Brit. J. Pharmacol. (1966), **26**, 120–141.

INTERACTIONS OF CHEESE AND OF ITS CONSTITUENTS WITH MONOAMINE OXIDASE INHIBITORS

BY

B. BLACKWELL AND E. MARLEY

From the Institute of Psychiatry, Maudsley Hospital, London, S.E.5

(*Received April* 26, 1965)

Patients treated with monoamine oxidase inhibitors sometimes experience severe and occasionally fatal hypertensive attacks after eating cheese (Blackwell, 1963). Other factors must modify this adverse reaction since many patients treated with monoamine oxidase inhibitors eat cheese with impunity. Some inhibitors appear more likely to interact with cheese in man (Marks, 1965) and earlier animal experiments showed that several inhibitors possess intrinsic sympathomimetic actions which may potentiate or obscure the blood pressure effects of cheese (Blackwell & Marley, 1964).

Preliminary studies in animals of interactions between cheese and amine oxidase inhibitors have been reported (Blackwell & Marley, 1964; Natoff, 1964) and an account of some of our experiments has been given to the British Pharmacological Society (Blackwell & Marley, July 1964). This paper reports the investigation of sympathomimetic effects due to cheese in three species with different dietary habits, together with the identification of responsible constituents and the factors which modify their actions on the blood pressure.

METHODS

Rat. Rats were injected with hyoscine (0.4 mg, subcutaneously) and anaesthetized with ether. The rat was pithed as described by Shipley & Tilden (1947) and artificially ventilated with oxygen delivered by a pump. Arterial blood pressure was recorded from a carotid artery with a Condon manometer writing on smoked paper. A cannula was tied into a jugular or femoral vein for intravenous injections, and all drugs were injected by this route unless otherwise stated. A midline abdominal incision was made, and a polyvinyl tube inserted into the stomach through an incision in the gastric greater curvature. The tube was passed into the duodenum and tied with the tip distal to the pyloric sphincter. The abdomen was closed, with the free end of the polyvinyl tube brought to the exterior.

Fowl. Adult fowls were anaesthetized with pentobarbitone (60 mg/kg intraperitoneally) and intubated by passing an endotracheal tube as far as the syrinx. The endotracheal tube was fixed to the beak with sticking plaster, and oxygen delivered through the tube usually sufficed to maintain adequate respiration. Arterial blood pressure was recorded from a carotid artery by a Condon manometer writing on smoked paper, and a cannula was tied into a jugular or ischiadic vein for intravenous injections. An abdominal incision was made 1 in. to the right of and parallel to the midline, the ascending or descending limb of the duodenum was opened, and a polyvinyl tube was inserted so that its tip pointed distally.

Cat. Cats were anaesthetized with chloralose (80 mg/kg intravenously) after induction with ethyl chloride and ether, and artificially ventilated except when respiration was recorded. In some cats the anaesthetic was stopped after the brain had been destroyed by an approach through the dorsal neck muscles and the atlanto-occipital membrane. Carotid arterial blood pressure, measured with a mercury manometer writing on a kymograph, was recorded from the opposite side to that which the responses of the nictitating membrane were being taken, and when responses were taken from both nictitating membranes, blood

pressure was measured from a femoral artery. A cannula was tied into a femoral vein for intravenous injections.

When responses of the nictitating membrane were measured the ipselateral superior cervical and nodose (vagal) ganglia were removed, except in experiments with bretylium when the ganglia were left *in situ*, and the cervical sympathetic postganglionic trunk was excited electrically through platinum electrodes (cathode distal) with supramaximal rectangular pulses of 0.5 msec duration at a frequency of 10 shocks/sec. Contractions of the nictitating membrane, after enucleation of the eyeball, were recorded with an auxotonic lever (Paton, 1957) of 16× magnification and 3 g loading. Identical matched levers were used when recording from both membranes. Respiration was measured with the apparatus described by Paton (1949).

A midline abdominal incision was made; a polyvinyl tube was inserted into the stomach through an incision in the gastric greater curvature, passed into the duodenum and tied with the tip distal to the pyloric sphincter. In selected experiments the adrenal glands were either removed or excluded from the circulation by ligatures. The abdomen was closed, the free end of the polyvinyl tube being brought to the exterior.

In other experiments, the blood-bathed isolated-organ technique of Vane (1958) was used. The cat was injected intravenously with heparin (10 mg/kg) and blood was drawn at 10 to 15 ml./min through silicone-rubber tubing by a roller pump (Saxby, Siddiqi & Walker, 1960), driven by a Servomex motor controller, from the carotid artery on the side opposite to that from which the responses of the nictitating membrane were being recorded. The blood was warmed in a water-jacket at 40° C before superfusing a rat isolated stomach strip (Vane, 1957), then collected in a nylon organ-bath and returned through silicone-rubber tubing to the jugular vein. The tone of the strip was recorded with an auxotonic lever of 16× magnification with a load of 1.5 or 2.0 g.

For assay of tyramine, 5.0 ml. of femoral arterial blood was removed from heparinized cats, placed in a chilled siliconed graduated centrifuge tube and immediately centrifuged at 3,000 rev/min for 10 min at 0° C. The plasma was drawn into siliconed pipettes and transferred to sealed siliconed bottles and kept on ice. For assay the plasma was extracted with ether at an alkaline *p*H, as described by Spector, Melmon, Lovenberg & Sjoerdsma (1963), the amino acids including tyrosine which would interfere with the assay being left in the residue. The extracted amine was returned to an acid phase and assayed after conversion to fluorophor by reaction with nitrosonaphthol. The fluorescence obtained corresponded with the native fluorescence in each case.

In two experiments the superior cervical and nodose ganglia were removed under aseptic conditions using halothane (Fluothane; I.C.I.) anaesthesia. These cats were allowed to recover and 10 to 14 days later were again anaesthetized with chloralose and prepared as described.

Preparation of the cheese. The cheeses were bought locally. Special preparation of the cheese was not crucial since effects were obtained with homogenates made up in saline or acidified with 0.1 N-hydrochloric acid. Acidification was intended to simulate the condition in which the cheese would be expelled from the stomach and to convert the tyramine present to an ionic form, but proved a disadvantage as the curd formed made injection difficult. Cheese was injected through the stomach tube into the duodenum. For intravenous injection cheese was prepared by centrifuging the homogenate at 5,000 rev/min for 30 min at −5° C to remove the fat and particles. The supernatant fluid was then tested.

Drugs. These (with molecular weights of salts in parentheses) were the hydrochlorides of chlorpromazine (355), cocaine (340), 3,4-dihydroxyphenylethylamine (dopamine) (190), α-methylbenzylhydrazine (170), nialamide (329), pargyline (193), phenethylamine (157), hydroxyphenethylamine (174), pheniprazine (176), pivhydrazine (245), tryptamine (190), and tyramine (174). In addition, the hydrochlorides were prepared of cadaverine (142), isocarboxazid (230), phenoxybenzamine (340), and the L-amino acids alanine (125), arginine (211), cysteine (158), cystine (275), glutamine (181), histidine (192), leucine (166), isoleucine (166), lysine (183), phenylalanine (200), 3,4-dihydroxyphenylalanine (dopa) (232), proline (150), serine (140), threonine (154), tryptophan (239), tyrosine (216), diodotyrosine (468) and valine (152). Also used were bretylium tolysate (414), chlorpheniramine dimaleate (391), L-α-methyldopamine hydrobromide (248), histamine acid phosphate (307). Hydergine (the methanesulphonate of the dihydro derivative of ergotoxine) hyoscine hydrobromide (438), iproniazid phosphate (277), methysergide (470), (−)-noradrenaline bitartrate monohydrate (337), phenelzine hydrogen sulphate (390), phenoxypropazine hydrogen maleate (276) and (±)-tranylcypromine sulphate (360). The doses are given in μmole/kg, except for Hydergine which is a mixture of ergot derivatives.

RESULTS

Rat

Effects of cheese on the blood pressure

Intravenous injection of cheese extract. As shown in Fig. 1 intravenous injection of an extract of cheddar cheese raised the blood pressure of the pithed rat. The extract assayed for its pressor activity against that of tyramine and 1 ml. of cheese extract was found to contain the equivalent of 1 μmole of tyramine, that is 255 μg of tyramine base per g of cheese. The same cheese extract was then injected into a rat previously treated with α-methylbenzylhydrazine (30 μmole/kg, 90 min previously). A dose of 0.8 ml. of the extract, which without the amine oxidase inhibitor produced a rise in blood pressure of about 30 mm Hg lasting 6 min (Fig. 1,*a*), now produced a rise of 40 mm Hg lasting 22 min (Fig. 1,*b*) until it was antagonized by phenoxybenzamine (2.5 μmole/kg).

The possibility that cheeses contained different amounts of pressor substances was studied by testing the saline extracts from four different cheeses. 10 g of the cheese was boiled with 10 ml. of 0.9% saline and centrifuged at 4,500 rev/min for 30 min. The supernatant fluids were assayed for their pressor activity against that of tyramine. Two of the cheeses (a stilton and a Canadian cheddar) contained the equivalent pressor activity of about 6 μmoles of tyramine per g of cheese. The other two (emmental and an English cheddar) contained less than the equivalent of 0.6 μmole of tyramine per g of cheese.

