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Histamine and Tyramine Content of Yeast Products

SUMMARY—Chromatographic estimations of tyramine and histamine in "Marmite" and four other yeast extracts showed a tyramine content from 0.1 to 1.6 mg/g and a histamine content of 0.2 to 2.8 mg/g. "Marmite" contained the largest amounts of both amines, but three samples of this extract showed a wide range in amine content. These variations in amine content of yeast extracts are discussed in terms of their manufacture. Pharmacological activity in one of the yeast extracts ("Marmite") on the rat's blood pressure and on the intact bronchioles and isolated ileum of the guinea pig was due to the presence of tyramine and histamine. The clinical relevance of a high histamine content is discussed.

INTRODUCTION

YEAST EXTRACTS are ingredients of foods such as canned soups, sauces, relishes, brawns or moulded meat products (Lyall, 1963) and at least five extracts are sold in Britain as sandwich spreads or beverages. The original manufacturing process for a yeast extract "Marmite" was developed "with the ultimate object of obtaining valuable nutritious and medicinal substances rich in enzymes and vitamins" (Weizman, 1934) and for this reason "Marmite" is commonly given to hospital patients.

Tests on animals to study the hypertensive effects caused by the yeast extract "Marmite" in patients taking monoamine oxidase inhibitors indicated that it contained tyramine and histamine in large quantities (Blackwell *et al.*, 1965b). Tyramine is not normally absorbed from the intestine but histamine given in large amounts or when liver function is impaired, reaches the systemic circulation in sufficient quantity to stimulate gastric secretion and appear in the urine (Irvine *et al.*, 1959).

Because of the possible danger of histamine absorption in susceptible patients, we have made the first chromatographic estimation of amine content in five commonly eaten yeast products, and performed tests in animals to determine whether the biological activity of "Marmite" could be ascribed to its content of tyramine and histamine.

METHODS

Chromatographic estimations

Five grams of yeast extract were dissolved in 40 ml warm water, boiled to prevent further enzyme activity, centrifuged, and the supernatant filtered. In some instances the extract was filtered through activated carbon (British Ceca Co.). The filtrate was stirred with Amberlite IRA 400 ion exchange resin and the slurry poured on to a column of the same resin to separate basic from non-basic constituents as described for cheese (Blackwell *et al.*, 1965a). The eluted basic constituents were chromatographed 1-dimensionally using butanol-acetic acid-water (4:1:5 v/v) or ethanol-ammonia-water (8:1:1 v/v) as solvent. Chromatograms were sprayed with either sulphanilic acid solution for histamine (Smith, 1960) or with ninhydrin solution for tyramine. When required, fractions were obtained from the chromatogram by eluting with 10% aqueous acetic acid.

For quantitative work the ethanol-ammonia solvent was used. Spot densities were measured using a "Chromograph" (Joyce, Loebel & Co. Ltd.) and compared with those obtained using known amounts of histamine or tyramine. The histamine and tyramine contents of known concentrations in aqueous solution when determined by the ion exchange and paper chromatographic methods were within ± 10% of the true values.

The amino acid content of various "Marmite" preparations was analyzed by the manufacturers. The substances were separated chromatographically using butanol acetic-acid solvent, located with ninhydrin spray, and measured photodensometrically after elution.

Pharmacological estimation

Yeast extracts were prepared for intravenous injection by mixing with saline and centrifuging to remove particles. The pressor activity of the supernatant fluid was determined in pithed rats and compared with that of intravenous injections of known quantities of tyramine. The histamine content of supernatants was determined by assay on a guinea-pig's isolated ileum suspended in an organ bath at 37°C and gassed with 95% O_2 and 5% CO_2.

Tests of histamine activity were also made in intact guinea-pigs by recording resistance of the lungs to inflation (Konzett *et al.*, 1940). The animals were anaesthetized with urethane (10 ml of 25% solution/kg i.p.) and artificially ventilated by a pump after insertion of a tracheal cannula. Rectal temperature was maintained at 35°–40°C. A cannula was tied into the jugular vein for intravenous injections.

