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THIOXANTHINES WITH POTENT BRONCHODILATOR AND CORONARY DILATOR PROPERTIES

BY

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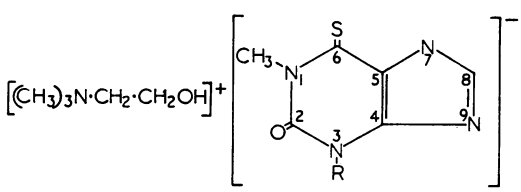
Some of the pharmacological properties of two new compounds, choline 6-thiotheophyllinate and the choline salt of 3-isobutyl-1-methyl-6-thioxanthine (M&B 5924), are described. Both these 6-thioxanthines are structurally related to theophylline and pharmacologically they are very similar to that compound, the main differences in their actions being essentially quantitative. They were more potent than choline theophyllinate as bronchodilators on the isolated guinea-pig tracheal ring preparation and as coronary dilators on the dog heart–lung preparation. Choline 6-thiotheophyllinate was about as effective as its oxygen analogue in protecting guinea-pigs against the lethal effects of a bronchoconstrictor aerosol; M&B 5924 was more effective in this respect, but the relative bronchodilator activity was much less than on the isolated preparation. Both thioxanthines were less potent than choline theophyllinate as diuretics. One outstanding qualitative difference in their properties was in their effect on the voluntary motor activity of mice; choline theophyllinate in low doses was stimulant whereas the thioxanthines were either inactive at low doses or sedative at higher doses. In dogs, on the other hand, there were indications that M&B 5924 had a stimulant action.

In the course of testing some new soluble theophylline derivatives, our colleague Mrs. S. Brazier discovered that the choline salt of 6-thiotheophylline (I, R = $-CH_3$) was about four times as active as its oxygen analogue in relaxing the bronchial muscle of the isolated guinea-pig tracheal ring preparation. 6-Thiotheophylline was also found to be a more potent coronary dilator than theophylline. As a result of these observations, other thioxanthines were synthesized and tested pharmaco-



(I)

logically in the hope of finding a compound which was highly active as a bronchodilator or as a coronary dilator, and which was well tolerated and absorbed when given orally. The present paper describes some of the pharmacological properties of such a compound, the choline salt of 3-isobutyl-1-methyl-6-thioxanthine, or M&B 5924 [I, R = $-CH_2.CH(CH_3)_2$].

METHODS

Bronchodilator activity

Isolated guinea-pig tracheal ring preparation. The activity of the compounds as bronchodilators was estimated *in vitro* on the isolated guinea-pig tracheal ring preparation, set up as described by Castillo & de Beer (1947). Changes in muscle tone were recorded on a kymograph by means of a suitably weighted frontal-writing lever. In preparations that rapidly lost tone and did not contract again when the dilator substance was removed from the bath, a low concentration of histamine (10^{-7} to 10^{-6} g/ml. of acid phosphate) was added to restore the tone to its original level. In some experiments, the effect of adding the dilator substance at the height of a histamine-induced contraction was observed. The presence of histamine did not appear to affect the quantitative assay.

Protection against bronchoconstrictor aerosols. In vivo bronchodilator activity was determined by observing whether the compounds would protect guinea-pigs from the bronchospasm induced by exposure to an acetylcholine or histamine aerosol. The guinea-pigs were placed in an anaesthetic box and aerosols were introduced by means of a compressor and a Collison inhaler. Guinea-pigs exposed to a bronchoconstrictor aerosol in this way behaved in a very characteristic manner and showed progressive signs of difficulty in breathing, leading to convulsions and finally death. With little experience the time until signs appeared of a convulsion being imminent (defined by Herxheimer (1952) as the pre-convulsion time) could be judged with considerable accuracy merely by observation of the breathing and behaviour of the guinea-pig. This pre-convulsion time was remarkably constant, if the guinea-pigs were not exposed too frequently. The protection was assessed quantitatively in a similar way to that described by Herxheimer & Rosa (1953). The percentage protection for each guinea-pig was calculated as $(1-\frac{T_1}{T_2})\times 100$, where T_1 is the mean of the control pre-convulsion times two days before and two days after the administration of bronchodilator and T_2 is the test pre-convulsion time determined after administration of the bronchodilator substance. Initially the protective effect of choline theophyllinate and choline thiotheophyllinate was investigated in 8 guinea-pigs each, exposing them 20 min after intraperitoneal injection to either a 2% histamine or 4% acetylcholine aerosol.

Experiments were later performed to see whether M&B 5924 and choline theophyllinate when given orally protected guinea-pigs and for approximately how long such protection lasted. Thirty-two guinea-pigs, deprived of food overnight, were next morning given the compounds by stomach tube, and subjected to a 4% acetylcholine aerosol in groups of 4, at 8 time intervals ranging from 0.5 to 6 hr. The degree of protection obtained by pretreatment with each compound was assessed in the way already described.

Protective effect in the anaphylactic microshock of the guinea-pig. The method used has been described by Herxheimer (1952). Guinea-pigs were sensitized by injection of egg albumen (0.7 ml. of a 5% solution intramuscularly) and 3 weeks later exposed to a 5% egg albumen aerosol. Sensitized animals showed typical symptoms of dyspnoea, whereas normal animals were unaffected. The protective effect of M&B 5924 was assessed by dosing the guinea-pigs orally with M&B 5924 1 hr before exposure to the antigen aerosol.

Microscopic examination of lung sections. The method used was that of Sollman & Gilbert (1937). A rabbit was killed, and the lungs and trachea were excised and filled with warm Ringer solution containing 10% gelatin. The gelatin was allowed to harden in the refrigerator and thin sections of lung were then cut by hand, pinned to a ring of cork and placed in a Petri dish containing Ringer solution at 37° C. After 30 min the gelatin had melted and the preparation had usually recovered from trauma. If the sections of lung contained a nearly transverse section of a bronchus or bronchiole it was possible to observe constriction of the bronchial musculature by addition of acetylcholine or histamine, and relaxation by addition of adrenaline or a theophylline compound. No attempt was made in these experiments to observe more than gross changes and the observer was always aware which drug was being applied to the tissue.

Antihistamine and anti-acetylcholine action on guinea-pig ileum

A piece of ileum was suspended in a 50 ml. bath containing Tyrode solution at 37° C and the tissue was oxygenated with a mixture of 95% O_2 and 5% CO_2. The composition of the Tyrode solution was Na^+ 149 mM, K^+ 2.7 mM, Ca^{++} 1.8 mM, Mg^{++} 1.0 mM, Cl^- 145 mM, $H_2PO_4^-$ 0.4 mM, HCO_3^- 12 mM and dextrose 0.1% (w/v). pA_2 M&B 5924-histamine and pA_2 M&B 5924-acetylcholine were determined with the antagonist in the bath for 14 min as described by Schild (1947). Choline theophyllinate and choline 6-thiotheophyllinate were tested only qualitatively for antihistamine and anti-acetylcholine action.

Cardiovascular studies

Effects on blood pressure. Cats and dogs were anaesthetized with chloralose (80 mg/kg). Blood pressure was recorded from a carotid artery and injections were made into a jugular vein.