Intraduodenal injection of cheese. In two control experiments, camembert cheese (7.5 and 12 g/kg) given intraduodenally was ineffective on the blood pressure. However, as shown in Fig. 1,*c*, camembert cheese (12 g/kg) given intraduodenally after α-methylbenzylhydrazine (120 μmoles/kg, intraperitoneally 5 hr previously and 60 μmoles/kg intravenously 20 min beforehand) raised the blood pressure 45 mm Hg. The rise in blood pressure began 5 min after injecting the cheese and lasted over 1 hr. As seen in Fig. 1, the response was antagonized by chlorpromazine (2.8 μmoles/kg) which restored the blood pressure to normal although cocaine, methysergide and hyoscine, in equimolar doses to chlorpromazine, had been ineffective as antagonists.

In other tests, rises in blood pressure due to camembert cheese were abolished or reduced by Hydergine (80 μg/kg). Rises in blood pressure due to cheese were also obtained after treatment with tranylcypromine provided 12 hr elapsed after the last dose to avoid tachyphylaxis.

Amines in cheese

Cheese contains several amines formed by bacterial action during its manufacture (Silverman & Kosikowski, 1956). Those which might be expected to affect the blood pressure were tested to see if their absorption from the gut and action on blood pressure were modified by monoamine oxidase inhibition.

Pressor amines. (1) *Tyramine.* As shown in Fig. 1,*d*, similar effects to those with cheese were elicited by injecting tyramine (30 μmoles/kg) into the duodenum 90 min after α-methylbenzylhydrazine (30 μmoles/kg intraperitoneally). As with cheese, the blood pressure effects of tyramine were completely antagonized by chlorpromazine (2.8 μmoles/kg) although cocaine, methysergide and hyoscine, in equimolar doses to chlorpromazine, had

been ineffective as antagonists. The same dose of tyramine placed in the duodenum of an untreated rat was ineffective on the blood pressure.

(2) *Phenethylamine.* In an untreated rat, phenethylamine (0.7 μmole/kg) injected intravenously was almost as potent as an equimolar dose of tyramine, but 1 hr after

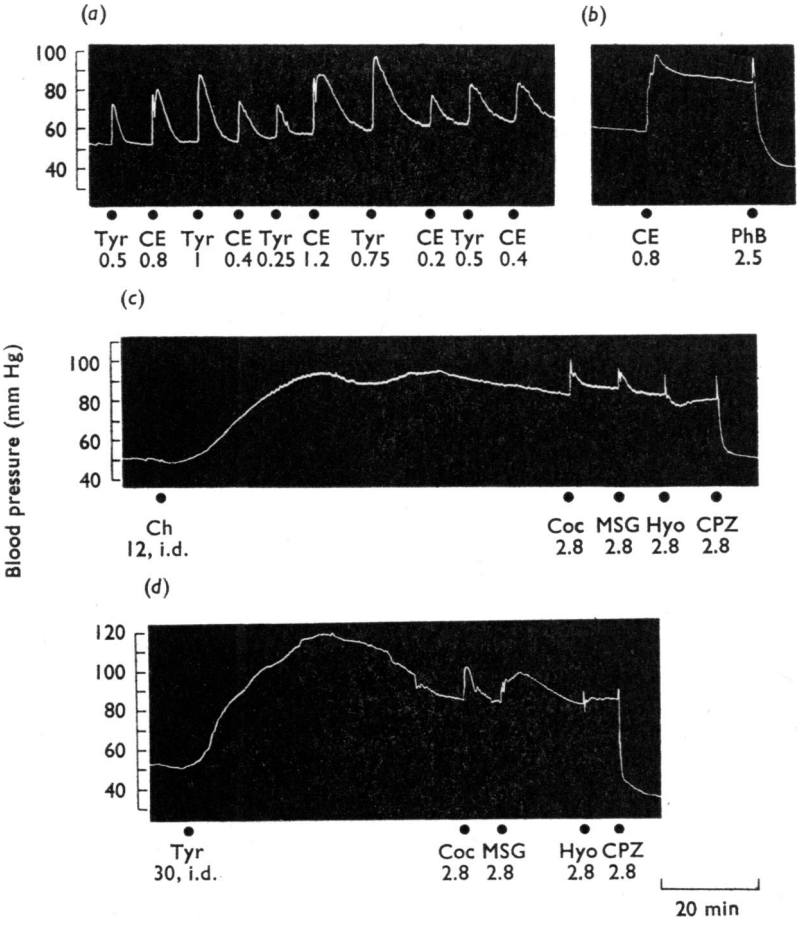

Fig. 1. Responses of the blood pressure in pithed rats to show the pressor activity of cheese or tyramine injected intravenously or intraduodenally. (*a*) Pithed rat, pressor activity of various intravenous doses of a saline extract of cheese (CE, in ml.) compared with those of intravenous tyramine (Tyr, in μmole). (*b*) Pithed rat given α-methylbenzylhydrazine (30 μmoles/kg, intravenously) 90 min previously. Cheese extract injected intravenously produced rise of blood pressure lasting 22 min until antagonized by intravenous phenoxybenzamine (PhB, in μmoles). (*c*) Pithed rat given α-methylbenzylhydrazine (120 μmoles/kg, intraperitoneally 5 hr previously and 60 μmoles/kg, intravenously 20 min beforehand). Cheese (Ch, in g) injected into the duodenum produced a rise of blood pressure completely antagonized by intravenous chlorpromazine (CPZ, in μmoles). Cocaine (Coc), methysergide (MSG) and hyoscine (Hyo) were ineffective (all doses in μmoles, intravenously). (*d*) Pithed rat given α-methylbenzylhydrazine (30 μmoles/kg, intraperitoneally) 90 min previously. Tyramine injected into the duodenum produced a rise in blood pressure completely antagonized by intravenous chlorpromazine. Intravenous cocaine, methysergide and hyoscine were ineffective.

α-methylbenzylhydrazine (30 μmoles/kg intraperitoneally) the pressor effect of phenethylamine was doubled whilst that due to tyramine was increased fourfold. Consequently, unless the cheese contained substantially larger quantities of phenethylamine, tyramine would contribute mostly to the pressor activity after amine oxidase inhibition. Phenethylamine (30 μmoles/kg) placed in the gut 90 min after α-methylbenzylhydrazine (30 μmoles/kg intraduodenally) produced a sustained rise (40 mm Hg) in blood pressure.

(3) *Tryptamine.* Tryptamine (1.5 μmoles/kg) injected intravenously produced a brief fall then a rise in blood pressure which were slightly potentiated by α-methylbenzylhydrazine (30 μmoles/kg) given intravenously 90 min previously. Tryptamine (30 μmoles/kg) placed in the gut 90 min after α-methylbenzylhydrazine (30 μmoles/kg intraduodenally) produced a sustained rise in blood pressure of 20 mm Hg.

Depressor amines. Cadaverine and histamine. The variability in pressor activity of different cheeses previously noted could have been due to the presence of depressor substances. For example, cadaverine and putrescine are present in relatively large amounts in cheese. When cadaverine (60 μmoles/kg) was injected intravenously in a dose which was greater than that found in cheese, it lowered the blood pressure slightly, but the effect was unchanged by amine oxidase inhibition. Histamine is rarely present in cheese and then only in small quantities. When histamine was given intraduodenally in a much larger amount (150 μmoles/kg) than that likely to be present in the quantity of cheese injected, it had no effect on the blood pressure even after previous treatment of the rat with an amine oxidase inhibitor.

Amino acids in cheese

The amino acids listed in Methods were tested. In addition to those present in casein, dopa was also tested, since some extracts of cheese examined spectrophotometrically (Mabbitt, 1955) gave a peak in the region corresponding to dopa.

With the exception of dopa, the amino acids were inactive on the blood pressure even after amine oxidase inhibition. The pressor effect of dopa given intravenously or intraduodenally was potentiated by amine oxidase inhibition. As shown in Fig. 2,*a* dopa (6 μmoles/kg) given intravenously elicited a 10 mm Hg rise in blood pressure lasting 8 min but after α-methylbenzylhydrazine (30 μmoles/kg, intraperitoneally 90 min previously) the same dose of dopa raised the blood pressure 35 mm Hg for over 45 min (Fig. 2,*b*). Dopa (50 μmoles/kg) injected into the duodenum produced a rise in blood pressure of 25 mm Hg in a control rat (Fig. 2,*c*), whereas the same dose injected in a rat previously treated with α-methylbenzylhydrazine (30 μmoles/kg intraperitoneally 90 min previously) produced a sustained rise of 70 mm Hg (Fig. 2,*d*). The effect of dopa on the blood pressure may be due to its conversion after absorption to dopamine, the pressor activity of which is enhanced by amine oxidase inhibition (compare Fig. 2,*e* and *f*). This may well be relevant for, as shown in Fig. 2,*h*, the pressor effect of dopa (25 μmoles/kg) injected intravenously was much reduced after treating the rat with α-methyldopa (2,000 μmoles/kg, intraperitoneally 4 hr previously), compared with its effect in an untreated rat (Fig. 2*g*). The pressor effect of dopa (20 μmoles/kg) injected intravenously was completely antagonized by Hydergine (500 μg/kg, intravenously.)