Yeast extracts

Proprietary brands of the various yeasts were obtained at random in the London and Reading areas. Salt free "Marmite" and extracts subject to modifications in manufacture given in Table 2 were supplied by Bovril Ltd.

RESULTS

Chromatography

Yeast extracts contained histamine and tyramine. Fig. 1 shows chromatograms prepared with butanol-acetic acid solvent of the basic fraction of "Marmite" (Sample 1, Table 1). Two intense areas were revealed by the imidazole spray (Fig. 1: fractions 2 and 4).

Pharmacological tests (see below) showed that the

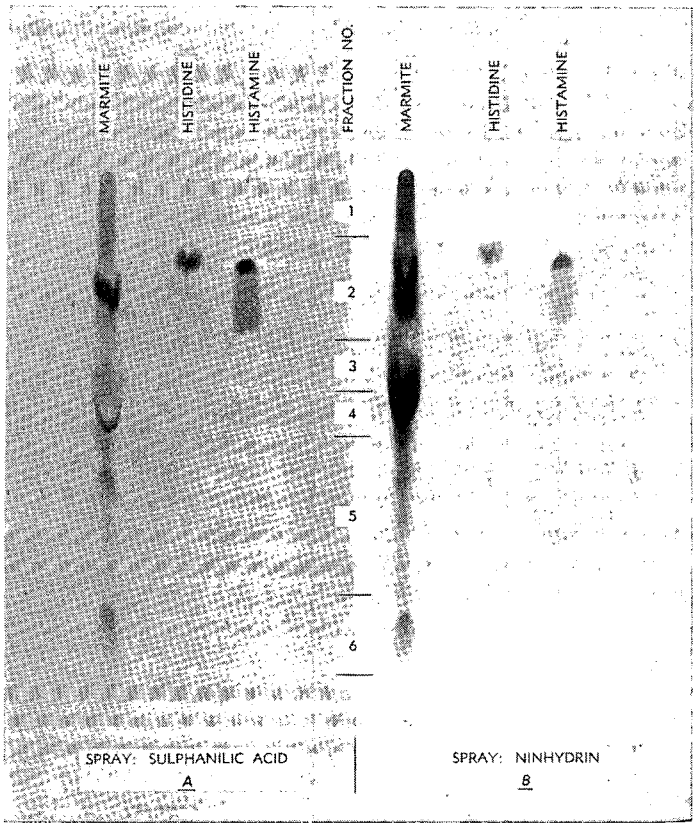

Fig. 1. One-dimensional chromatogram of the basic fraction of the yeast extract "Marmite." Solvent, butanol-acetic acid-water (4:1:5 v/v). Fractions 1–6 were eluted from a similar chromatogram and tested for pharmacological activity. A, sprayed with sulphanilic acid; B, sprayed with ninhydrin.