Effects on the coronary vessels of the dog heart-lung preparation. The heart-lung preparation of the dog, with a Morawitz cannula in the coronary sinus, was used for measuring effects on coronary blood flow. The preparation was made as described by Burn, Vaughan Williams & Walker (1955). The venous inflow on the right side of the heart was maintained at a constant head of pressure by means of an overflow reservoir, and the excess blood was collected and returned to the reservoir by a Dale-Schuster pump. Changes in the flow of blood from the coronary sinus were recorded by the method of Stephenson (1949) and this coronary blood was then returned to the venous reservoir. The preparation was supplied with oxygen from a partially filled Douglas bag: the Douglas bag was connected to an oxygen cylinder via a flowmeter and the flow adjusted so that oxygen input exactly balanced oxygen consumption. These elaborations were made since changes in oxygen tension and venous pressure are known to affect coronary blood flow (Anrep, 1926). Injections were made into the rubber tubing attached to the cannula in the superior vena cava.

Effects on the vessels of the dog hind leg perfused with heparinized blood. The preparation was made as described by Burn and Rand (1958). Arterial resistance and venous outflow were recorded, the latter by means of a Stephenson recorder (1949).

Effects on the rate and amplitude of the isolated perfused rabbit heart and isolated rabbit atria. The heart was removed from a freshly killed rabbit and perfused with a modified Locke solution (McEwen, 1956) at 32° C by the Langendorff method. The composition of this solution was Na^+ 156 mM, K^+ 5.6 mM, Ca^{++} 2.2 mM, Cl^- 140 mM, $H_2PO_4^-$ 1.2 mM, HCO_3^- 25 mM, dextrose 0.2% (w/v) and sucrose 0.45% (w/v), and it was gassed with 95% O_2 and 5% CO_2. The heart was perfused for about 30 min until the rate and amplitude were steady; by turning a tap the perfusion was then continued with a solution containing a known concentration of M&B 5924.

In the experiments on isolated atria, the atria were carefully dissected from ventricular muscle and as much fat and connective tissue as possible removed, particular care being taken to avoid damage to the atria in the pacemaker region. They were suspended in a 50 ml. bath containing oxygenated double-dextrose Locke solution at 29° C. The composition of the Locke solution was Na^+ 160 mM, K^+ 5.6 mM, Ca^{++} 2.2 mM, Cl^- 164 mM, HCO_3^- 6.0 mM and dextrose 0.2% (w/v), and it was gassed with 100% O_2. Contractions were recorded by a straw lever writing on a smoked drum.

Diuretic activity

In conscious rats. Eight groups of 4 male rats, each weighing approximately 200 g, were used. They were deprived of food overnight and the following morning were given 25 ml./kg of 0.9% saline by stomach tube. Initially, all 8 groups were given saline alone and served as controls; 2 days later they were given orally the test compound dissolved in the appropriate quantity of saline. The animals in each group were then placed in a metabolism cage and the urine was collected and measured at hourly intervals in a graduated centrifuge tube for 5 and in some cases 7 hr. During the test period the rats were left without food and water.

The total volume of urine excreted by each group (V_1) was measured and the % urinary excretion (U) as defined by Lipschitz, Hadidian & Kerpscar (1943) was calculated for each group. If V_0 was the volume of saline originally given to each group, then $U = \frac{V_1}{V_0} \times 100$. There was considerable variation in U among the 8 groups, though the mean value for any given treatment was remarkably constant. The diuretic effects of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 were investigated at only one dose, namely, 20 mg/kg, as this dose was shown in a preliminary screening experiment to cause optimal diuresis. Urinary sodium and potassium concentrations were determined with a flame photometer and chloride concentrations were determined volumetrically by Volhard's method.

In anaesthetized rabbits. The method used has been described by Burn (1952). Rabbits of either sex were anaesthetized with urethane. The bladder was cannulated and urine flow was measured with a Thorp impulse counter.

Central effects in mice

Effects on the voluntary locomotor activity of mice were determined using the light-box method of Dews (1953). The dose causing a 50% increase or decrease in motor activity was determined.

Acute toxicity in mice

Intravenous and oral LD50 figures in albino mice were determined by the method of de Beer (1945). In the oral toxicity experiments the mice were deprived of food overnight and deaths were recorded for 2 to 3 days.

Effects on cats and dogs

Choline theophyllinate and M&B 5924 were given orally and intravenously to dogs, and orally to cats, to see whether they caused vomiting or other undesirable effects.

RESULTS

Bronchodilator activity

Isolated guinea-pig tracheal ring preparation. Preliminary experiments on choline theophyllinate showed that over a wide range of concentrations the relaxation of the bronchial muscle was linearly related to the log. concentration of theophylline derivative in the bath. For a quantitative assay, it was therefore considered adequate to determine the relaxation produced by only two concentrations of any compound and to assume a linear relationship between relaxation and concentration. The further assumption was made that the dose-response curves of choline theophyllinate and the thioxanthines were parallel and relative activity was calculated in terms of choline theophyllinate = 1, so that the larger the figure

TABLE 1

RELATIVE ACTIVITY OF TWO THIOXANTHINES TO CHOLINE THEOPHYLLINATE (=1) ON THE ISOLATED GUINEA-PIG TRACHEAL RING PREPARATION

Compound	Mean relative bronchodilator activity ± s.e.	Number of experiments
Choline theophyllinate	1	—
Choline 6-thiotheophyllinate	5 ± 0·4	5
M&B 5924	57 ± 6	12

the greater the activity. In assessing relative activity, no account was made of the fact that the mol. wt. of the three compounds were not all the same, but ranged from 283 for choline theophyllinate to 341 for M&B 5924.

The relative activity of choline theophyllinate and M&B 5924 varied considerably in different experiments. A mean of twelve determinations for M&B 5924 and

five determinations for choline 6-thiotheophyllinate (shown in Table 1) showed them to be respectively 57 times and 5 times more active than choline theophyllinate in relaxing the bronchial muscle of the isolated guinea-pig tracheal ring preparation. Part of an experiment which clearly illustrates the potency of M&B 5924 as a bronchodilator is shown in Fig. 1. Here it is seen that M&B 5924 in a concentration of 1.5 μg/ml. produced a relaxation of approximately similar size to that produced by choline theophyllinate in a concentration of 100 μg/ml.

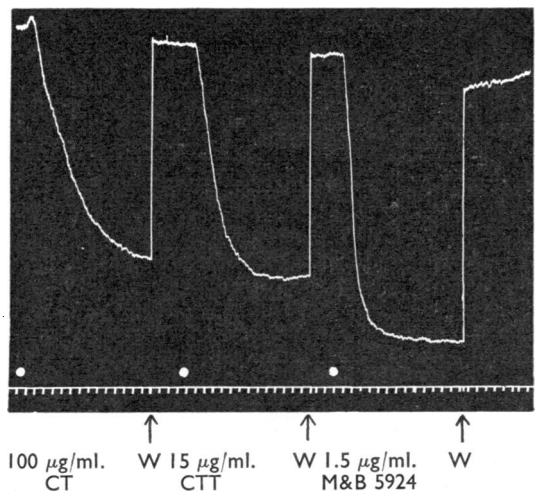


Fig. 1. Isolated guinea-pig tracheal ring preparation showing relaxations of the bronchial muscle of approximately similar size produced by choline theophyllinate (CT), choline 6-thiotheophyllinate (CTT) and M&B 5924. Time marker=60 sec. At W, the bath was washed out several times and the drum stopped until the preparation regained its original tone. Choline theophyllinate sometimes caused a small initial constriction before the dilatation as shown in this figure. This was possibly due to the high concentration of choline. When any of the three compounds were added to the bath, there was always a short latent period before the bronchial muscle relaxed.