The possibility remained that other amino acids were decarboxylated by organisms in the gut to form pressor amines. This was not very likely, for tyrosine, phenylalanine and

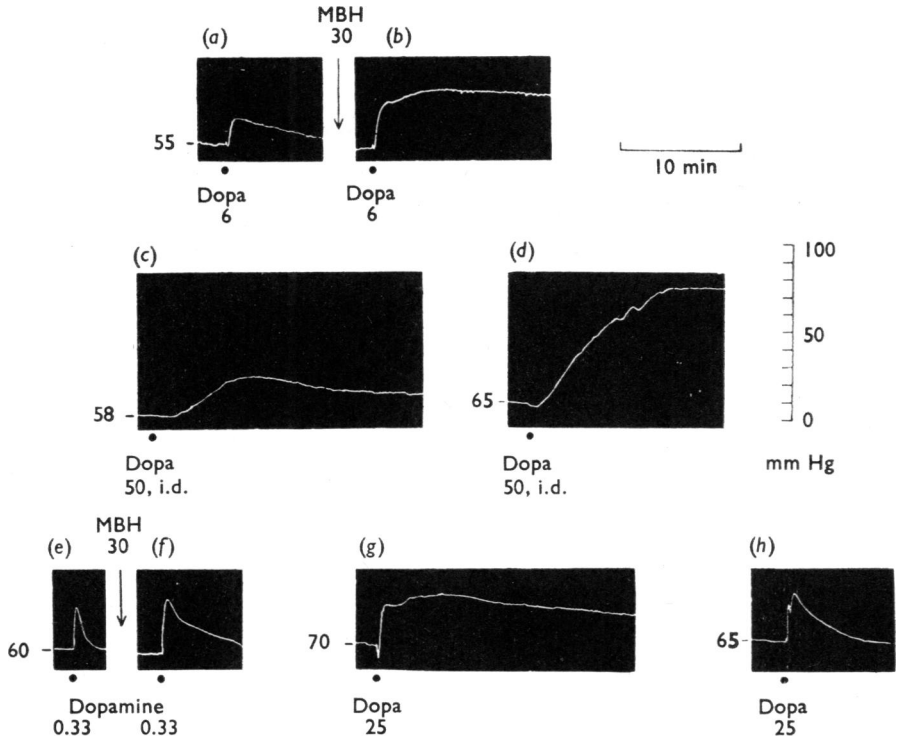

Fig. 2. Responses of the blood pressure in pithed rats to show the pressor activity of dopa and dopamine and modified by inhibition of monoamine oxidase or dopa decarboxylase. (*a*) and (*b*) Pithed rat. Rise in blood pressure evoked by dopa injected intravenously, enhanced (*b*) 90 min after the intravenous injection of α-methylbenzylhydrazine (MBH), (*c*) Pithed rat. Rise in blood pressure produced by dopa injected intraduodenally. (*d*) Pithed rat. Enhanced pressor effect of dopa injected intraduodenally after intraperitoneal injection of α-methylbenzylhydrazine 90 min previously. (*e*) and (*f*) Pithed rat. Rise in blood pressure evoked by dopamine injected intravenously enhanced (*f*) 60 min after the intravenous injection of α-methylbenzylhydrazine. (*g*) Pithed rat. Rise in blood pressure produced by dopa injected intravenously and (*h*) smaller pressor effect of same dose of dopa injected intravenously in a rat treated with α-methyldopa (2,000 μmoles/kg, intraperitoneally 4 hr previously). All doses in μmoles/kg.

tryptophan, amino acid precursors of the three amines in cheese with greatest pressor activity, were ineffective on the blood pressure after large amounts (250 to 1,000 μmoles/kg) had been placed in the duodenum of a rat previously treated with an amine oxidase inhibitor (α-methylbenzylhydrazine, 100 μmoles/kg, intraperitoneally, 3 to 5 hr previously).

Interactions between different amine oxidase inhibitors and tyramine

Apart from variations in the composition of cheese other factors which would modify its effects on blood pressure were studied. Tyramine, the principle amine in cheese, was administered to rats previously treated with different amine oxidase inhibitors given in single or repeated doses by various routes.

Route of administration of tyramine. Tyramine (60 μmoles/kg) injected into the stomach after α-methylbenzylhydrazine (30 μmoles/kg, intraperitoneally 90 min previously) was

ineffective on the blood pressure. When the same dose of tyramine was placed in the caecum or duodenum, rapid absorption occurred as shown by marked rises in blood pressure.

Effects with single doses of different inhibitors. Four monoamine oxidase inhibitors were tested, with single intraduodenal doses of each over a range from 0.25 to 30 μmoles/kg; 90 min later tyramine (60 μmoles/kg) was injected into the duodenum and the effect on blood pressure recorded. As shown in Fig. 3,*a* the pressor effect of intraduodenal tyramine

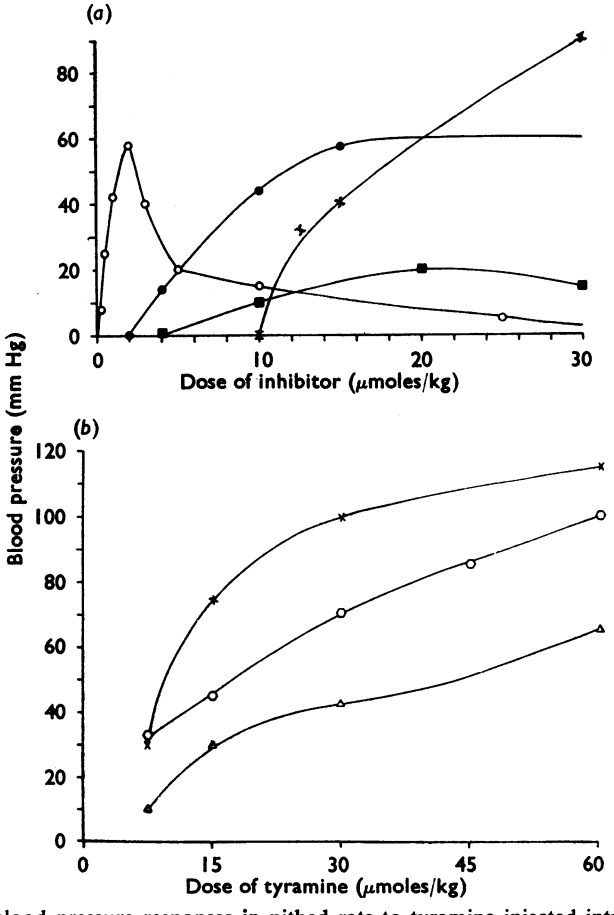

Fig. 3. Graphs of blood pressure responses in pithed rats to tyramine injected intraduodenally showing effects with type of, and route of administration of inhibitor. Each point represents an experiment in one rat. (*a*) Pithed rats blood pressure responses in mm Hg to tyramine (60 μmoles/kg) placed in the duodenum 90 min after different amine oxidase inhibitors in increasing dosage (0.25 to 30 μmoles/kg, intraduodenally). Tranylcypromine O——O; phenelzine ●——●; nialamide ■——■; α-methylbenzylhydrazine ×——×. (*b*) Pithed rats blood pressure responses in mm Hg to tyramine (7.5, 15, 30, 45 and 60 μmoles/kg) given intraduodenally after treatment with α-methylbenzylhydrazine (30 μmoles/kg each dose) in three different ways. O——O: one dose of α-methylbenzylhydrazine orally (via stomach tube) 18 to 24 hr previously, followed by an intraduodenal dose 90 min before tyramine. ×——×: two doses of α-methylbenzylhydrazine as above, but given intraperitoneally. Δ —— Δ: single intraperitoneal dose of α-methylbenzylhydrazine 90 min before tyramine.

was maximally enhanced after small amounts of tranylcypromine or phenelzine (2 and 15 μmoles/kg, intraduodenally, respectively), doses not much greater than those used therapeutically in man (0.5 μmole/kg by mouth). Larger doses of α-methylbenzylhydrazine (30 μmoles/kg, intraduodenally) were required than with tranylcypromine or phenelzine and only small rises in blood pressure were elicited with tyramine after a single dose of nialamide. Larger doses of monamine oxidase inhibitor were required to permit absorption of tyramine from the gut than to potentiate the effects of tyramine injected intravenously. Thus 90 min after a single does of α-methylbenzylhydrazine (5 μmoles/kg, intraduodenally) intraduodenal tyramine (50 μmoles/kg) was ineffective on the blood pressure whereas the response to intravenous tyramine (0.5 μmoles/kg) was considerably enhanced and prolonged.

Tranylcypromine and phenelzine had pressor activity of their own, that of tranylcypromine being the more prolonged. After large intraduodenal doses of tranylcypromine, tachyphylaxis developed to tyramine given intraduodenally or intravenously. This can be seen in Fig. 3,*a*, which shows that the pressor effect of tyramine was much less with an intraduodenal dose of tranylcypromine of 4 to 6 μmoles/kg. With doses of tranylcypromine larger than 6 μmoles/kg the pressor effects obtained with tyramine became progressively smaller. This phenomenon was not observed with the other amine oxidase inhibitors in the doses used.

To avoid tachyphylaxis in subsequent experiments, α-methylbenzylhydrazine, a potent inhibitor with negligible sympathomimetic properties, was used.