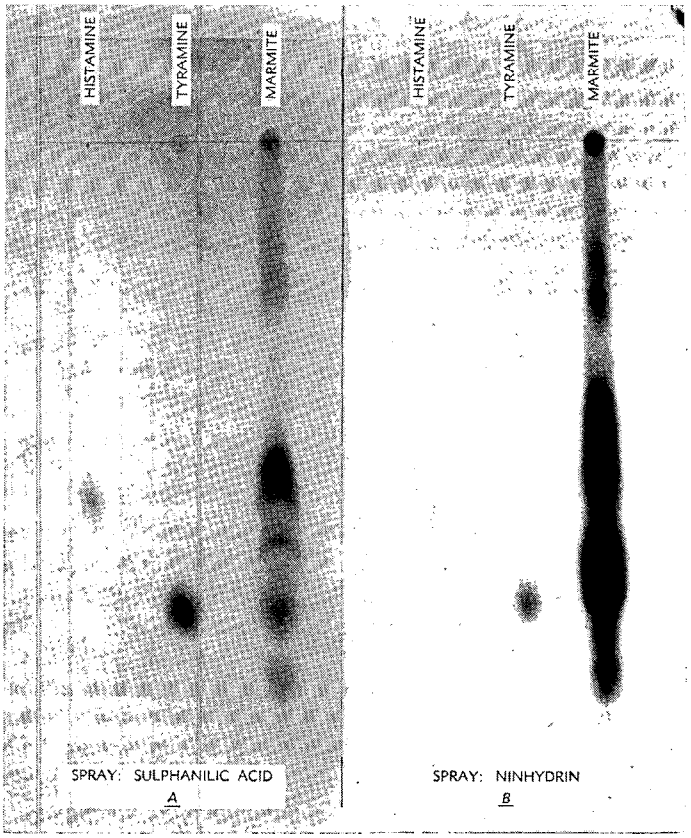

Fig. 2. One-dimensional chromatogram of the basic fraction of the yeast extract "Marmite." Solvent, ethanol-ammonia-water (8:1:1 v/v). A, sprayed with sulphanilic acid; B, sprayed with ninhydrin.

eluted Fraction 4 contained almost all the histamine activity, yet the position of this fraction on the chromatogram was different from that of the control histamine spot. It was clear from the appearance of the chromatogram that inorganic ions were running along with the imidazole-positive Fraction 4 and were influencing the R_f. A similar alteration in R_f of histamine was noted in an analysis of a meat extract (Wood *et al.*, 1957). The following observations confirmed this.

When Fraction 4 was eluted from the chromatogram and rechromatographed in the same solvent, the R_f of the imidazole was the same as that for the histamine control. Further, when histamine was added to the basic fraction of "Marmite" and the mixture chromatographed in butanol-acetic acid-water solvent only the intensity of the spot in the position of Fraction 4 was increased.

When sodium chloride was added to the histamine control before chromatography, the resulting R_f was identical with that of Fraction 4. When the sample was chromatographed under alkaline conditions using ethanol-ammonia-water solvent, there was no interference with the R_f of histamine (Fig. 2). Thus the chemical evidence supported the pharmacological findings. "Marmite" also contained histidine (Fig. 1, 2), the precursor of histamine, but this substance was inactive in the pharmacological tests used, so distinguishing it from histamine.

Table 1 lists the tyramine and histamine content of eight samples of yeast extracts purchased at random including four different samples of "Marmite."

Table 1 shows that the tyramine and histamine content of five different yeast extracts varied from 0.1 to 1.6 mg per g extract. The mean histamine and tyramine contents of three samples of "Marmite" (1–3 in Table 1) were four times greater than the mean tyramine or histamine contents of the four other yeast extracts. The three different batches of "Marmite" varied from each other by ± 18% in tyramine content and by ± 47.5% in histamine content, differences which considerably exceed the error of the method (± 10%).

Table 1. Tyramine and histamine content of yeast extracts estimated chromatographically (ethanol-ammonia solvent).

Proprietary yeast extracts	Tyramine HCl	Histamine HCl
	mg per g	
Marmite (1)	1.64	2.83
Marmite (2)	1.44	1.95
Marmite (3)	1.09	0.98
Salt-free Marmite	0.19	1.66
Yex	0.51	1.34
Befit	0.42	0.27
Barmene	0.15	0.21
Yeastrel	0.10	0.26

Pharmacological activity

Identification of active substances. In order to ascertain which regions on the chromatogram contained substances with pharmacological activity, a 1-dimensional chromatogram of the total basic fraction from 5 g "Marmite" (No. 2 in Table 1) was divided into six strips (in the manner shown in Fig. 1). The constituents of each strip were eluted and freeze-dried. The fractions were labelled 1–6 and tested for their pharmacological activity. The results are illustrated in Fig. 3.

Assay for activity on the blood pressure of a pithed rat (A) revealed that only fraction 6 was pressor (suggesting the presence of tyramine) but 4 and 5 were depressor, (compatible with the presence of histamine). Further assay (B) of fraction 6 against the pressor activity of tyramine in another rat showed that the eluted spot contained the equivalent of 0.01 mg tyramine HCl per mg Fraction 6 (Fig. 3C). Since 60 mg of fraction 6 were obtained as the acetate from 1 g of original extract, the estimated tyramine hydrochloride content of the total "Marmite" was 1 mg per g of extract. The pressor activity of tyramine and of fraction 6 were both antagonized by a pharmacological antagonist, phenoxybenzamine.