Each compound was left in the bath for 15 min, by which time a maximum effect had been attained. Recovery to the original tone after washing out the bath usually took about 30 min and all three compounds appeared to be washed out with equal facility. Recovery time, however, was not measured accurately. An experiment in which choline theophyllinate (CT), choline 6-thiotheophyllinate (CTT) and M&B 5924 were assayed together on a single preparation is shown in Fig. 2.

Protection against bronchoconstrictor aerosols. Control pre-convulsion times were usually between 45 and 90 sec, and if the test pre-convulsion time was greater than 10 min the protection was assumed to be 100%. If the guinea-pigs were accidentally exposed to the bronchoconstrictor aerosol for too long, they convulsed and sometimes died. This happened occasionally with both normal and treated guinea-pigs. If the test pre-convulsion time (T_2) of a guinea-pig that subsequently died had already been determined, then the % protection was

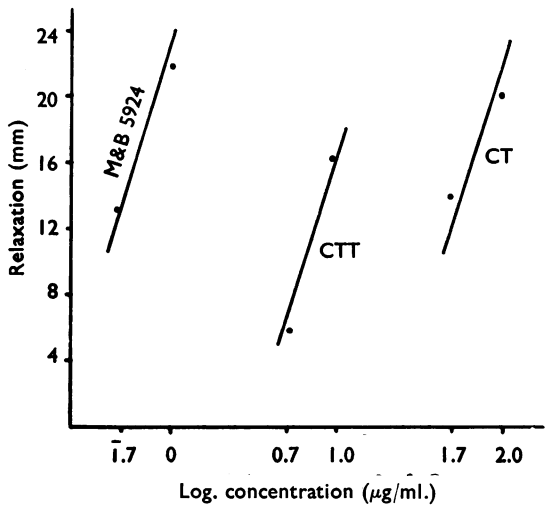


Fig. 2. Quantitative assessment of bronchodilator activity in an experiment on isolated guinea-pig tracheal ring preparation. The mean slope of the three lines was calculated and the three lines were then drawn parallel. Note that the observed relaxations lie very close to the calculated dose/response curves. CT=choline theophyllinate; CTT=choline 6-thiotheophyllinate. Abscissae: log. concentration (μg/ml.). Ordinates: relaxation (mm).

TABLE 2

PROTECTION AGAINST BRONCHOCONSTRICTOR AEROSOLS

Guinea-pigs were injected intraperitoneally with one of the compounds and after 20 min exposed to either a 2% histamine or a 4% acetylcholine aerosol. The mean % protection was calculated as described in the text

Compound	Dose (mg/kg)	Mean % protection ± s.e.	
		Acetylcholine	Histamine
Choline theophyllinate	50	30±6	19±8
	100	61±3	69±8
Choline 6-thiotheophyllinate	50	27±5	26±6
	100	57±3	65±4
Promethazine hydrochloride	0·5	14±8	69±18
	1	23±4	100±0
Atropine sulphate	1	80±14	2±12
	3	100±0	39±3

calculated using only one control pre-convulsion time, and this figure was used in the calculation of the mean % protection of the group.

The results of the experiments on choline theophyllinate and choline 6-thiotheophyllinate, in which protection was assessed 20 min after intraperitoneal administration, are shown in Table 2. The protective effect of choline theophyllinate was almost identical with that of choline 6-thiotheophyllinate against histamine and acetylcholine aerosols. Also included in Table 2 are promethazine and atropine, showing that promethazine gave good protection against histamine aerosols and poor protection against acetylcholine aerosols, whereas the opposite occurred with atropine.

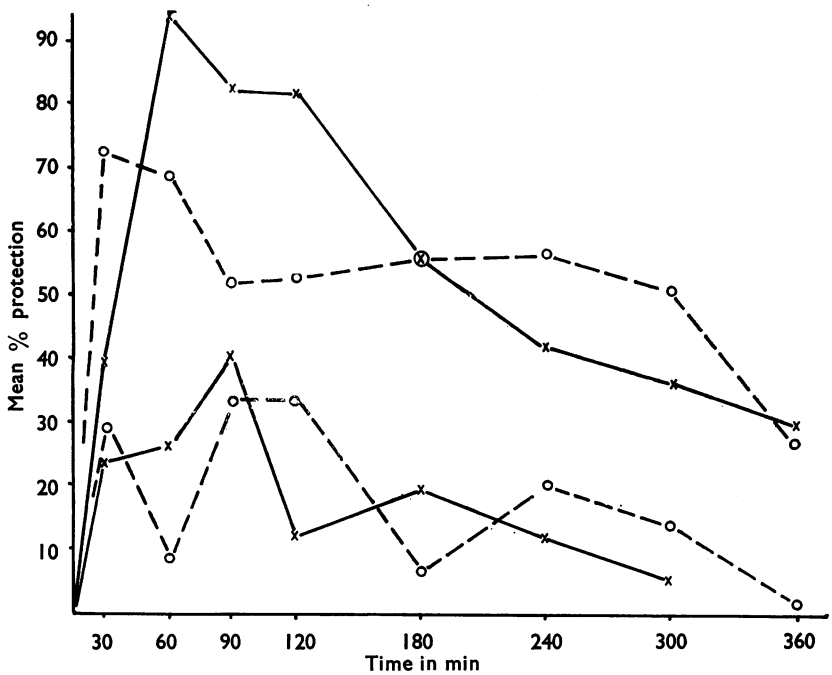


Fig. 3. The protection of choline theophyllinate and M&B 5924 in guinea-pigs exposed to an acetylcholine aerosol. Abscissae: time in min. Ordinates: mean % protection (see text). Each point represents observations on four guinea-pigs. X——X=M&B 5924 (upper curve 100 mg/kg and lower curve 25 mg/kg). O - - - O=choline theophyllinate (upper curve 200 mg/kg and lower curve 50 mg/kg). Note that there was good protection with the high dose of both compounds for the first 2 hr, and still some protection after 6 hr.

M&B 5924 was not tested in this way but was compared with choline theophyllinate for its ability when given by mouth to protect guinea-pigs against acetylcholine aerosols. The results of these experiments are shown graphically in Fig. 3. The lower dose of 50 mg/kg choline theophyllinate corresponds to a dose of 3.5 g in man, which is rather more than is generally given in one day. Although only 4 guinea-pigs were used for each observation, the results clearly show that maximum protection occurred after 1 hr and that there was still some protection at the higher doses after 6 hr. The protection with M&B 5924 (100 mg/kg) appeared to be more complete than with choline theophyllinate (200 mg/kg), but the results were not examined statistically. The high dose of each compound approximated to the maximum single tolerated dose.

Protective effect in the anaphylactic microshock of the guinea-pig. The mean pre-convulsion time of 8 untreated and sensitized guinea-pigs exposed to a 5% aerosol of egg albumen was 61 sec, whereas for 8 other guinea-pigs pretreated with M&B 5924 (100 mg/kg) the pre-convulsion time for all 8 was greater than 4 min. At this dose, the protection was therefore assumed to be complete.

Microscopic examination of lung sections. Qualitative observations only were made, and the concentration of choline theophyllinate required to dilate the

bronchioles by a given amount was as much as 50 times greater than the concentration of M&B 5924 required to cause what was judged to be a similar dilatation.