Effects of repeated doses of monoamine oxidase inhibitor. The various schedules of treatment are given in Table 1. The inhibitor was given by stomach tube for 1 or 3 days or by

TABLE 1

BLOOD PRESSURE RESPONSE TO TYRAMINE (60 μMOLES/KG) INJECTED INTRADUODENALLY IN RATS TREATED WITH α-METHYLBENZYLHYDRAZINE (30 μMOLES/KG BY MOUTH OR INTRAPERITONEALLY)

Additional intraduodenal injections of α-methylbenzylhydrazine (30 μmoles/kg) were given, when indicated, 90 min before the tyramine.

	α-Methylbenzylhydrazine		
Route	Pretreatment (days)	Additional injection	Pressor response to tyramine (mm Hg)
Oral	0	+	100
	1	+	105
	3	+	105
Intraperitoneal	1	+	115
	9	+	105
	9	−	105

intraperitoneal injection for 1 or 9 days followed in all but one experiment by an intraduodenal dose of α-methylbenzylhydrazine 90 min before intraduodenal tyramine (60 μmoles/kg). Maximal blood pressure rises of 100 to 115 mm Hg ensured whether the rat had been treated for 1 or 9 days. This means that, provided dosage is adequate, reactions in the rat occur just as readily with few as with many doses of inhibitors.

Route of administration of amine oxidase inhibitor. The above experiments suggested that amine oxidase inhibition was equally satisfactory whether the inhibitor was given by

mouth or intraperitoneally. The importance of the route of administration was tested further in three ways: a dose of α-methylbenzylhydrazine (30 μmoles/kg) was given by stomach tube 18 to 24 hr previously followed after pithing the rat by an intraduodenal dose (30 μmoles/kg) 90 min before tyramine; the same procedure was followed except that the drugs were injected intraperitoneally; a single intraperitoneal dose of α-methylbenzylhydrazine (30 μmoles/kg) was injected 90 min before the tyramine.

As shown in Fig. 3,*b* large pressor effects of tyramine were obtained whether the inhibitor was given orally or parenterally.

Effects of a second dose of inhibitor. The results in Fig. 3,*b*, also show a large response to tyramine following a second dose of inhibitor irrespective of its route of administration.

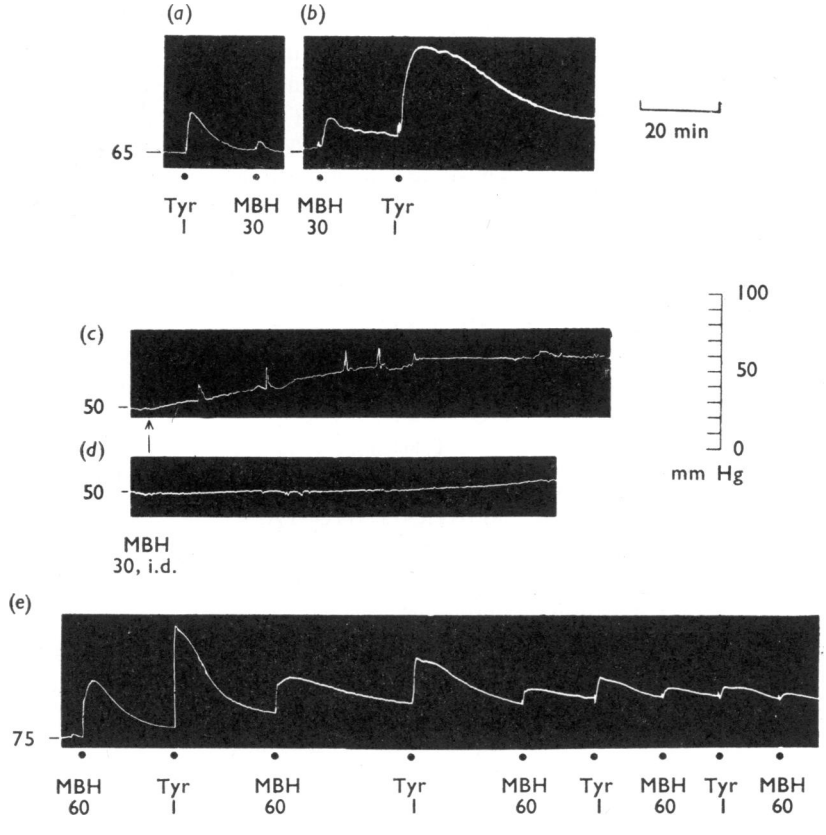

Fig. 4. Blood pressure responses in pithed rats showing potentiation and cross-tachyphylaxis to tyramine and α-methylbenzylhydrazine after amine oxidase inhibition. (*a*) and (*b*) Pithed rat. (*a*) Pressor effects due to tyramine (Tyr) and α-methylbenzylhydrazine (MBH); (*b*) enhanced effects in same rat 90 min after previous dose of α-methylbenzylhydrazine. All injections intravenous. (*c*) and (*d*) Pithed rats. Blood pressure effect of α-methylbenzylhydrazine given intraduodenally in untreated rat (*d*), compared with (*c*) same dose of α-methylbenzylhydrazine given 18 hr after intraperitoneal injection of the same dose of the same inhibitor. (*e*) Pithed rat. Blood pressure effects of intravenous alternating doses of α-methylbenzylhydrazine (60 μmoles/kg) and tyramine (1 μmole/kg) in a rat treated with α-methylbenzylhydrazine (30 μmoles/kg, intraperitoneally) 18 hr previously. Cross-tachyphylaxis developed to these amines as shown by diminishing pressor effects. All doses in μmoles/kg.

The possibility that the second dose of inhibitor enhanced the effects of tyramine by some mechanism other than monoamine oxidase inhibition was studied by testing the effects of the inhibitor on the blood pressure after inactivation of monoamine oxidase. The effects of tyramine (1 μmole/kg) and of α-methylbenzylhydrazine (30 μmoles/kg) injected intravenously into an untreated rat are shown in Fig. 4,*a* and compared with those in the same rat 90 min later (Fig. 4,*b*) when the pressor effects of both substances are increased two- to fourfold in amplitude and considerably prolonged. The potentiation of tyramine and of α-methylbenzylhydrazine was presumably due to the first dose of α-methylbenzylhydrazine. As shown in Fig. 4,*c* similar potentiation occurred on intraduodenal injection of α-methylbenzylhydrazine. α-Methylbenzylhydrazine (30 μmoles/kg) injected intraduodenally was without effect on the blood pressure, but the same dose in a rat given α-methylbenzylhydrazine 18 hr previously (30 μmoles/kg, intraperitoneally) produced a sustained rise of 35 mm Hg (Fig. 4,*c*). When repeated alternating doses of α-methylbenzylhydrazine and tyramine were given to a rat in which amine oxidase has been inhibited by treatment with a single dose of the inhibitor the initial phase of potentiation was followed by tachyphylaxis to both substances. Fig. 4,*e* shows this effect in a rat treated with α-methylbenzylhydrazine (30 μmoles/kg, intraperitoneally 18 hr previously). A large pressor response was obtained with the first intravenous injection of α-methylbenzylhydrazine (60 μmoles/kg) and of tyramine (1 μmole/kg), but with repeated injections of the two substances the responses grew progressively smaller.

Fowl

As shown in Fig. 5, a Canadian cheddar cheese (10 g/kg) injected intraduodenally 90 min after α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally) produced a rise in blood pressure of 50 mm Hg reaching its maximum in about 20 min. Similar blood

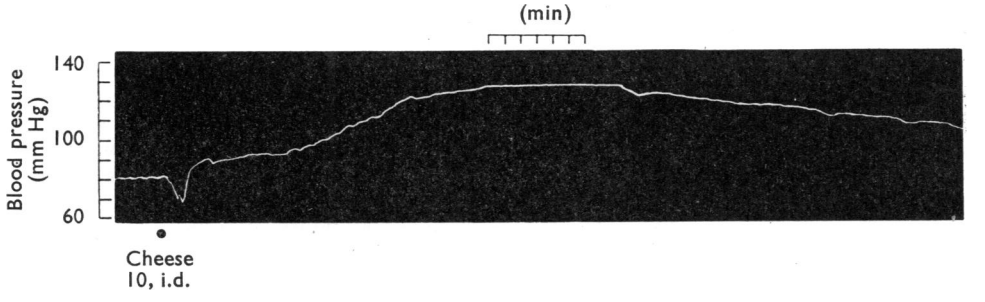

Fig. 5. Response of the blood pressure in a 1.5 kg fowl anaesthetized with pentobarbitone to homogenized Canadian cheddar cheese (10 g/kg) injected into the duodenum. The fowl had been treated with α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally 90 min previously).

pressure changes were obtained after the intraduodenal administration of tyramine (50 μmoles/kg) in another fowl previously treated with α-methylbenzylhydrazine. The same intraduodenal doses of cheese or of tyramine were ineffective on the blood pressure in the absence of previous treatment with α-methylbenzylhydrazine.