Tests in the intact guinea-pig (D) showed that mainly fraction 4, and to a slight extent fraction 5, contained a substance which like histamine increased the resistance of the lungs to inflation by constricting the tracheobronchial musculature. Tests on the isolated guinea-pig ileum (E) which contracts to histamine, confirmed that most of the histamine-like activity was present in fraction 4. These histamine-like effects were antagonized by the histamine antagonist mepyramine. Assay of the total basic fraction in another experiment showed that the "Marmite" contained 0.616 mg histamine HCl per g "Marmite."

Correlations between the pharmacological and chromatographic results (Table 1) were not made until tests were complete. Fraction 6 corresponded with the region occupied by tyramine and fraction 4 with that occupied by histamine. The pharmacological estimates of activity were below the chromatographic figures possibly due to modifications in response brought about by a mixture of substances or to incomplete elution of spots from the chromatogram.

Method of manufacture and pharmacological activity. The pharmacological activity of several samples of "Marmite" was estimated to determine the effect of some modifications in the manufacturing process. These were:

a. selection of a yeast with high tyrosine and histidine content.
b. treatment with "activated carbon" in an attempt to preferentially remove aromatic compounds such as tyramine and histamine.
c. the control of pH during autolysis at a higher than normal level.

The results of these tests are set out in Table 2.

Selection of a yeast with a high tyrosine and histidine content increased the tyramine but not the histamine content of the extract. The attempt to remove these amino acids or amines by carbon treatment failed. A small increase in pH during autolysis halted the formation of histamine but not tyramine.

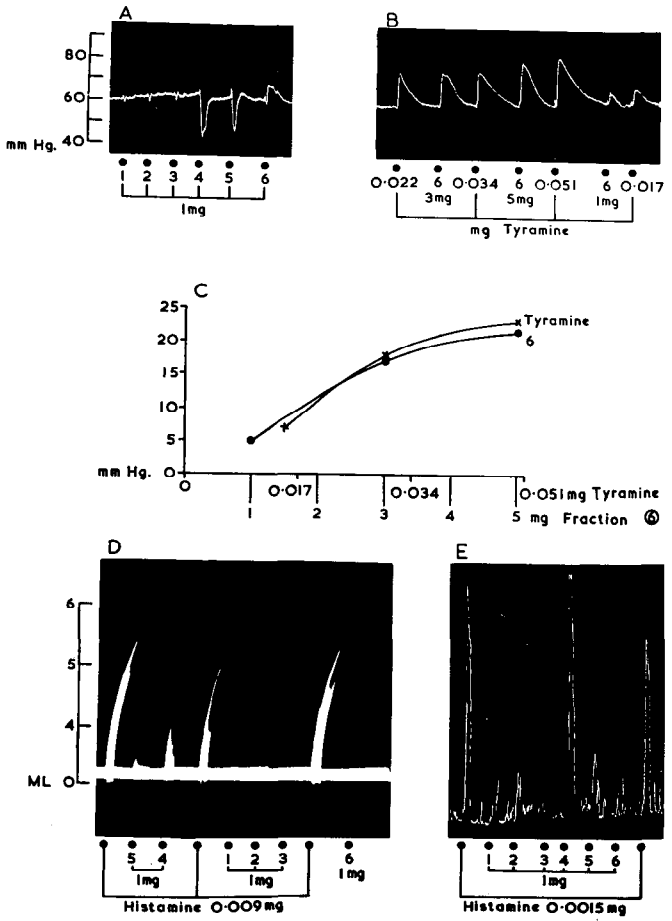

Fig. 3. Tests for pharmacological activity in yeast extract, fractions 1–6 eluted from a chromatogram (see Fig. 1). A. Blood pressure changes in pithed rats after intravenous injection of fractions 1–6. B. Blood pressure changes in pithed rat after intravenous injections of different doses of fraction 6 and tyramine. C. Blood pressure responses in pithed rat to intravenous injection of fraction 6 and graded amounts of tyramine. D. Resistance of guinea-pig lungs to inflation in vivo *(measured as overflow volume in ml), following intravenous injection of fractions 1–6 and histamine. E. Contraction of isolated guinea-pig ileum by histamine and fractions 1–6 in the presence of hyoscine (10^{-7}). Doses represent total amounts placed in 20 ml bath.*

Table 2. Effects of modifications in manufacture on amine content of "Marmite." (Tyramine and histamine were estimated pharmacologically; amino acid content estimated by manufacturer).

