richer in protein, if we assume that a similar proportion of the total nitrogen of each sample is non-protein nitrogen. Weight for weight, brewer's yeast is also richer in the sulphur-containing amino-acids, having 38 % more total cystine and 14 % more methionine than baker's yeast. The same proportion of total cystine is represented by glutathione in both yeasts, therefore brewer's yeast contains 38 % more glutathione than baker's yeast. If their amino-acid compositions calculated on the basis of total nitrogen are compared, the yeasts appear to be roughly similar except that brewer's yeast contains more arginine and threonine and less phenylalanine than baker's yeast.

SUMMARY

1. One sample of baker's yeast and one sample of brewer's yeast were compared with regard to their contents of nitrogen, sulphur, amino-acids and glutathione.

2. Separate estimations were made on the 75 % ethanol extracts and on the ethanol-insoluble residues.

3. Some modifications of the technique of estimating amino-acids by paper chromatography were used.

4. The behaviour of glutathione on paper chromatograms is described.

We are indebted to Dr P. György for the sample of brewer's yeast, to Sir Charles Harington, F.R.S., for a specimen of synthetic glutathione and to Dr R. Consden for thiazolidine carboxylic acid. We acknowledge technical assistance from Mr R. Denman and Mr F. Grover. One of us (O.L.) is in receipt of a Research Grant from the Medical Research Council.

REFERENCES

Astrup, T. & Øhlenschläger, Y. (1948). *Biochem. J.* **42**, 211.
Awapara, J. (1948). *Arch. Biochem.* **19**, 172.
Barton-Wright, E. C. (1949). *Biochim. Biophys. Acta*, **3**, 679.
Block, R. J. (1948). *Science*, **108**, 608.
Block, R. J. & Bolling, D. (1945). *Arch. Biochem.* **7**, 313.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1946). *Biochem. J.* **40**, 580.
Crumpler, H. R. & Dent, C. E. (1949). *Nature, Lond.*, **164**, 441.
Dent, C. E. (1947). *Biochem. J.* **41**, 314.
Dent, C. E. (1948). *Biochem. J.* **43**, 169.
Dohan, J. S. & Woodward, G. E. (1939). *J. biol. Chem.* **129**, 393.
Edwards, L. E., Sealock, R. R., O'Donnell, W. W., Partlett, G. R., Barclay, M. B., Tully, R., Tybout, R. H., Box, J. & Murlin, J. R. (1946). *J. Nutrit.* **32**, 597.
Fisher, R. B., Parsons, D. S. & Morrison, G. A. (1948). *Nature, Lond.*, **161**, 764.
György, P. (1948). *Trans. 3rd Conf. on Biological Antioxidants. Josiah Macy, Jun. Foundation*, p. 71.
György, P. & Goldblatt, H. (1949). *J. exp. Med.* **89**, 245.
Himsworth, H. P. & Glynn, L. E. (1944*a*). *Clin. Sci.* **5**, 93.
Himsworth, H. P. & Glynn, L. E. (1944*b*). *Clin. Sci.* **5**, 133.
Himsworth, H. P. & Lindan, O. (1949). *Nature, Lond.*, **163**, 30.
Hock, A. & Fink, H. (1943*a*). *Hoppe-Seyl. Z.* **278**, 136.
Hock, A. & Fink, H. (1943*b*). *Hoppe-Seyl. Z.* **279**, 187.
Landua, A. J. & Awapara, J. (1949). *Science*, **109**, 385.
Lindan, O. & Work, E. (1951). *Biochem. J.* **48**, 344.
Lugg, J. W. H. (1938). *Biochem. J.* **32**, 114.
Martin, A. J. P. (1949). *Ann. Rep. Chem. Soc.* **45**, 267.
Martin, A. J. P. & Mittelmann, R. (1948). *Biochem. J.* **43**, 353.
Masters, M. (1939). *Biochem. J.* **33**, 1313.
Matet, A., Matet, J. & Fridenson, O. (1947). *J. Physiol., Paris*, **39**, 381.
Naftalin, L. (1948). *Nature, Lond.*, **161**, 763.
Pratt, J. J. & Auclair, J. L. (1948). *Science*, **108**, 213.
Polson, A. (1948). *Biochim. Biophys. Acta*, **2**, 575.
Schwarz, K. (1944). *Hoppe-Seyl. Z.* **281**, 101, 109.
Weichselbaum, T. E. (1935). *Quart. J. exp. Physiol.* **25**, 363.
Woiwod, A. J. (1948). *Nature, Lond.*, **161**, 169.
Work, E. (1949). *Biochim. Biophys. Acta*, **3**, 400.
Work, E. (1950). *Nature, Lond.*, **165**, 74.

The Nutritive Properties of Two Yeasts used to Produce Massive Dietetic Liver Necrosis in Rats

By O. LINDAN and ELIZABETH WORK
Medical Unit and Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

(*Received* 7 *July* 1950)

Dietetic liver necrosis in rats has been produced by feeding diets deficient both in protein and in tocopherol (Himsworth, 1950). Such diets containing suboptimal amounts of casein, soya-bean meal or yeast, produce liver necrosis within periods varying from a few weeks to several months; when extra cystine or methionine is added to the diets the lesion is prevented (Weichselbaum, 1935; Hock & Fink, 1943*a*; Himsworth & Glynn, 1944*b*; Matet, Matet & Fridenson, 1947; György & Goldblatt, 1949). A deficiency in the sulphur-containing amino-acids relative to other amino-acids in the diet thus appears to be one of the factors responsible for the development of liver necrosis. This theory is supported by experiments of Glynn, Himsworth & Neuberger (1945) in which dietetic liver necrosis was produced in rats by substituting the dietary protein by a synthetic mixture containing all the essential amino-acids except methionine and cystine. Hock & Fink (1943*b*), following an investigation of the necrogenic properties of diets in which the major part of the protein was provided by different types of yeasts, associated the ease of development of liver necrosis with the relative amounts of sulphur and nitrogen in the diet. When the sulphur:nitrogen ratio was 1:30 the incidence of necrosis was high, but there was no necrosis with a ratio of 1:13. It is obvious that the authors thought that this ratio