Antihistamine and anti-acetylcholine action on guinea-pig ileum

The protective effect of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 against acetylcholine and histamine aerosols could be explained by an antihistamine or anti-acetylcholine action, although in view of the similar protection against both types of aerosol this did not seem very likely. Experiments were therefore made on the guinea-pig ileum to see if the compounds had a specific antihistamine or anti-acetylcholine action. Large concentrations of all three compounds were required to reduce the stimulant effect of acetylcholine or histamine. Whilst choline theophyllinate was in the bath the effects of acetylcholine and histamine were actually potentiated. After the choline theophyllinate had been washed out of the bath, however, the effects of acetylcholine and histamine were reduced. With choline 6-thiotheophyllinate and M&B 5924 the phase of potentiation was not seen. All three compounds had very little antihistamine and anti-acetylcholine activity. pA_2 values were determined only for M&B 5924, the pA_2 (histamine) being 4.8 and pA_2 (acetylcholine) being 4.2.

Cardiovascular studies

Effects on blood pressure. In the anaesthetized cat and dog, choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 all caused a transient fall in blood pressure, which in the case of choline theophyllinate was preceded by a transient rise. Doses of the order of 1 mg/kg choline theophyllinate produced a fall in blood pressure similar to that caused by 0.05 to 0.1 mg/kg M&B 5924.

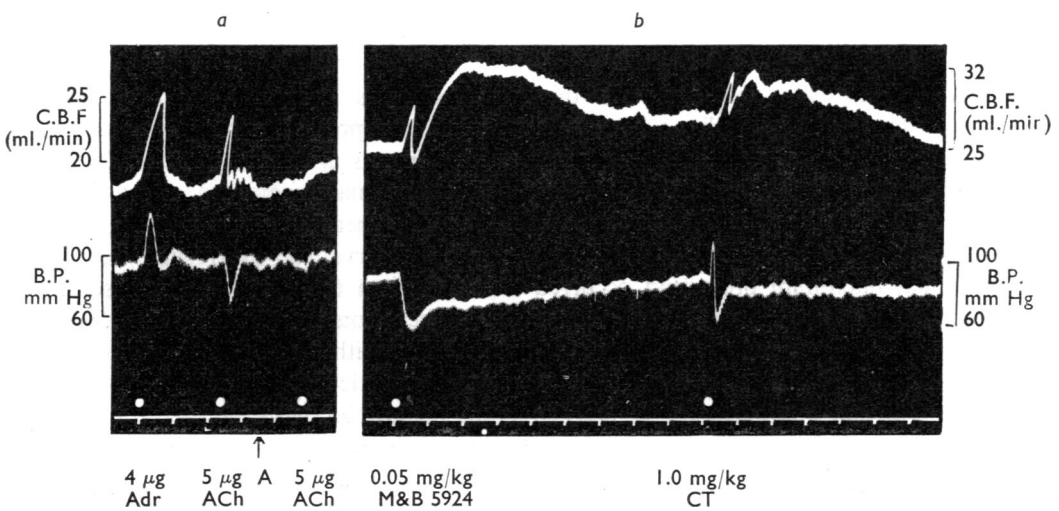


Fig. 4. Dog, 10 kg, chloralose anaesthesia. Upper record: coronary blood flow; lower record: blood pressure in carotid artery. Adr=adrenaline, ACh=acetylcholine, A=1 mg atropine sulphate and CT=choline theophyllinate. Time marker=60 sec. The time interval between (*a*) and (*b*) was 17 min. Note that in spite of the prolonged fall in systemic blood pressure caused by M&B 5924, there was a prolonged increase in coronary blood flow. (A small part of the blood pressure record in (*b*) has been retouched.)

The lower tracing (Fig. 4) shows the effect of 0.05 mg/kg M&B 5924 and 1.0 mg/kg choline theophyllinate on the blood pressure of a dog under chloralose anaesthesia. Choline 6-thiotheophyllinate was more effective than choline theophyllinate in lowering the blood pressure, but less effective than M&B 5924. These falls in blood pressure were only very slightly reduced by atropine and mepyramine.

Effects on the coronary vessels of the dog heart–lung preparation. Coronary blood flow in this paper indicates the blood flowing into the coronary sinus, which Evans and Starling (1913) showed to be about three-fifths of the total blood passing through the coronary arteries.

The coronary blood flow at the start of an experiment was usually about 20 to 30 ml./min. At this stage, the preparation was sensitive to small doses (1 mg and less) of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 injected close to the superior vena cava, the volume of circulating blood being approximately one litre. Such doses sometimes caused a 50% increase in coronary flow, without causing any appreciable effect on the heart rate or output. An experiment in which the coronary blood flow was unusually steady is shown in Fig. 5. It is seen that 1.5, 3 and 6 mg of choline 6-thiotheophyllinate caused respectively greater increases in coronary blood flow than 1.5, 3 and 6 mg of choline theophyllinate. Left cardiac output, recorded continuously in this experiment by means of a Stephenson recorder, was not greatly affected by any of these injections and the blood pressure in the lower tracing (=resistance in the artificial circuit) was steady throughout. The coronary blood flow in the dog heart–lung preparation increases slowly but

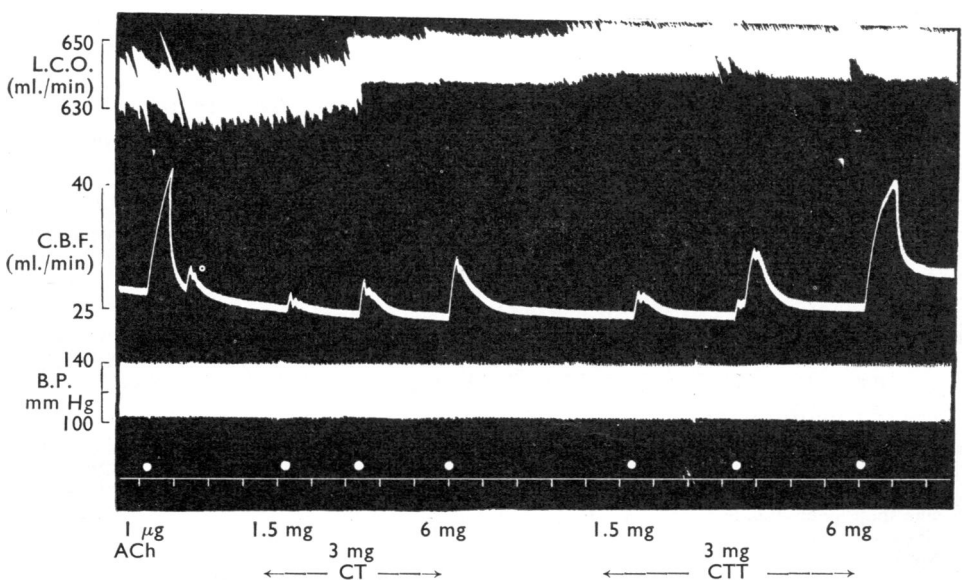


Fig. 5. Dog heart–lung preparation showing left cardiac output (upper record), coronary blood flow (middle record) and blood pressure (lower record). The calibration is shown at the left-hand side of the tracing. ACh=acetylcholine, CT=choline theophyllinate and CTT=choline 6-thiotheophyllinate. All injections were made close to the superior vena cava. Time marker=60 sec.