Modification	Amino acid content g/100 g yeast tyrosine	histidine	Tyramine content	Histamine content
			mg per g extract	
None (Marmite 2 in Table 1)	1.21	1.17	1.00	0.62
Selected yeast of high amino acid content	1.85	1.9	1.50	0.50
Autolysate carbon treated	Not known	Not known	1.40	0.40
Controlled autolysis increased pH (+0.5)	0.91	1.58	0.85	0

DISCUSSION

THESE TESTS showed that the pharmacological activity of yeast extracts could be accounted for by the presence of tyramine and histamine. The amounts of these amines in different products or in separate samples of the same product varied considerably. A similarly wide range in the tyramine content of various cheeses and different samples of the same variety (Cheddar cheese) has been attributed to differences in manufacture (Blackwell *et al.*, 1965a).

Yeast extracts are prepared by a process which involves plasmolysis and autolysis (Weizman, 1934; Acraman, 1966). Waste yeast from the brewing industry is soaked in water containing 5% ethyl acetate and salt which act as plasmolyzing agents to disrupt the yeast cells, liberating protein and enzymes without damaging the latter. During autolysis the liberated yeast protein is converted to amino acids by proteases, polypeptidases and dipeptidases so that most of the protein is reduced to soluble form within 24 hr. Conditions are carefully controlled to preserve maximum enzyme activity; the pH is kept between 6.3 and 6.6 by continuous addition of 7% trisodium phosphate buffer and temperature is maintained at 36°C. After 24 hr the digest is heated to 70°C for 2 hr to terminate enzyme activity and improve flavor.

The amine content of foodstuffs is determined both by the amount of amino acids available and the extent to which they are converted to amines by tissue or bacterial decarboxylating enzymes.

Amino acid content

Different yeast strains vary in their initial amino acid composition (Holden, 1964) and a high initial tyrosine or histidine content might increase the amount of corresponding amine. Selection of a yeast with a high content of these two amino acids (Table 2) increased the tyramine but not the histamine in the final product. The extent of these changes was insufficient to account for the wide variation in tyramine content of the different products or the range of histamine content in the three samples of "Marmite." It therefore seems unlikely that the amino acid composition in the original crude yeast is the major factor in determining the final amine composition.

Amino acids are also produced by degradation of yeast protein during autolysis and manufacturing conditions profoundly affect this process. An increase in alkalinity destroys the sensitive dipeptidase enzyme, while a fall in pH to 5 due to unbuffered carbon dioxide production halts most enzyme activity and reduces the amino acid yield to below a quarter (Weizman, 1934). This difficulty is avoided in the manufacture of "Marmite" by constant additions of buffer but may differ in other commercial processes.

Attempts to remove aromatic amino acids or amines by treatment with "activated carbon" failed since the amount of tyramine and histamine in the final product was unchanged.

Amino acid decarboxylation

Little is known about decarboxylation enzymes in fungi and yeasts which might produce amines during their autolysis. Cochrane (1958) discussed the literature on amine formation in fungi and noted that *Claviceps purpurea* contains tyramine and histamine. Werle (1941) examined several fungi, including a yeast strain *Torula utilis* and found no histidine decarboxylase activity at a pH range from 3 to 8 during 18 hr incubation.