Cat

Absorption of a sympathomimetic substance from cheese

Tests were made to demonstrate that, after an amine oxidase inhibitor, a sympathomimetic substance was absorbed into the systemic circulation from cheese placed in the intestine. Vane's (1957) superfusion method was used in which a rat stomach strip suspended in a cat's extracorporeal circulation relaxes to sympathomimetic amines. A cat was first treated with α-methylbenzylhydrazine (60 μmoles/kg intraperitoneally 24 hr previously). As shown in Fig. 6 marked relaxation of the stomach strip developed within 12 min of placing cheese

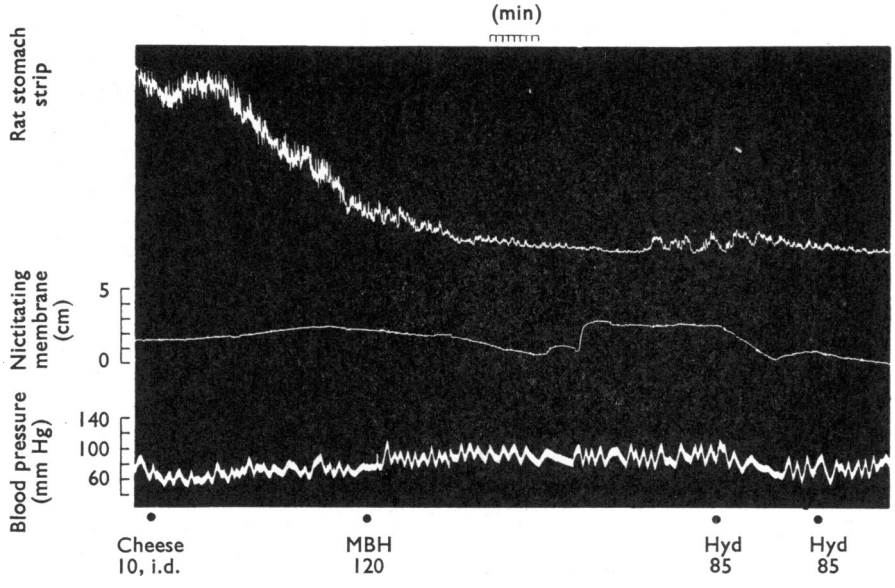

Fig. 6. Cat 3.0 kg; chloralose anaesthesia. Response of a rat stomach strip to superfused blood from the cat's carotid artery, of the cat's nictitating membrane and of the cat's blood pressure to homogenized camembert cheese (10 g/kg) injected into the duodenum. The cat had been treated with α-methylbenzylhydrazine (60 μmoles/kg, intraduodenally 20 hr previously). Relaxation of the rat stomach strip began 5 min after injecting cheese and was maximal at 55 min. Contraction of the acutely denervated nictitating membrane developed 10 min after injecting cheese but waned after 60 min. Blood pressure rose 30 mm Hg, 55 min after injecting cheese. A further dose of α-methylbenzylhydrazine (MBH, 120 μmoles/kg, intraduodenally) was given 40 min after injecting the cheese. Subsequent contraction of the nictitating membrane was abolished by Hydergine (Hyd, 85 μg/kg, intravenously).

(10 g/kg) in the duodenum. There was a minimal contraction of the nictitating membrane and rise in blood pressure. As the effect on the nictitating membrane waned, α-methylbenzylhydrazine (120 μmoles/kg) was injected intraduodenally. Some 30 min later the nictitating membrane again contracted. This may have been due to more of the sympathomimetic substance in cheese being absorbed, or to sympathomimetic effects of α-methylbenzylhydrazine developing after treatment with an amine oxidase inhibitor. The contraction of the nictitating membrane was abolished by Hydergine.

The plasma was tested from another cat given α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally 2 hr previously). It did not contain any tyramine-like substance but 60 min after giving cheese (10 g/kg), when the nictitating membrane had contracted, the plasma contained 0.009 μmoles/ml. of a substance with the fluorescent characteristics of tyramine or its β-hydroxylated derivative, octopamine.

Blood pressure and nictitating membrane effects due to cheese

Cheese homogenate (10 g/kg) injected intraduodenally into two untreated cats was without effect on the blood pressure or the nictitating membrane. Tests were next made with cheese given intraduodenally to sixteen cats, 90 or 120 min after an intraduodenal dose of an amine oxidase inhibitor. The effects on the blood pressure were variable in degree, onset and duration. There was a rise in blood pressure in eight cats, a fall in three, and a negligible change in five. Figs. 8 and 9 show that the usual rise in blood pressure was 30 to 60 mm Hg; the maximum rise was 90 mm Hg commencing immediately after giving the cheese and lasting for 1 hr (until antagonized by Hydergine); the fall in blood pressure was not greater than 20 to 30 mm Hg; and the effect of intraduodenal cheese on the blood pressure was immediate, although delays of up to 20 min occurred in other experiments. Larger rises in blood pressure were swifter in onset than smaller ones. The delay in onset may have been due to a low concentration of pressor amine in the cheese or to slow absorption of the amine because of fatty constituents in cheese. When the cheese homogenate had been centrifuged to remove the fatty particles and the supernatant fluid was injected into the duodenum, the rise in blood pressure was immediate (Fig. 10,*c*).

The effects of cheese on the nictitating membrane were more consistent than those on the blood pressure, and contraction developed in fourteen of the sixteen cats. This began 10 to 20 min after injecting the cheese, ranged from 1 to 9 cm (mean 3.5 cm) and lasted 2 to 3 hr. The effects on the nictitating membrane and on the blood pressure were directly related, a large rise in blood pressure accompanying considerable increase in tone of the membrane.

Effects of cheese after various amine oxidase inhibitors

The sympathomimetic effects were obtained with cheese given intraduodenally after a single dose of various amine oxidase inhibitors (Table 2, including the amines tranylcypromine and pargyline, the hydrazines α-methylbenzylhydrazine, phenelzine, pheniprazine and phenoxypropazine, and the hydrazides isocarboxazid, pivhydrazine, iproniazid and nialamide. The dose given and other details are shown in Table 2. The intraduodenal dose required with most of the inhibitors ranged from 72 to 174 μmoles/kg but the dose with tranylcypromine was smaller (2 to 14 μmoles/kg), indicating its potency.

Localization of the site of action of the sympathomimetic substances in cheese

Brain, spinal cord and superior cervical ganglion. The sympathomimetic effects of cheese were obtained in cats previously treated with amine oxidase inhibitors and in which the brain had been destroyed (Table 2) or the cervical sympathetic trunks or the superior cervical and nodose ganglia had been acutely removed. The effects of the cheese substance on the nictitating membrane were not therefore primarily due to an action on sympathetic

TABLE 2

SYMPATHOMIMETIC RESPONSES DUE TO CHEESE GIVEN INTRADUODENALLY IN CATS TREATED WITH A SINGLE INTRADUODENAL DOSE OF ONE OF VARIOUS MONOAMINE OXIDASE INHIBITORS

N.M. = nictitating membrane; B.P. = blood pressure; + = contraction of nictitating membrane or pressor response; − = no effect. The cheese was camembert in a dose of 10 g/kg

	Monoamine oxidase inhibitors			Intrinsic sympathomimetic action	Response to cheese	
Preparation	Chemical type	Name	Dose (μmole/kg)		N.M.	B.P.
Chloralose	Amine	Tranylcypromine	2.8	++	+	−
			5.6	++	+	+
			14.0	++	+	−
Chloralose		Pargyline	260	++	+	+
Chloralose	Hydrazine	α-Methylbenzyl-hydrazine	120	−	+	+
			120	−	+	+
			120	−	+	+
Spinal		Phenelzine	72	+	+	−
Chloralose		Pheniprazine	80	++	−	−
			40	+	+	−
Chloralose		Phenoxypropazine	35	+	+	−
Chloralose	Hydrazide	Isocarboxazid	174	+	+	+
Chloralose		Pivhydrazine	100	−	+++	+
Chloralose		Iproniazid	90	−	+	+
Chloralose		Nialamide	38	−	+	+
			76	−	+	+

centres in the brain-stem or spinal cord, or to a nicotine-like action on the superior cervical ganglion.

Cervical sympathetic postganglionic nerve. (1) *Experiments with bretylium.* Bretylium selectively depresses responses to stimulation of sympathetic postganglionic nerves. As shown in Fig. 7, after an intravenous dose of bretylium (25 μmoles/kg) the contraction of the nictitating membrane on electrical stimulation of the cervical sympathetic postganglionic trunk (Fig. 7,*a*) was abolished after 85 min (Fig. 7,*d*). However, contractions of the membrane were still obtained with intravenous injections of cheese extract (1 mg/kg) or of tyramine (2 μmoles/kg). The responses were larger (Fig. 7,*d*) than before injecting bretylium and were greater still 75 min later (Fig. 7,*e*). The pressor activities of cheese extract and of tyramine were also enhanced after bretylium. These findings suggest that cheese extract and tyramine produce their sympathomimetic effects by acting on chromaffine tissue in or beyond the sympathetic nerve terminal. The effects of the cheese extract (1 mg/kg, intravenously) on the nictitating membrane were further prolonged 30 min after injecting α-methylbenzylhydrazine (60 μmoles/kg intravenously). Consequently it was possible to inactivate the intraneuronal monoamine oxidase after abolition of adrenergic synaptic transmission.