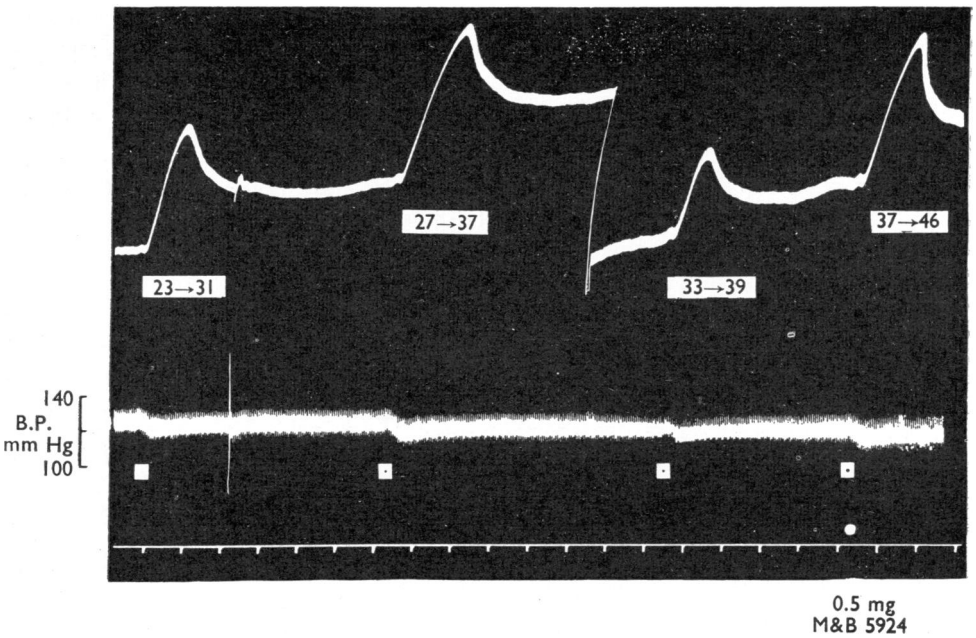


Fig. 6. Record as in Fig. 5, except that left cardiac output was not recorded. At each dot, a xanthine or thioxanthine was injected into the superior vena cava, the fourth compound being M&B 5924 (0.5 mg). Time marker=60 sec. Note that the coronary blood flow gradually increased in this experiment, from 23 ml./min initially to 46 ml./min 20 min later. With each injection there was an increase in coronary blood flow, but only partial recovery to the level before the injection.

spontaneously even when no dilator substances are injected. In the experiment shown in Fig. 5, the coronary blood flow was unusually steady; the more usual course is shown in Fig. 6.

In this experiment, in the course of 20 min, during which two xanthines and two thioxanthines, including M&B 5924, were injected into the superior vena cava, the resting coronary blood flow increased from 23 to 46 ml./min. When the

TABLE 3

CORONARY VASODILATOR ACTIVITY (CDA) ON THE DOG HEART-LUNG PREPARATION AND VASODILATOR ACTIVITY (VDA) ON THE PERFUSED VESSELS OF THE DOG LEG EXPRESSED RELATIVE TO CHOLINE THEOPHYLLINATE=1

Compound	Mean relative CDA	Range of CDA and no. of expts. in parentheses	Mean relative VDA	Range of VDA and no. of expts. in parentheses
Choline theophyllinate	1	—	1	—
Choline 6-thiotheophyllinate	2·5	2–4·5 (6)	2·5	— (1)
M&B 5924	15	8–24 (14)	14	12–16 (3)

coronary blood flow was continually increasing as in this experiment, it was difficult to get more than a rough estimate of coronary dilator activity, because the sensitivity to a given dose of any compound gradually diminished. The coronary dilator activities quoted in Table 3 are therefore only approximations and assessed

from small coronary dilator effects. Doses as high as 10 or 15 mg choline theophyllinate were sometimes required to match the dilator effects of much smaller doses of M&B 5924. Doses of choline theophyllinate as high as this caused noticeable stimulation of the heart, and, whereas the flow reached a peak and then fell to a steady level with small doses of choline theophyllinate, the flow increased and remained high with larger doses. The figures in Table 3 are expressed as the ratios of the dose of choline theophyllinate producing a given increase in coronary blood flow to the dose of test compound producing a similar increase in flow. In some of the experiments on M&B 5924, however, the coronary dilator activity of this compound was not compared directly with choline theophyllinate but with a compound whose activity relative to choline theophyllinate had been determined in previous experiments. The duration of the effect, which was usually short and less than 5 min, is therefore not included in the assessment of coronary dilator activity. The useful " life " of the preparation was about 1 hr ; by this time the coronary blood flow was usually 3 or 4 times greater than at the beginning of the experiment.

Small doses of M&B 5924 (less than 1 mg) had very little effect on the heart rate and no noticeable effect on the output of the left side of the heart. Output was measured by interrupting the flow of blood from the brachiocephalic artery to the reservoir, in the artificial circuit, and collecting the blood in a measuring cylinder for 15 sec. When the dose of M&B 5924 was increased to 5 mg or 10 mg, the heart rate sometimes increased by as much as 20% and the coronary vessels became fully dilated, giving a coronary blood flow of up to 120 ml./min or an increase of about 100 ml./min over the initial value. At this stage, the left cardiac output was invariably less than at the start of the experiment by an amount approximately equal to the increase in coronary blood flow. In spite of these changes in blood flow, M&B 5924 therefore has no effect on the *total* output of the heart.

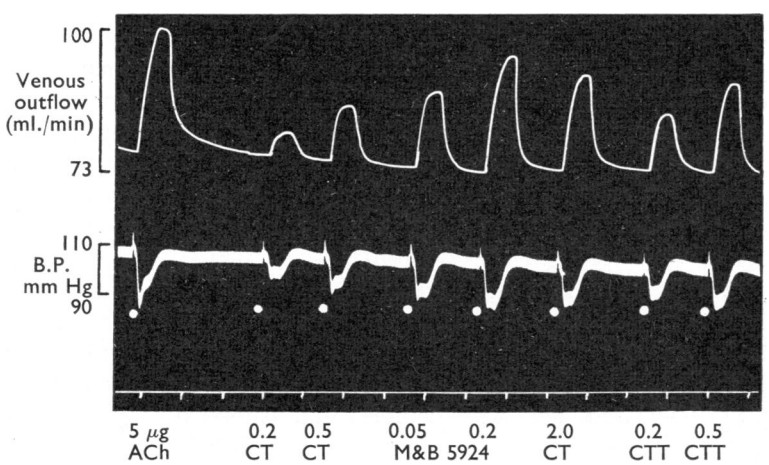


Fig. 7. Perfused vessels of the dog hind leg showing venous outflow (upper record) and arterial resistance=B.P. (lower record). The calibration is shown at the left-hand side of the tracing. ACh=acetylcholine, CT=choline theophyllinate and CTT=choline 6-thiotheophyllinate. All doses were injected intra-arterially, the figures with CT, M&B 5924 and CTT referring to the dose in mg. Time marker=60 sec.

In the anaesthetized animal, M&B 5924 also increased the coronary blood flow (Fig. 4, upper tracing).

Effects on the vessels of the dog hind leg perfused with heparinized blood. The vasodilator activity of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 was compared on the vessels of the dog hind leg perfused with heparinized blood. Fig. 7 shows the dilator effects of 0.2 mg, 0.5 mg and 2.0 mg choline theophyllinate (CT), 0.05 mg and 0.2 mg M&B 5924, and 0.2 mg and 0.5 mg choline 6-thiotheophyllinate (CTT). The bottom tracing shows a fall in arterial resistance and the upper tracing an increase in venous outflow, when injections were made into the arterial cannula. The vasodilator activity of the three compounds on this preparation was assessed by bracketing dilator responses caused by different doses, and was almost identical with the coronary dilator activity in the heart–lung preparation (Table 3). Relative activity was calculated in terms of choline theophyllinate = 1.