As in the manufacture of cheese, decarboxylation of amino acids could also occur due to the enzymic action of contaminating bacteria. Yeast products are frequently incorporated in media for bacterial culture. Their high amino acid composition, and rich content of codecarboxylase factor (Gale, 1946) would favor the presence of amine-producing organisms. During autolysis the controlled pH and constant temperature of 36°C provide an ideal medium for bacterial growth. There is little knowledge concerning organisms present during manufacture of yeast extracts, but brewer's yeast is often contaminated with lactobacilli (Bhandari *et al.*, 1954). Lagerburg *et al.* (1952) found that 3 out of 33 strains of this organism decarboxylated tyrosine but only one (Type 1) produced histamine. Streptococci may also be present, and may be more potent decarboxylating organisms (Blackwell *et al.*, 1965a).

The environmental requirements in pH, temperature and co-factors for bacterial decarboxylases are often specific (Gale, 1946) and may account for the finding in this study that when the pH was increased by 0.5 during autolysis, histamine was absent while the tyramine content remained high (Table 2). This agrees with Gale's finding that a change of pH in the medium from 5 to 7 reduced tyramine formation to 40% but histamine to 3%. Absence of salt during plasmolysis greatly reduced the tyramine but not the histamine content (Table 1).

If amine production primarily depends upon bacterial action this could be halted either by carrying out autolysis at a higher temperature or by adding antiseptics (Jorgensen, 1948). Neither of these steps has previously been taken in the commercial process, although in the original patent (Weizman, 1934) toluene was given as an effective antiseptic which did not hinder autolysis.

Clinical implications

The significance of a high tyramine and histamine content of yeast extracts in relationship to monoamine oxidase inhibition has been reported (Blackwell *et al.*, 1966). In rats, cats and fowl, tyramine was only absorbed from the intestine after amine oxidase inhibition and in cats histamine absorption was facilitated. However, when histamine was placed in the small intestine in untreated cats and guinea-pigs, some absorption occurred. The possibility that histamine may be absorbed from the intestine in untreated subjects must be considered.

The amount of histamine in "Marmite" exceeds tenfold that found in naturally occurring foods since animal tissues rarely contain more than 0.28 mg/g (Tabor, 1954) although some preserved fish foods such as herring and sardine contain up to 3 mg/g (Kimata, 1961). Fortunately, the high salt content and savory flavor of yeast extracts limit the amount consumed and even if taken as a beverage the total quantity is unlikely to exceed 10 g, containing not more than 60 mg of histamine.

In dogs, at least 100 mg histamine placed in the jejunum were required to stimulate gastric secretion although free histamine appeared in the urine after smaller amounts (Irvine *et al.,* 1959). In man, up to 225 mg histamine by mouth may have been well tolerated although 10 mg given intravenously produced profound effects (Sollman, 1957). One of us has taken 250 mg of histamine by mouth without change in pulse rate, blood pressure, or other effects.

Susceptible subjects with allergic complaints, asthma or peptic ulcers might be adversely affected by quantities smaller than 1 mg of circulating histamine. Patients with impaired liver function less able to metabolize histamine would also be a risk. Allergic complaints were not exacerbated in patients on monoamine oxidase inhibitors some of whom ate yeast extracts (Blackwell *et al.,* 1965c). However, deterioration in nearly half of asthmatics receiving different inhibitors was reported by Mathov (1964).

Children sometimes experience circumoral irritation or urticaria after eating "Marmite" and cases of asthma, angioneurotic oedema and "intestinal spasm" have previously been attributed to "allergic hypersensitivity" to foods which contain baker's or brewer's yeast (Urbach *et al.,* 1946). In an intensive review of food allergy, Withers *et al.* (1956) refer to a patient who developed urticaria and angioneurotic oedema after eating stewed but not roast meats and in whom patch-testing with proprietary substances used in gravies, including "Marmite," gave positive reactions.

There is sufficient evidence to suggest that patients vulnerable to histamine should be carefully observed to detect possible adverse effects due to yeast extracts.

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Ms. rec'd 7/20/67; revised 5/6/68; accepted 10/14/68.

This research was supported in part by a Fellowship from the Mental Health Research Fund and partly supported by the Bethlem Royal and Maudsley Hospitals' Research Fund.

Supplies of "Marmite" and various extracts subjected to modifications in manufacture were provided by "Bovril" Ltd., to whom we are grateful for amino acid estimations of the products.