(2) *Degeneration of the cervical sympathetic postganglionic nerve.* If the sympathomimetic substance in cheese is tyramine, it would act by releasing noradrenaline from sympathetic postganglionic nerve terminals but would be ineffective on tissues in which the noradrenaline had disappeared after degeneration of the terminals. The effect of cheese was compared on the acutely and on the chronically denervated nictitating membrane after the adrenal glands had been excluded from the circulation by ligatures to prevent release of catechol

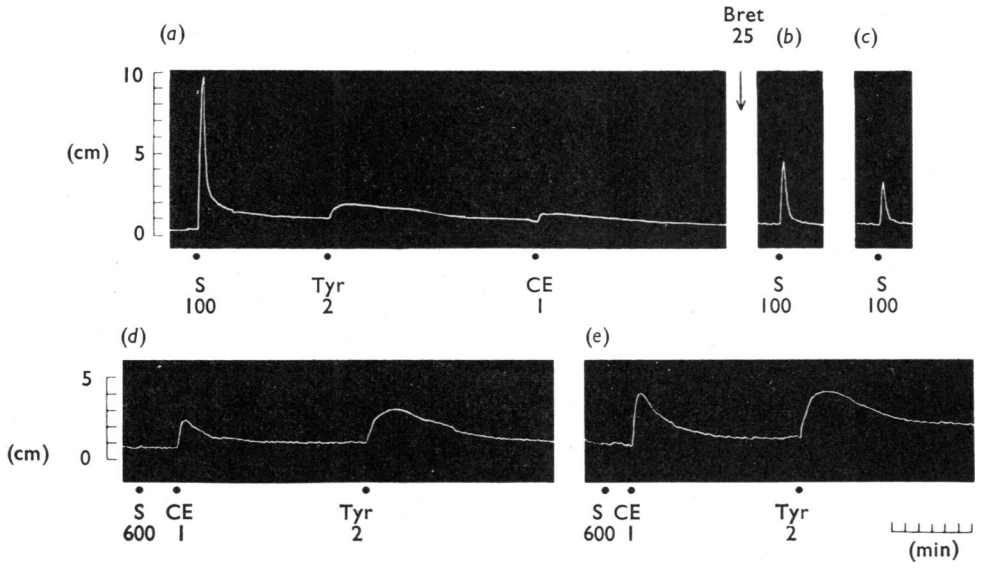

Fig. 7. Response of the nictitating membrane in a 2.0 kg cat anaesthetized with chloralose to stimulation of the cervical sympathetic postganglionic nerve, to tyramine and to a saline extract of Canadian cheddar cheese before and after bretylium. (*a*) Contraction of the nictitating membrane on electrical excitation (S) of the postganglionic trunk (100 supramaximal shocks at 10 shocks/sec) and intravenous injections of tyramine (Tyr, in μmoles/kg) and cheese extract (CE, in mg/kg). Between (*a*) and (*b*) bretylium (Bret, 25 μmoles/kg) was injected intravenously. There was diminished response of the membrane on stimulating the postganglionic trunk at (*b*), 30 min, and (*c*), 60 min, and at (*d*) (85 min) there was no response with 600 supramaximal shocks (S). In contrast, the amplitude and duration of the contraction produced by intravenous injection of cheese extract and of tyramine were enhanced, and had increased again (*e*) 160 min after bretylium.

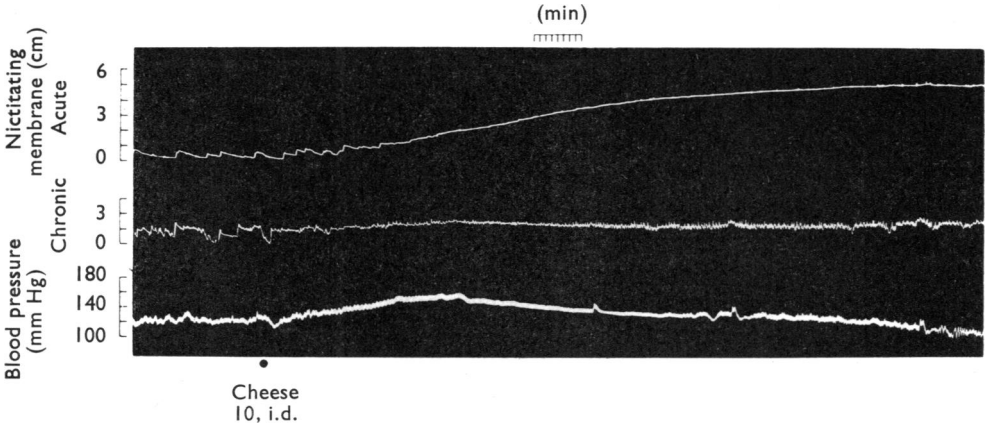

Fig. 8. Responses of both nictitating membranes and blood pressure in a 2.3 kg cat anaesthetized with chloralose to homogenized camembert cheese (10 g/kg) injected into the duodenum. The cat had been treated with α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally 2 hr previously). The acutely denervated nictitating membrane (uppermost trace) contracted after the injection of cheese but the chronically denervated membrane (middle trace), with the superior cervical and nodose ganglia removed 13 days previously, did not respond. Blood pressure (lowest trace) rose 40 mm Hg. The adrenal glands had been excluded from the circulation by ligatures.

amines. The chronically denervated nictitating membrane showed considerable spontaneous activity, still present (Fig. 8) when cheese was given 3 hr after the operative procedure.

As seen in Fig. 8, administration of cheese (10 g/kg, intraduodenally) 2 hr after α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally) elicited sustained contraction of the acutely denervated (uppermost trace) but had no effect on the chronically denervated nictitating membrane (middle trace); the blood pressure rose by 40 mm Hg (lowest trace). The difference in effects on the acutely and chronically denervated membranes indicated that the cheese substance acts upon the intraneuronal noradrenaline. The failure of the chronically denervated membrane to contract meant that these effects were not due to absorption of catechol amines from cheese or their indirect liberation from other organs.

Experiments with drug antagonists

If the sympathomimetic effects of cheese are due to the absorption and circulation of tyramine or of phenethylamine, they should be diminished or abolished by antagonists at α-receptors or by cocaine. Tyramine or a near congener was found in the blood-stream in amounts of 0.009 μmole/ml. at a time when the blood pressure was raised and the nictitating membrane had contracted after intraduodenal cheese. Assuming the blood volume of a cat to be 150 ml., then 1 to 2 μmoles of the pressor substance are circulating at the height of the response. Accordingly, at least 1 μmole/kg of the antagonist injected intravenously would be required for effective antagonism. In fact, larger intravenous doses of antagonists than these were required, and the effects on the blood pressure were easier to antagonize than those on the nictitating membrane.

Phenoxybenzamine (9 μmoles/kg) and chlorpromazine (2.8 to 6 μmoles/kg) completely abolished the rise in blood pressure produced by cheese; Hydergine (300 to 500 μg/kg) either partially or completely abolished the effects on blood pressure. Cocaine (3 to 10 μmoles/kg) was only partly effective as an antagonist. Methysergide (1.25 to 1.6 μmoles/kg), chlorpheniramine (5 μmoles/kg) and hyoscine (4.5 to 5 μmoles/kg) were ineffective. After an amine oxidase inhibitor, methysergide and chlorpheniramine in these doses sometimes had pressor activity. The only effective antagonists to the action on the nictitating membrane were phenoxybenzamine or chlorpromazine and the doses required were larger than those effective on the blood pressure. Partial reduction of the effect on the membrane was obtained with Hydergine (300 to 500 μg/kg). The effects of some of these antagonists, all of which were given intravenously, are shown in Fig. 9,*a* and *b*.

A dose of tyramine (50 μmoles/kg), comparable to that present in some cheeses, was placed in the duodenum 90 min after α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally). It produced sympathomimetic effects similar in duration and magnitude to those obtained with cheese and which were also antagonized by intravenous phenoxybenzamine (4 μmoles/kg) or chlorpromazine (4 μmoles/kg).

Failure to obtain sympathomimetic effects with cheese

With marked sympathomimetic effects of a single dose of an amine oxidase inhibitor. Many of the inhibitors in the doses used (Table 2) had marked sympathomimetic actions on the blood pressure and nictitating membrane which obscured the effects produced by cheese. Those responsible were tranylcypromine, pargyline, the hydrazines tested except for α-methylbenzylhydrazine, and the hydrazide, isocarboxazid; all were given intra-