Effects on the rate and amplitude of the isolated perfused rabbit heart and isolated rabbit atria. When the isolated rabbit heart was perfused with a solution containing 10^{-6} g/ml. M&B 5924, the rate increased by a mean of 22% in 4 different experiments. Effects on the amplitude were variable. Choline theophyllinate and choline 6-thiotheophyllinate were not tested on this preparation, but Schachter, Kimura, Nowarra & Mestern (1954) stated that choline theophyllinate increases the rate and amplitude of the isolated perfused rabbit heart only in concentrations of the order 5×10^{-5} g/ml. On two isolated rabbit atrial preparations, choline theophyllinate (10^{-4} g/ml.) was without any effect apart from a slight slowing; on the other hand, choline 6-thiotheophyllinate (10^{-5} g/ml.) and M&B 5924 (10^{-6} g/ml.) both caused an increase in rate and amplitude.

Diuretic activity

Conscious rats. The results of the experiments are shown in Fig. 8 and Table 4. Chlorothiazide and choline theophyllinate both caused an increase in urine excretion in the first 5 hr, but the diuresis of the rats treated with choline 6-thiotheophyllinate and M&B 5924 closely followed that of the controls. Between 4 and 7 hr, however, choline 6-thiotheophyllinate and M&B 5924 caused a diuresis similar to that caused by choline theophyllinate in the first 4 hr. The effects of these thioxanthines on the excretion of Na^+, K^+, and Cl^- in the first 5 hr are shown in Table 4, together with similar data for chlorothiazide and choline theophyllinate.

In a dose of 20 mg/kg, choline theophyllinate caused a 100% increase in excretion of sodium; choline 6-thiotheophyllinate and M&B 5924 caused increases of 11% and 38% respectively. Chlorothiazide in a dose of 10 mg/kg increased sodium excretion by more than 100%. With all three compounds potassium excretion was increased, and with choline 6-thiotheophyllinate and M&B 5924 chloride excretion was depressed. Although choline 6-thiotheophyllinate and M&B 5924 have been studied in detail only at the one dose level of 20 mg/kg, it is evident that they are of no interest as diuretics.

Anaesthetized rabbits. On this preparation the diuretic activity of the compounds was compared by giving an intravenous infusion in 40 ml. of physiological saline

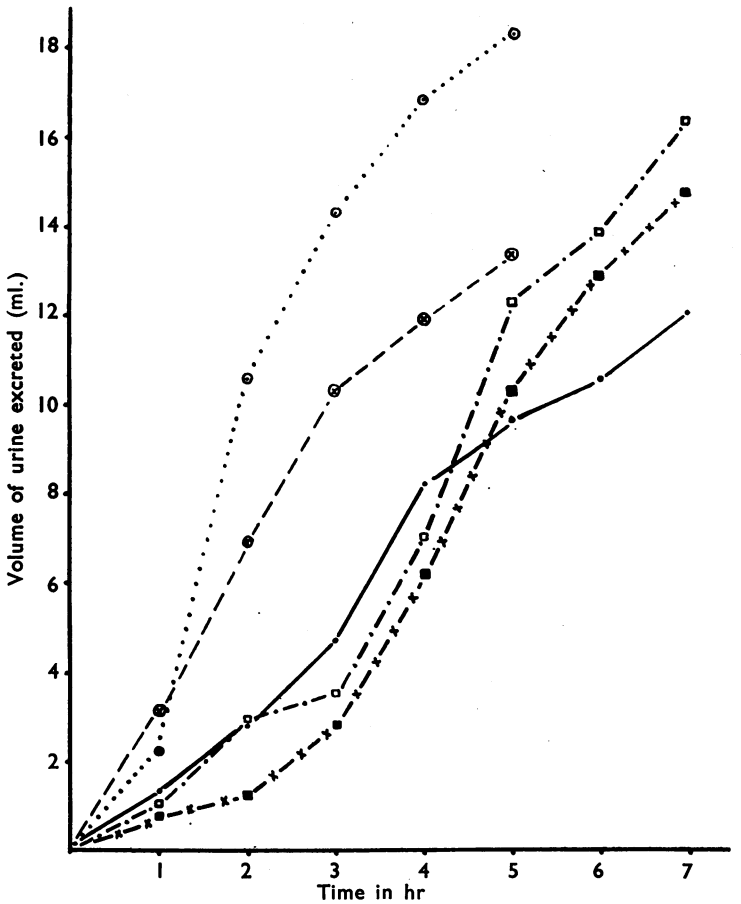


Fig. 8. Diuresis in conscious rats. Abscissae: time in hr. Ordinates: volume of urine excreted in ml. ○ . . . ○=chlorothiazide ; ⊗ – – – ⊗=choline theophyllinate ; □–·–□ =choline 6-thiotheophyllinate ; ■–×–×–■=M&B 5924 ; [and ●———●=controls. Note the powerful and rapid diuretic action of chlorothiazide and the delayed diuresis caused by the thioxanthines.

TABLE 4

URINE AND ELECTROLYTE EXCRETION IN RATS

Compound	Dose (mg/kg)	% urinary excretion	Ionic excretion μeq/100 g/5 hr			pH of urine	Na^+/K^+
			Na^+	K^+	Cl^-		
Controls	—	32	89	62	152	6·0	1·44
Choline theophyllinate	20	50	180	102	239	7·0	1·76
Choline 6-thio-theophyllinate	20	40	99	109	96	7·1	0·91
M&B 5924	20	37	123	79	132	6·8	1·56
Chlorothiazide	10	66	201	90	288	6·2	2·24