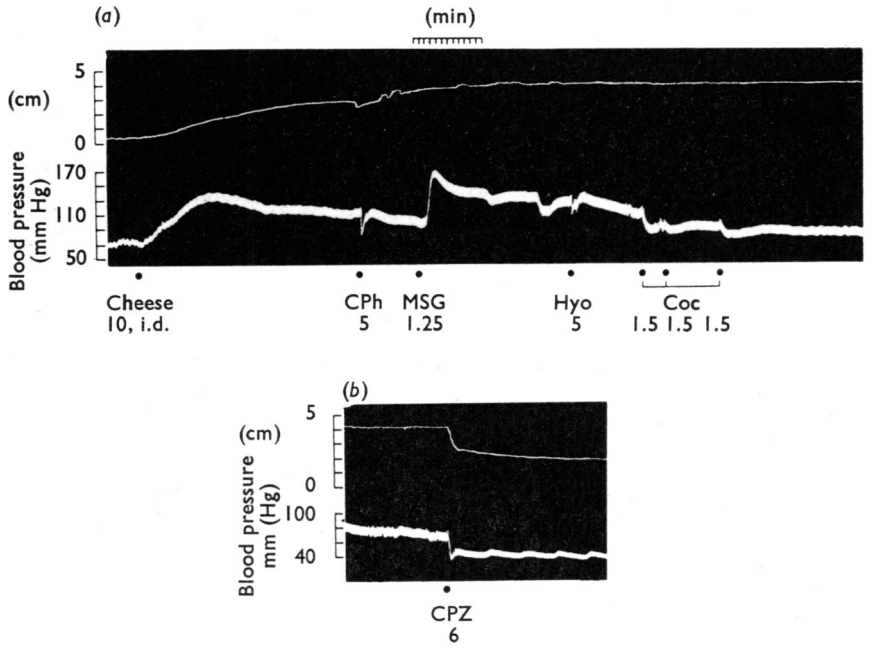

Fig. 9. Responses of the nictitating membrane and blood pressure in a 3.4 kg cat anaesthetized with chloralose to homogenized camembert cheese (10 g/kg) injected into the duodenum. The cat had been treated with α-methylbenzylhydrazine (120 μmoles/kg, intraduodenally 2 hr previously). (*a*) The acutely denervated nictitating membrane contracted and the blood pressure rose 70 mm Hg after the injection of cheese. Chlorpheniramine (CPh), methysergide (MSG) and hyoscine (Hyo) were ineffective on the nictitating membrane. Chlorpheniramine and hyoscine were ineffective on the blood pressure, but methysergide had a pressor action. There was a modest antagonistic action of cocaine (Coc) on the blood pressure. Drugs were injected intravenously and doses are in μmoles/kg. (*b*) The contraction of the nictitating membrane was abolished and blood pressure was lowered by chlorpromazine (CPZ, 6 μmoles/kg, intravenously).

TABLE 3

EFFECTS OF CHEESE INJECTED INTRADUODENALLY IN CATS TREATED WITH A MONOAMINE OXIDASE INHIBITOR

I.p. = intraperitoneal; i.d. = intraduodenal; i.v. = intravenous; N.M. = nictitating membrane; B.P. = blood pressure; + = contraction of nictitating membrane or pressor response; * no response to cheese, tachyphylaxis to intravenous phenethylamine, tyramine and hydroxyphenethylamine. The cheese was camembert in a dose of 10 g/kg

	Monoamine oxidase inhibitor			Response to cheese	
Preparation	Name	Dose (μmole/kg)	Additional treatment (μmole/kg)	N.M.	B.P.
Chloralose	Tranylcypromine	28, orally, 3 days	–	+	+
Spinal	Tranylcypromine	38, orally, 8 days	28, i.d., 2 hr before cheese	*	*
Chloralose	α-Methylbenzyl-hydrazine	120, i.p., 1 day	120, i.v., 1 hr before and 120, i.d., 10 min before cheese	+	+
Chloralose	α-Methylbenzyl-hydrazine	60, i.p., 1 day and 120, i.p., 3 days previously	120, i.v., 2 hr before cheese	+	+

duodenally. The lack of effect of cheese in these circumstances could be attributed to the sympathomimetic effects of the inhibitors rather than to inadequacy of monoamine oxidase inhibition. Thus the effects of cheese were not obtained after a single large dose of tranylcypromine (28 μmoles/kg) which had marked sympathomimetic actions, but were elicited with smaller doses of tranylcypromine (2.8 to 14 μmoles/kg). After a much larger dose of α-methylbenzylhydrazine (120 μmoles/kg) which has little sympathomimetic activity, the same amount (10 g/kg) of the cheese which had been ineffective with tranylcypromine now produced a rise of blood pressure and contraction of the nictitating membrane.

With repeated doses of monoamine oxidase inhibitors. Cheese was also ineffective after repeated doses of some amine oxidase inhibitors, although inhibition of the enzyme was likely to be maximal. This occurred when inhibitors had marked sympathomimetic properties, and was apparently related to the interval between the last dose of the inhibitor and giving the cheese. Details of representative experiments are given in Table 3. In the first of these, tranylcypromine (28 μmoles/kg) had been given daily by stomach tube for 3 days. The cat was then anaesthetized and camembert cheese (10 g/kg) was injected intraduodenally 24 hr after the last dose of the inhibitor. A moderate rise of blood pressure and contraction of the nictitating membrane ensued. Another cat was given tranylcypromine (38 μmoles/kg) daily by stomach tube for 8 days. It was then anaesthetized, the brain destroyed, and camembert cheese (10 g/kg) was injected intraduodenally 2 hr after a further dose of tranylcypromine (28 μmoles/kg, intraduodenally). In contrast to the previous experiment, cheese (10 g/kg) was ineffective on the blood pressure or the nictitating membrane. Cross-tachyphylaxis between tranylcypromine and sympathomimetic substances

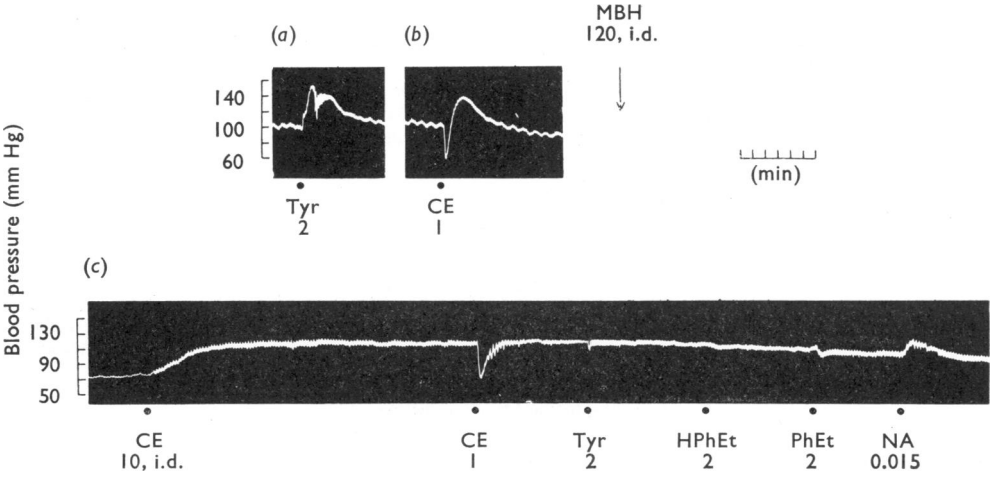

Fig. 10. Response of blood pressure in a 2.2 kg cat, anaesthetized with chloralose, to tyramine (Tyr), and to a saline extract of cheddar cheese (CE, in g/kg) injected intravenously or intraduodenally. There was a rise in blood pressure with tyramine (*a*) and the saline extract of cheddar cheese (*b*) injected intravenously. Between (*b*) and (*c*) α-methylbenzylhydrazine (120 μmoles/kg) was injected into the duodenum. (*c*) 50 min later, a saline extract of the same cheddar cheese (10 g/kg) was injected into the duodenum and produced a rise in blood pressure of 40 mm Hg. An intravenous injection of 1 g/kg of the saline extract of cheddar cheese was now depressor, and tyramine, β-hydroxyphenethylamine (HPhEt) and phenethylamine (PhEt) were ineffective. Noradrenaline (NA) injected intravenously produced a small increase in blood pressure. All doses of drugs in μmoles/kg.

including those in cheese was suggested by the finding that phenethylamine, tyramine and β-hydroxyphenethylamine given intravenously in doses of 2 μmoles/kg were also ineffective on the blood pressure and nictitating membrane.

Two cats were next treated for 1 to 3 days with much larger doses of α-methylbenzylhydrazine than had been given with tranylcypromine (Table 3). These doses were followed by injections of α-methylbenzylhydrazine up to a total of 240 μmoles/kg intravenously and intraduodenally in the 2 hr before injecting cheese (Table 3). In contrast to the experiments with tranylcypromine in which the last dose of the inhibitor had been injected 2 hr before cheese, the effects on the blood pressure and nictitating membrane were still obtained.

During the sustained rise of blood pressure produced by cheese. The pressor effect of the cheese extract injected intravenously was abolished during the rise in blood pressure elicited by cheese. As shown in Fic. 10,*c*, once the blood pressure had been raised by the intraduodenal injection of cheese, the intravenous injection of 1.0 g/kg of the same cheese extract elicited a fall of blood pressure whereas initially it had depressor followed by pressor actions (Fig. 10,*b*). The intravenous injection of tyramine (2 μmoles/kg) which originally raised the blood pressure (Fig. 10,*a*) was now ineffective. The intravenous injections of β-hydroxyphenethylamine and phenethylamine in doses of 2 μmoles/kg, which would normally produce a rise in blood pressure, were also ineffective (Fig. 10,*c*) suggesting that tachyphylaxis had developed. Noradrenaline (0.015 μmole/kg) injected intravenously elicited a small pressor response.

DISCUSSION

To explain the hypertensive crisis which sometimes occurs after eating cheese in patients taking monoamine oxidase inhibitors (Blackwell, 1963) it was necessary to show that a substance present in cheese was capable of raising the blood pressure in animals and that this substance was only absorbed from the intestine into the systemic blood-stream after monoamine oxidase inhibition. It was also necessary to identify the substance, its site of action and the various factors which modified its effects on blood pressure.

Intravenous injections of a saline extract of cheddar cheese raised the blood pressure of the pithed rat and anaesthetized cat. In both species the effects were potentiated by previous treatment with a monoamine oxidase inhibitor which suggested that the pressor substance was inactivated by oxidative deamination.

In control experiments cheese injected intraduodenally produced no change in the blood pressure of three species, the rat, fowl and cat, but after treatment with an amine oxidase inhibitor, rises in mean arterial pressure of 30 to 60 mm Hg ensued. This is comparable to the mean blood pressure rise (calculated from the systolic and diastolic blood pressures) of patients during hypertensive crises. Blood pressure rises in excess of this figure were obtained in the pithed rat and have been reported in man.

The presence of a circulating sympathomimetic amine absorbed from intraduodenal cheese after treatment with an monoamine oxidise inhibitor was shown by relaxation of a rat isolated stomach strip suspended in a cat's extracorporeal circulation. At the same time tyramine was detected in the plasma in a pharmacologically active concentration. Pharmacological evidence was sought to confirm that the cheese pressor substance absorbed from the gut was tyramine. Cheese and equivalent amounts of tyramine placed in the duodenum

produced similar effects on the blood pressure of the rat and cat and nictitating membrane of the cat; in each case the effects were abolished by identical doses of the same drug antagonists. The experiments showed that the sympathomimetic effects due to tyramine and the cheese pressor substance were mediated through the α-receptors in blood vessels, and experiments on the nictitating membrane after chronic denervation or after bretylium indicated that this effect on α-receptors was mediated indirectly through noradrenaline release. It was difficult to demonstrate antagonism of the sympathomimetic effects due to cheese with cocaine, since treatment with a monoamine oxidase inhibitor appeared to interfere with its competition for the noradrenaline store. Further evidence that the cheese pressor substance might be tyramine was obtained when cross-tachyphylaxis was demonstrated between the cheese pressor substance and tyramine as well as other indirectly acting sympathomimetic amines. The effects of tyramine given by mouth on the blood pressure are potentiated after amine oxidase inhibitors in man (Horwitz, Lovenberg, Engelman & Sjoerdsma, 1964) and in rats (Tedeschi & Fellows, 1964).

There is indirect evidence to suggest that tyramine is the principal pressor substance in cheese, which is known to contain amines formed by bacterial decarboxylation of amino acids in casein (Silverman & Kosikowski, 1956). Tyrosine, from which tyramine is formed, was first isolated from cheese (Liebig, 1846). Tyramine metabolites appear in the urine of untreated subjects after eating cheese which contains large amounts of tyramine (Asatoor, Levi & Milne, 1963). However, it cannot be assumed that the pressor effects of cheese are always due to tyramine alone since certain cheeses also contain phenethylamine or tryptamine and our experiments would not have distinguished between the effects of these substances. Tryptamine is seldom present in cheeses and had limited pressor activity when injected into the duodenum after an amine oxidase inhibitor, but phenethylamine has marked pressor actions and cheese sometimes contains quite large amounts.

The tyramine equivalent of different cheeses is very variable. Extracts from four different cheeses assayed against tyramine in the blood pressure of the pithed rat showed a tenfold variation in pressor content from an equivalent of 0.6 to 6 μmoles of tyramine per g of cheese and these figures agree with estimates of tyramine present in cheese by chromatographic methods (Horwitz *et al.*, 1964; Blackwell & Mabbitt, 1965). This wide range in the tyramine content of different cheeses could account for the variability in sympathomimetic effects due to intraduodenal cheese in the cat and the observation that patients taking amine oxidase inhibitors often eat cheese with impunity. A further modifying factor could be the occurrence of depressor substances in cheese. Cadaverine was tested but its depressor action and absorption from the gut were not significantly altered by amine oxidase inhibition, so that its presence was unlikely to modify the effects due to tyramine.

Other factors which might modify the absorption or action of tyramine or the pressor substance in cheese were studied. Adequate inhibition of monoamine oxidase in the gut and liver was essential to allow tyramine from cheese in the intestine to enter the systemic circulation. Potentiation of tyramine injected intravenously occurred after doses of the monoamine oxidase inhibitor which did not allow absorption of tyramine from the intestine. Tyramine was absorbed from the caecum and duodenum after amine oxidase inhibition but not from the stomach. Effects due to cheese could therefore be modified by factors affecting time and rate of gastric emptying. All the inhibitors tested in single doses by various routes of administration permitted the absorption of cheese amines but tranylcy-

promine and phenelzine were most effective and these are the two inhibitors responsible for 90% of hypertensive crises in humans (Marks, 1965). Prolonged administration of inhibitors was unnecessary and raised tissue concentrations of amines which might accumulate in these circumstances are not therefore essential for the reaction. Moreover, in the cat, the noradrenaline stores are not increased by amine oxidase inhibitors (Euler & Hellner-Bjorkman, 1955).

Tranylcypromine had marked sympathomimetic properties. Other amine oxidase inhibitors, such as α-methylbenzylhydrazine, had little pressor activity when first injected but after inactivation of monoamine oxidase second or subsequent doses produced marked rises of blood pressure. With tranylcypromine and second or subsequent doses of other inhibitors, tachyphylaxis readily developed to tyramine. These interactions between amine oxidase inhibitors and amines have not been previously reported and are being studied further. They would obviously modify the effects of tyramine or allied amines in cheese.

The results of these experiments in animals explain the occurrence of hypertension due to cheese after monoamine oxidase inhibition in man and animals, their more frequent occurrence with tranylcypromine and phenelzine in patients, and the variability in the sympathomimetic effects due to cheese. They also throw further light on the function of monoamine oxidase. This enzyme is widely distributed in the body and appears to serve a single biological role by limiting the effects of endogenous and exogenous amines in several ways. In the postganglionic sympathetic nerve it abbreviates the action of intraneuronal noradrenaline released by nerve impulses and also inactivates amines known to release noradrenaline; in the intestine and liver it deaminates potentially active metabolites.

The function of monoamine oxidase in the intestinal wall has appeared least significant and remained longest in doubt although the enzyme is often present in high concentration. The theory of intestinal amine absorption leading to adverse effects was elaborated in the " ptomaine " theory (Metchnikoff, 1905) but there has been little supportive evidence that this can occur. These experiments in three species eating various diets demonstrate the logical purpose of high concentrations of monoamine oxidase in the intestinal tract. The ingestion of preformed pressor amines in putrefied food such as cheese may be largely the prerogative of a sophisticated omnivore such as man, but in the carnivorous and herbivorous species tested amino acids in food might be decarboxylated by micro-organisms of the intestinal flora to form amines. At least one amino acid (dopa) had pressor activity which appeared to be due partly to its conversion to dopamine and which was potentiated after amine oxidase inhibition, as previously noted by Blaschko (1952).

SUMMARY

1. Saline extracts of cheese injected intravenously into the rat and cat elicited a rise in blood pressure which was prolonged after the intravenous injection of a monoamine oxidase inhibitor. The pressor activity varied tenfold between different cheeses.

2. After treatment of the animal with a monoamine oxidase inhibitor intraduodenal injection of homogenized cheese generally raised the blood pressure in the rat, fowl and cat and contracted the nictitating membrane in the cat.

3. The pressor effects of intraduodenally administered amine and amino acid constituents of cheese, tyramine, phenethylamine, tryptamine and dopa, were potentiated by monoamine

oxidase inhibition in the rat but depressor effects of cadaverine and histamine were unchanged.

4. The sympathomimetic effects produced by cheese were due to the presence of an indirectly acting amine whose effects were abolished by pharmacological antagonists at sympathetic α-receptors or by chronic sympathetic postganglionic denervation.

5. Tyramine in a pharmacologically active concentration was detected in the plasma after intraduodenal injection of cheese into a cat previously treated with an amine oxidase inhibitor.

6. The intrinsic pressor activity of monoamine oxidase inhibitors was enhanced after inactivation of monoamine oxidase. Tachyphylaxis sometimes developed between the amine oxidase inhibitors, tyramine and the pharmacologically active substances on cheese.

7. Sympathomimetic effects due to cheese were obtained in the cat after treatment with amine oxidase inhibitors of the amine, hydrazine and hydrazide varieties. Absorption of tyramine from the intestine depended on adequate inhibition of monoamine oxidase; this was obtained in the rat with smaller doses of tranylcypromine and phenelzine than with other inhibitors tested. Single or repeated doses of amine oxidase inhibitors administered by several different routes enhanced the sympathomimetic effects of tyramine placed in the gut. There was no clear advantage in prolonged or oral treatment.

One of us (B. B.) would like to acknowledge support from the Mental Health Research Fund. The other (E. M.) is grateful for support from the Bethlem Royal and Maudsley Hospitals Research Fund. A grant from Smith Kline & French Laboratories is gratefully acknowledged. Our thanks are due to Dr W. G. Dewhurst for the spectrofluorimetric analyses. We are grateful to Mr D. Allen and Mr M. Wallis for expert technical assistance and to Mrs C. Sutton for typing the manuscript. We are indebted to Abbott Laboratories for pargyline, Burroughs Wellcome for bretylium, I.C.I. for halothane and α-methylbenzylhydrazine, Pfizer for nialamide, Roche Products for iproniazid, isocarboxazid and pivhydrazine, Sandoz Products for methysergide, Smith Kline & French Laboratories for phenoxybenzamine and (±)-tranylcypromine, Smith & Nephew for phenoxypropazine and William Warner for phenelzine.

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