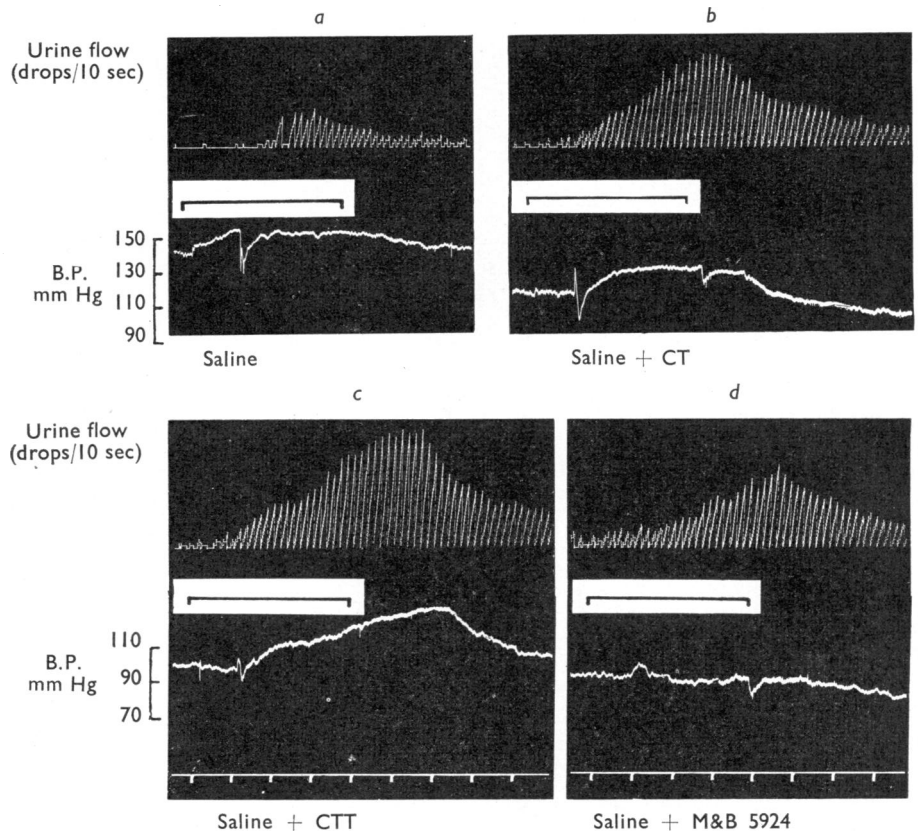


Fig. 9. Diuresis in the anaesthetized rabbit, showing flow of urine in drops recorded by mean of a Thorp impulse counter (upper record) and blood pressure (lower record). In each section an intravenous saline infusion was given over 4 min, as shown by the continuous line; in sections (*b*), (*c*) and (*d*) the saline contained the calculated amount of compound to give a dose of 10 mg/kg. The time interval between the sections was about 20 min. CT=choline theophyllinate; CTT=choline 6-thiotheophyllinate. Time marker=60 sec. Note that the diuresis caused by M&B 5924 was considerably less than that due to choline theophyllinate or choline 6-thiotheophyllinate.

during 4 min. An experiment of this type is shown in Fig. 9. In (*a*) is shown the control diuretic effect of 40 ml. of physiological saline alone. In (*b*) a much larger and prolonged diuresis was caused by 10 mg/kg choline theophyllinate (CT). Choline 6-thiotheophyllinate (CTT) in the same dose was more active than choline theophyllinate (*c*), whereas in (*d*), M&B 5924 caused an effect less than that of choline theophyllinate but greater than that of the control infusion of saline. The sensitivity to any substance and even to the saline alone was liable to change during the experiment, and when the blood pressure fell below 70 mm Hg diuresis sometimes stopped. Effects on the blood pressure were variable though there was usually a slight rise followed by a very slow fall.

In four experiments, choline theophyllinate and choline 6-thiotheophyllinate were found to be about equiactive, whereas M&B 5924 was consistently less active than both these compounds.

Central effects in mice

In doses up to 20 mg/kg choline theophyllinate increased the voluntary locomotor activity of mice. A 50% increase was produced by 12 mg/kg. Choline 6-thiotheophyllinate and M&B 5924, however, in doses from 5 to 80 mg/kg either decreased motor activity or had no effect. A 50% decrease in motor activity was produced by 32 mg/kg choline 6-thiotheophyllinate and by 30 mg/kg M&B 5924.

Acute toxicity in mice

The acute oral and intravenous LD50 figures in mice of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 are shown in Table 5. The low values of the ratio B/A indicate that all three compounds were well absorbed from the gastro-intestinal tract. Toxic doses of choline theophyllinate caused intense excitement and convulsions, whereas toxic doses of the thioxanthines caused sedation. Death in all cases was due to respiratory failure.

TABLE 5

ACUTE TOXICITY IN MICE

LD50 figures (mg/kg) in terms of active component and limits ($P=0{\cdot}05$) shown in parentheses

Compound	Intravenous (A)	Oral (B)	Oral/intravenous (B/A)
Choline theophyllinate	74 (70–80)	337 (308–368)	4·5
Choline 6-thiotheophyllinate	39 (34–45)	260 (210–322)	6·7
M&B 5924	51 (47–54)	163 (143–186)	3·2

Effects on cats and dogs

Observations were first made on four dogs, which were given the compounds daily by stomach tube for one week. Choline theophyllinate in a dose as high as 120 mg/kg caused no ill-effects ; M&B 5924, however, caused vomiting and retching at 60 and 80 mg/kg and these effects lasted for about 1 hr after dosing. During this time, the dogs were very quiet, but they had fully recovered within 2 or 3 hr. At 20 mg/kg, M&B 5924 was without effect, and though 40 mg/kg did not make them vomit, this dose obviously caused them distress.

M&B 5924 was given intravenously to 5 dogs in doses up to 3 mg/kg. This dose caused vomiting and retching in 2 of the dogs within 10 min of dosing. Other symptoms included excitation and restlessness, in contrast to the sedation seen in mice. Lower doses were usually without behavioural effects and did not cause vomiting. It was noticed that after an injection of M&B 5924, the dogs sometimes had diarrhoea. Choline theophyllinate, however, in intravenous doses up to 5 mg/kg did not affect 3 other dogs in any way.

The effects of choline theophyllinate and M&B 5924 given to cats in gelatin capsules were also investigated. Both compounds caused salivation (not seen in dogs), but whereas M&B 5924 caused vomiting and retching in similar doses to those tried in dogs, much higher doses of choline theophyllinate were without effect.

DISCUSSION

The diuretic, bronchodilator and coronary dilator properties of theophylline have been known for many years (Goodman & Gilman, 1955). Theophylline itself, however, is an almost insoluble compound which is poorly absorbed and in addition

is highly irritating to the gastric mucosa. Aminophylline, a double salt of theophylline with ethylenediamine, is much more soluble and is a useful and effective drug when given intravenously in the treatment of bronchial and cardiac asthma and Cheyne Stokes respiration, possibly on account of its ethylenediamine content. When given orally, however, aminophylline is relatively poorly absorbed and in sufficiently high doses to produce a therapeutically effective blood level is poorly tolerated. Choline theophyllinate is a theophylline preparation that is claimed to be better tolerated and absorbed than aminophylline after oral administration (Gagliani, de Graff & Kupperman, 1954). Duesel & Fand (1954) have suggested that the reason for the better tolerance of choline theophyllinate is that it is a true chemical compound, stable in the acid secretions of the stomach, whereas aminophylline is merely an addition compound and is unstable under these conditions. The rationale for the better tolerance of choline theophyllinate does not seem very convincing and whether the choline moiety is important seems dubious. Nevertheless, the thioxanthines were tested as their choline salts, since this salt for both compounds could be prepared in a crystalline form which was readily soluble in water and was therefore very suitable for pharmacological study.

In most of their pharmacological properties, choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 differed only quantitatively. Both the thio-compounds were more active than choline theophyllinate as bronchodilators and coronary dilators, but less active as diuretics. Choline theophyllinate, however, showed stimulant properties in increasing the voluntary motor activity of mice, whereas its thio-analogue and M&B 5924 both decreased motor activity.

In view of the high bronchodilator activity of M&B 5924 on the isolated guinea-pig tracheal ring preparation, the protection this compound gave to guinea-pigs exposed to bronchoconstrictor aerosols was surprisingly low. This was unexpected since the ratio of oral LD50: intravenous LD50 in mice (Table 5) was low, suggesting that oral absorption was good. It is possible, however, that oral absorption in guinea-pigs was not as good as in mice.

The extremely weak antihistamine and anti-acetylcholine action of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 on the guinea-pig ileum and the almost identical protection that choline theophyllinate and choline 6-thiotheophyllinate afforded against acetylcholine and histamine aerosols suggested that the protection was due to a genuine bronchodilator action and not due to an anti-acetylcholine or antihistamine action. Aminophylline in these experiments was incidentally found to be very similar in activity to choline theophyllinate. Herxheimer & Rosa (1953) investigated the protective effect of sympathomimetic amines and of aminophylline in the anaphylactic microshock of the guinea-pig. With high doses of aminophylline (100 mg/kg) full protection was achieved, but the sympathomimetic amines, isoprenaline, adrenaline, noradrenaline and ephedrine, all caused toxic symptoms before full protection was achieved. In our experiments, M&B 5924 in the dose of 100 mg/kg also afforded full protection.

The observations on lung sections showed that the dilatation caused by M&B 5924 was not merely confined to the bronchial muscle of the trachea but acted presumably on all the smooth muscle of the bronchial tree. Our experiments indicate that M&B 5924 was more active than choline theophyllinate. Offset

against the increased activity, however, is the possibility that sufficiently high blood levels may not be achieved for a therapeutic effect.

The experiments on the dog heart–lung preparation clearly demonstrated that choline 6-thiotheophyllinate and M&B 5924 were more effective than choline theophyllinate in increasing the flow of blood through the coronary vessels. Under the conditions of the heart–lung experiments, the venous pressure was constant, and, since the blood from the left side of the heart was diverted through a fixed peripheral resistance, generalized vasodilatation was not possible. Coronary dilator effects were observed with small doses of M&B 5924, which had no effect on heart rate, counted for 15 sec with a stop-watch, or cardiac output. It is therefore likely that the increased coronary blood flow was due to a direct action on the coronary vessels. Larger doses (5 to 10 mg) of choline theophyllinate and the thioxanthines, however, caused an increase in heart rate, and with M&B 5924 very large increases in coronary flow. A cardio-accelerator action was also observed on the isolated atrial preparation and was therefore probably due to an effect on the pacemaker.

In the whole animal, the effect on coronary blood flow was complicated by the fall in systemic blood pressure which these compounds caused. Nevertheless, in spite of the relatively prolonged hypotensive action of 0.05 mg/kg M&B 5924 in the dog (Fig. 4), there was also an increase in coronary blood flow. Le Roy & Speer (1940) have reported that various dialkylxanthines are more potent coronary dilators than theophylline in the anaesthetized dog, and Quimby, Aviado & Schmidt (1958) have reported the effects of aminophylline and other xanthines on the pulmonary circulation of the dog. Quimby *et al.* show the chemical structures of xanthines, including some thioxanthines, that caused non-selective vasodilatation and vasoconstriction. These authors state that replacement of oxygen in the 2- or 6- position did not affect dilator action, but it is not clear which thioxanthines were examined. 1,3-Dialkylthioxanthines, however, do not appear to be excluded from their study. We have not studied the effects of M&B 5924 on the pulmonary circulation, but in addition to dilating the coronary vessels it also dilated the vessels of the hind leg of the dog. M&B 5924 is therefore also likely to be a non-selective dilator and it is most likely that the main cause of its hypotensive action is vasodilatation of the peripheral vessels.

By comparison with chlorothiazide, the thioxanthines were relatively ineffective diuretics. When rats were given choline 6-thiotheophyllinate or M&B 5924 orally, the diuresis in the next 5 hr scarcely differed from that of control rats, although a marked diuresis usually occurred between 5 and 8 hr. One possible explanation of this delayed diuresis is that the $>C=S$ group is converted in the body to $>C=O$.

The only experimental comparison of the central properties of the thioxanthines with those of theophylline has been by observation of the effects on the voluntary locomotor activity of mice. It was interesting to find that both thio-compounds were weakly sedative whereas theophylline was weakly stimulant. One of the objects of this research was to find a compound which would relieve bronchospasm and consequent restlessness in the chronic asthmatic ; a compound with a sedative action as opposed to the stimulant action of theophylline would therefore be an

advantage. In dogs, however, M&B 5924 appeared to have a stimulant rather than a sedative effect. The fact that M&B 5924 caused vomiting in some dogs when given intravenously also suggests a central stimulant action. There is therefore the possibility that, when given orally, M&B 5924 may cause vomiting by a local irritant action in the stomach and after absorption by a central mechanism. The emetic properties of M&B 5924 and related compounds are being studied in more detail.

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REFERENCES

ANREP, G. V. (1926). The regulation of the coronary circulation. *Physiol. Rev.*, **6**, 596–629.

BURN, J. H. (1952). *Practical Pharmacology*, p. 53. Oxford: Blackwell.

BURN, J. H., VAUGHAN WILLIAMS, E. M. & WALKER, J. M. (1955). The effects of acetylcholine in the heart-lung preparation including the production of auricular fibrillation. *J. Physiol. (Lond.)*, **128**, 277–293.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol. (Lond.)*, **144**, 314–336.

CASTILLO, J. C. & DE BEER, E. J. (1947). The tracheal chain. I. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. *J. Pharmacol. exp. Ther.*, **90**, 104–109.

DE BEER, E. J. (1945). The calculation of biological assay results by graphic methods. The all-or-none type of response. *J. Pharmacol. exp. Ther.*, **85**, 1–13.

DEWS, P. B. (1953). The measurement of the influence of drugs on voluntary activity in mice. *Brit. J. Pharmacol.*, **8**, 46–48.

DUESEL, B. F. & FAND, T. I. (1954). Choline theophyllinate, a new stable theophylline derivative of enhanced solubility. *Int. Rec. Med.*, **167**, 245–247.

EVANS, C. L. & STARLING, E. H. (1913). The part played by the lungs in the oxidative processes of the body. *J. Physiol. (Lond.)*, **46**, 413–434.

GAGLIANI, J., DE GRAFF, A. C. & KUPPERMAN, H. S. (1954). Enhanced theophylline blood levels obtained after ingestion of choline theophyllinate as compared to aminophylline. With or without aluminium hydroxide. *Int. Rec. Med.*, **167**, 251–255.

GOODMAN, L. & GILMAN, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 340. New York: The Macmillan Company.

HERXHEIMER, H. (1952). Repeatable "microshocks" of constant strength in guinea-pig anaphylaxis. *J. Physiol. (Lond.)*, **117**, 251–255.

HERXHEIMER, H. & ROSA, L. (1953). The protective effect of sympathomimetic amines and of aminophylline in the anaphylactic microshock of the guinea-pig. *Brit. J. Pharmacol.*, **8**, 177–180.

LE ROY, G. V. & SPEER, J. H. (1940). A comparison of the coronary vasodilator activity of certain alkyl xanthines. *J. Pharmacol. exp. Ther.*, **69**, 45–51.

LIPSCHITZ, W. L., HADIDIAN, Z. & KERPSCAR, A. (1943). Bioassay of diuretics. *J. Pharmacol. exp. Ther.*, **79**, 97–110.

MCEWEN, L. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol. (Lond.)*, **131**, 678–689.

QUIMBY, C. W., AVIADO, D. M. & SCHMIDT, C. F. (1958). The effects of aminophylline and other xanthines on the pulmonary circulation. *J. Pharmacol. exp. Ther.*, **122**, 396–405.

SCHACHTER, R. J., KIMURA, E. T., NOWARRA, G. M. & MESTERN, J. (1954). Pharmacology of choline theophyllinate. *Int. Rec. Med.*, **167**, 248–250.

SCHILD, H. O. (1947). pA, a new scale for the measurement of drug antagonism. *Brit. J. Pharmacol.*, **2**, 189–206.

SOLLMAN, T. & GILBERT, A. J. (1937). Microscopic observations of bronchiolar reactions. *J. Pharmacol. exp. Ther.*, **61**, 272–285.

STEPHENSON, R. P. (1949). An apparatus for recording the output and coronary flow in the heart-lung preparation. *J. Physiol. (Lond.)*, **108**, 102–103.